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Changes in the Expression of Various Transporters as Influencing Factors of Resistance to Cisplatin

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Abstract. *Background: Changes in the expression of transporters have been reported as factors in resistance to cisplatin (CDDP). This study was designed to clarify whether CDDP-resistant strains isolated from a cell line had the same characteristics, and whether these characteristics could be therapeutic targets. Materials and Methods: Intracellular platinum levels were determined by the inductively-coupled plasma method. mRNA expression levels were determined using the real-time polymerase chain reaction. Results: Some CDDP-resistant HepG2 cell lines exhibited changes in the expression of copper transporter 1, multidrug resistant protein (MRP)2, and/or MRP3, resulting in decreased intracellular platinum amounts, while others showed no change in platinum accumulation. Expression of these transporters was not necessarily maintained in a constant direction within the cell population isolated from the same origin. Conclusion: These results suggest that the CDDP-resistant tumors caused by a decrease in intracellular platinum content consist of a heterogeneous cell population showing expression changes of several transporters.*

Cisplatin (CDDP) is an anticancer drug used for the treatment for various solid cancers. The antitumor effect of CDDP involves uptake into cells, binding to DNA, and ultimately inducing apoptosis. On the other hand, CDDP is a drug often prone to resistance, and various attempts to overcome CDDP resistance have been reported. The main resistance mechanisms against CDDP are a decrease in the intracellular platinum amount, enhancement of CDDP inactivating ability, and enhancement of DNA repairing ability. Among these, a decrease in the amount of intracellular platinum has been reported in some CDDP-resistant cells (1-3).

CDDP is a charge-free metal complex that passes easily through cell membranes by passive transport, and is also taken up through active transport. Reduction in the amount of intracellular platinum found in many CDDP-resistant cancer cells involves in a decrease in intracellular uptake and/or an increase in extracellular excretion. Therefore, fluctuations in the expression of the transporters are one of the most important factors of CDDP resistance.

In the family of the multidrug resistance-associated proteins (MRPs), MRP2 and MRP3 are the most well-known transporters associated with resistance to CDDP. Increased expression of MRP2 and MRP3 increases the extracellular efflux of CDDP and contributes to the acquisition of resistance (4-6). In addition, transporters involved in the cellular uptake of CDDP have been reported in many cell lines. For example, using cell-knockout models in yeast and mice, the copper transporter, CTR1, was identified as a major determinant governing CDDP resistance and accumulation (7). Investigations of CDDP-resistant cancer cell lines (small cell lung cancer SR2, ovarian cancer A2780cis, and cervical cancer HeLaCK) also confirmed the relevance CTR1 for CDDP sensitivity as there was a correlation between CTR1 levels, intracellular platinum concentrations, and cytotoxicity of CDDP (8, 9).

Yonezawa *et al*. reported that the organic cation transporter (OCT) was responsible for the CDDP-induced renal tubular toxicity (10). Moreover, they showed that cytotoxicity and accumulation of CDDP were enhanced in HEK293 cells transiently transfected with OCT1 or OCT2, which are expressed primarily in the liver or kidney, respectively (11). In previous work, we found that expression of the Na^+, K^+-ATPase α1 subunit was decreased in CDDP-resistant H4-II-E/CDDP cells (12). Andrews *et al*. reported that CDDP accumulation was partially Na^+ dependent and that the Na^+, K^+-ATPase, which maintains the Na^+ gradient, plays an important role in determining how much CDDP enters the cell (13).

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LAT1, one of the system L amino acid transporters, has been attracting much attention in the field of cancer research but cannot transport CDDP (14). Kaira *et al*. reported that LAT1 was a significant factor in predicting poor prognosis, and its expression was closely associated with resistance to platinum-based chemotherapy in non-small cell lung cancer (15).

There are many reports on changes in transporter expression associated with CDDP resistance. In addition to transporters, there is the possibility that other factors are involved in CDDP resistance. Furthermore, it is conceivable that expression of resistance factors is not uniform in cell populations of tumor tissue. In the current study, we selected CDDP-resistant cell populations from HepG2 cells and compared their CDDP sensitivity, intracellular platinum accumulation, and transporter expression. Our objective was to define the role of the transporter expression in CDDP resistance, and clarify if a specific transporter could be a target for enhancing the efficacy of CDDP treatment.

Materials and Methods

Cell culture. Human hepatocellular carcinoma HepG2 cells were purchased from Dainippon Pharmaceutical (Osaka, Japan). Cells were maintained in minimum essential medium containing 10% fetal bovine serum at 37˚C in humidified air with 5% CO_2.

Cytotoxicity assay. Cytotoxicity studies were carried out using a sulforhodamine B (SRB) microculture colorimetric assay. The SRB assay was also used to determine cell density, based on the measurement of cellular protein content. The assay is based on the ability of the protein dye SRB to bind electrostatically and in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Briefly, cells were plated at a density of 4.0×10^3 cells per well into 96-well flat bottom plates on day 0 and exposed to CDDP on day 1. The SRB assay was carried out 72 h after drug exposure. At the termination of incubation, the cells were fixed with 10% trichloroacetic acid *in situ*, washed, and dried. Thereafter, 0.4% SRB solution in 1% acetic acid was added and the cells were incubated at room temperature. The cells were washed again, the bound stain was solubilized with trisma base solution, and the absorbance measured at 570 nm in a microplate reader (Model 550, BIO-RAD, Tokyo, Japan). The data were transformed into the Microsoft Excel format and the surviving cell fraction at each concentration of CDDP was calculated relative to the control. All data represent the average value of five wells.

Intracellular platinum accumulation. Cells (1×10^6) were seeded into 75-cm^2 tissue culture flasks and incubated overnight. Immediately following a 4 h exposure to 30 μM CDDP exposure, the cells were harvested and washed three times with cold phosphate-buffered saline. A small portion (5%) of the cells was used to determine the protein content using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA). The resulting cell pellets were digested in a mixture of perchloric acid and 30% hydrogen peroxide (1:2) for 12 h at 65˚C. The amount of platinum in the samples was determined using an SPS 3100 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Hitachi High-Tech Science Corporation, Tokyo, Japan). The results are expressed as ng platinum per mg protein.

Table I. *Primers list for the real-time polymerase chain reaction*.

Gene	Primer sequence (5'-3')
MRP2	F: AGG CAC TCC AGA AAT GTG CT
	R: GAC TAT GGG CTG ATA TCC AGT GT
MRP3	F: AAT GGC TGC TTT CTC CTC CT
	R: GGC ACT GCT GAT TGA AGA CA
LAT1	F: TTC TGT AGG GGT TGA TCA TTT CC
	R: TCA AGG TCT GGA TCG AGC TGC TC
ATP1A1	F: CCT TTA GAT CAC TGC CGT GTA C
	R: GAA GTG CTG GAA TTA AGG TCA TC
CTR1	F: AGG CTC TCT CGG GCT ATC TT
	R: AGA TTC GGA GAG AGA GGT GCT
OCT1	F: ACT TCA TAG CGC CTG CAC TG
	R: TCC TCA TCT TAT GCC TGC TG
GAPDH	F: CCA TCA CCA TCT TCC AGG AG
	R: CCT GCT TCA CCA CCT TCT TG

RNA extraction and the real-time polymerase chain reaction (RT-PCR). Total RNA was extracted using Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan) in accordance with the manufacturer's protocol. The quality and concentration of RNA in each sample were confirmed by spectrophotometry using NanoDrop Lite (Thermo Fisher Scientific, Yokohama, Japan). cDNA was produced from total RNA by reverse transcription using a PrimeScript II First Strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan). Amplification was performed using Rotor-Gene Q (Qiagen, Tokyo, Japan) with a Rotor-Gene SYBR® Green PCR Kit (Qiagen). Glyceraldehyde 3-phospate dehydrogenase (GAPDH) was used for normalization of the relative expression levels. The primer sequences, synthesized by BEX (Tokyo, Japan), used to specifically amplify the genes of interest are shown in Table I. The PCR conditions were set as follow: 95˚C for 5 sec and 60˚C for 10 sec for 40 cycles. The cycle threshold (Ct) indicated the fractional cycle number at which the PCR product was first detected above a fixed threshold. Relative mRNA levels were determined using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis. Results are expressed as means±standard deviation. Statistical analysis was performed in Microsoft Excel using an analysis of variance by Dunnett's test for multiple comparisons. $p<0.05$ was considered significant.

Results

Isolation of CDDP-resistant cell lines. CDDP-resistant strains were isolated *via* a three-step CDDP exposure. Specifically, isolations were done following exposures to 1, 3, and 5 μM CDDP, respectively. First, parental HepG2 cells seeded in a petri dish were exposed to 1 μM CDDP. Twenty of the colonies that proliferated were transferred to flasks. Two fast growing colonies were selected as the first generation resistant lines (R1) and named R1-8 and R1-18. R1 cells were then exposed to 3 μM CDDP and the most fast

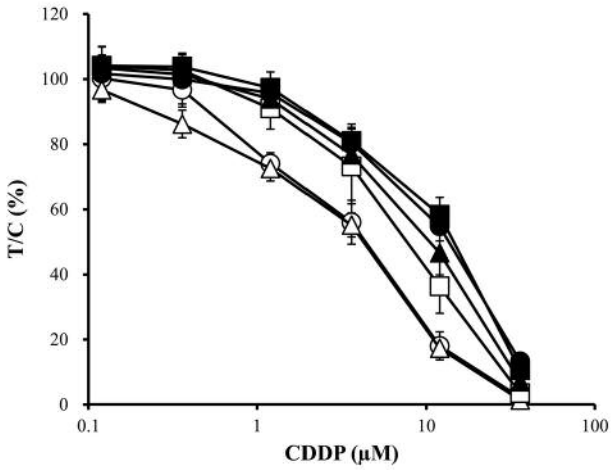

Figure 1. *Sensitivity of CDDP-resistant HepG2-R1-8 cell lines to CDDP. Cells were exposed to CDDP for 72 h. Cytotoxicity was assessed by the sulforhodamine B assay. Open circles: HepG2 cells, opened triangles: R1-8 cells, open squares: R2-8-2 cells, closed circles: R3-8-2-8 cells. Closed triangles: R3-8-2-9 cells, closed squares: R3-8-2-10 cells. Data are presented as the means±SD for 3-6 individual experiments.*

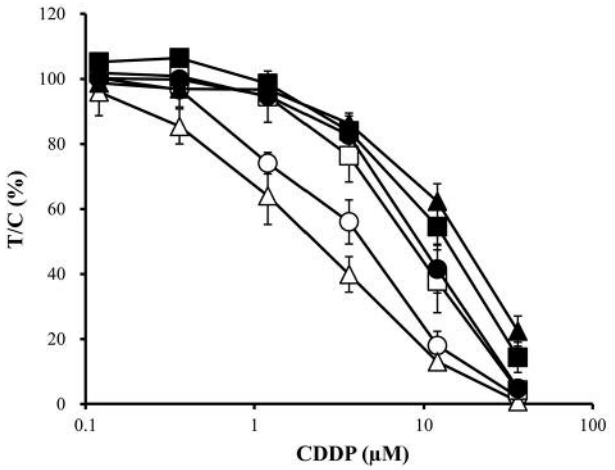

Figure 2. *Sensitivity of CDDP-resistant HepG2-R1-18 cell lines to CDDP. Cells were exposed to CDDP for 72 h. Cytotoxicity was assessed by the sulforhodamine B assay. Open circles: HepG2 cells, open triangles: R1-18 cells, open squares: R2-18-1 cells, closed circles: R3-18-1-6 cells, closed triangles: R3-18-1-9 cells, closed squares: R3-18-1-10 cells. Data are presented as means±SD for 3-6 individual experiments.*

growing strains were selected from each R1 line as the second generation resistant lines (R2) and named R2-8-2 (derived from R1-8) and R2-18-1 (derived from R1-18). R2 cells were then exposed to 5 μM CDDP and three growing colonies were selected from each R2 line as the third generation resistant lines (R3) and named R3-8-2-8, 9, and 10 (from R2-8-2) and R3-18-1-6, 9, and 10 (from R2-18-1).

CDDP-resistant cells were maintained without additional CDDP exposure. CDDP sensitivities were assessed by the SRB assay several times over the experimental period and no significant variation was noted in any line. The sensitivity profiles of CDDP-resistant cell lines to CDDP are shown in Figures 1 and 2, and the IC_{50}s in Table II. CDDP sensitivities of R1-8 and R1-18 cells were not different from that of parental HepG2 cells. The IC_{50} values of CDDP in the R2 and R3 cell lines increased gradually with the progression of CDDP-resistance, and showed a significant difference from that in HepG2 cells. Between the R1-8 and R1-18 cell lines, there was a significant difference in the IC_{50} values of CDDP. However, between the R2-8-2 and R2-18-1 lines, and among R3-8-2 and R3-18-1 lines there was no difference in the sensitivity to CDDP.

Table II. *CDDP IC_{50} values in CDDP-resistant HepG2 cell lines.*

Cell	IC_{50} (μM)	Resistance fold
HepG2	4.11±0.50	1.00
R1-8	4.24±0.45	1.03
R2-8-2	7.79±2.29*	1.90
R3-8-2-8	13.6±1.42*	3.31
R3-8-2-9	10.6±2.43*	2.58
R3-8-2-10	14.6±1.65*	3.55
R1-18	2.25±0.73	0.55
R2-18-1	8.56±2.53*	2.01
R3-18-1-6	9.56±1.92*	2.33
R3-18-1-9	16.6±3.74*	4.04
R3-18-1-10	13.6±1.99*	3.31

IC_{50} values are represented as means±SD for 3-6 individual experiments. Resistance fold was calculated by dividing the IC_{50} value in the resistant cell lines by the IC_{50} value in HepG2 cells. *$p<0.05$ vs. HepG2 cells.

Intracellular platinum accumulation. Following exposure to 30 μM CDDP for 4 h, the intracellular platinum level was measured by ICP-OES (Figures 3 and 4). The amounts of platinum in HepG2, R1, and R2 cells were similar. However, R3-8-2-10, R3-18-1-9, and R3-18-1-10 showed significantly lower intracellular platinum levels. Among the R3 cell lines derived from R1-8, there was an obvious difference in the intracellular platinum amount; 58-105% relative to HepG2 cells. All R3 cell lines derived from R1-18 showed lower intracellular platinum accumulation than R2-18-1 cells. However, there was no clear relationship between platinum accumulation and the extent of CDDP-resistance.

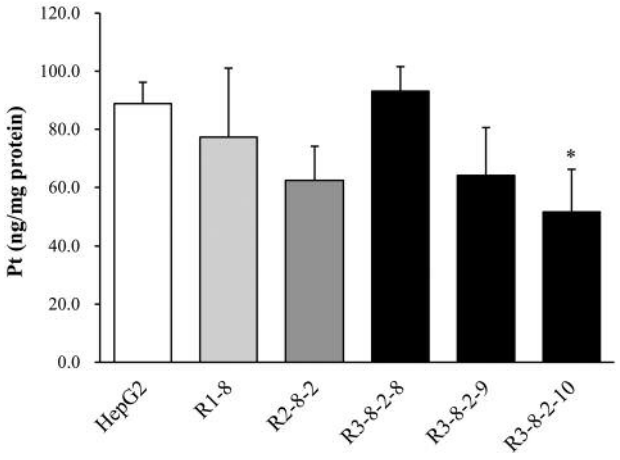

Figure 3. *Intracellular platinum accumulation by CDDP-resistant HepG2-R1-8 cell lines after a 4 h exposure to 30 μM CDDP. The amount of accumulated platinum was determined by inductively coupled plasma optical emission spectrometry. Data are presented as means±SD for 3-5 individual experiments. *p<0.05 vs. HepG2 cells.*

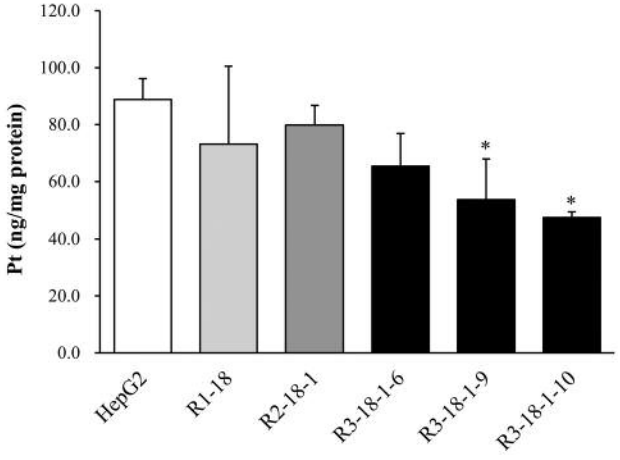

Figure 4. *Intracellular platinum accumulation by CDDP-resistant HepG2-R1-18 cell lines after a 4 h exposure to 30 μM CDDP. The amount of accumulated platinum was determined by inductively coupled plasma optical emission spectrometry. Data are presented as means±SD for 3-5 individual experiments. *p<0.05 vs. HepG2 cells.*

Expression of transporter genes. The mRNA expression levels of CDDP efflux transporters (MRP2 and MRP3), an inhibitory factor of CDDP activity (LAT1), and CDDP uptake transporters (ATP1A1, CTR1, and OCT1) were assessed (Figures 5 and 6). Among CDDP export transporter mRNAs in R3 cell lines derived from R1-8, only MRP3 was increased in R3-8-2-8 cells. Also, among CDDP uptake transporter mRNAs in R3 cell lines derived from R1-8, CTR1 was universally decreased. Decreased CTR1 expression was confirmed in almost all resistant lines derived from R1-8, and the relative expression level in the R1-8 resistant lines was likely to be related to the intracellular platinum amount.

In R3 cell lines derived from R1-18, the expression of MRP2 and MRP3 was increased. In contrast, none of the CDDP uptake transporters were decreased. The relative expression level of MRP2 in the R1-18 resistant lines appeared to be somewhat related to the relative intracellular platinum levels in these resistant lines. Comparing the resistant cell lines with HepG2 cells, none of the six transporters whose expression levels were increased or decreased in the order of R1, R2, and R3 were present: *i.e.*, there was no relationship between the progression of resistance and the change in expression levels of these transporters.

Discussion

In the past decades, various CDDP-resistant cell lines were established to elucidate the mechanisms of the action and resistance. Among them, significant attention was placed on the transporters that are required for CDDP activity (16, 17). Studies have been characterized individual cell lines resistant to CDDP by one of the transporters such as CTR1 or ATP1A1. However, cells expressing CDDP resistance in tumor tissues are a heterogeneous, not a single cell population (18, 19).

To obtain various CDDP-resistant cell populations, we increased the exposure concentration of CDDP to cells in stages, isolated cells showing tolerance in each stage, and investigated their CDDP sensitivity, intracellular platinum content, and transporter expression. Initially, the R1-8 and R1-18 cells showed no difference from HepG2 cells in CDDP sensitivity and intracellular platinum content. However, increased expression of MRP3, a transporter related to CDDP efflux, and decreased expression of ATP1A1 and OCT1, transporters related to CDDP uptake, were observed commonly in both cell lines. These expression fluctuations did not lead to a decrease in intracellular platinum levels.

Haga *et al*. reported that a glioma cell line transfected with MRP3 antisense was 3- to 5-times more sensitive to CDDP and accumulated twice as much platinum compared to the control (6). We reported previously that the CDDP-resistant rat hepatoma cell line, H4-II-E/CDDP, had a greatly reduced ATP1A1 level and about 6- to 12-times lower platinum accumulation compared to parental H4-II-E cells (12). Yonezawa *et al*. reported that HEK293 cells transfected with OCT1 were about 2-times more sensitive to CDDP and had twice the platinum accumulation compared to the control (11).

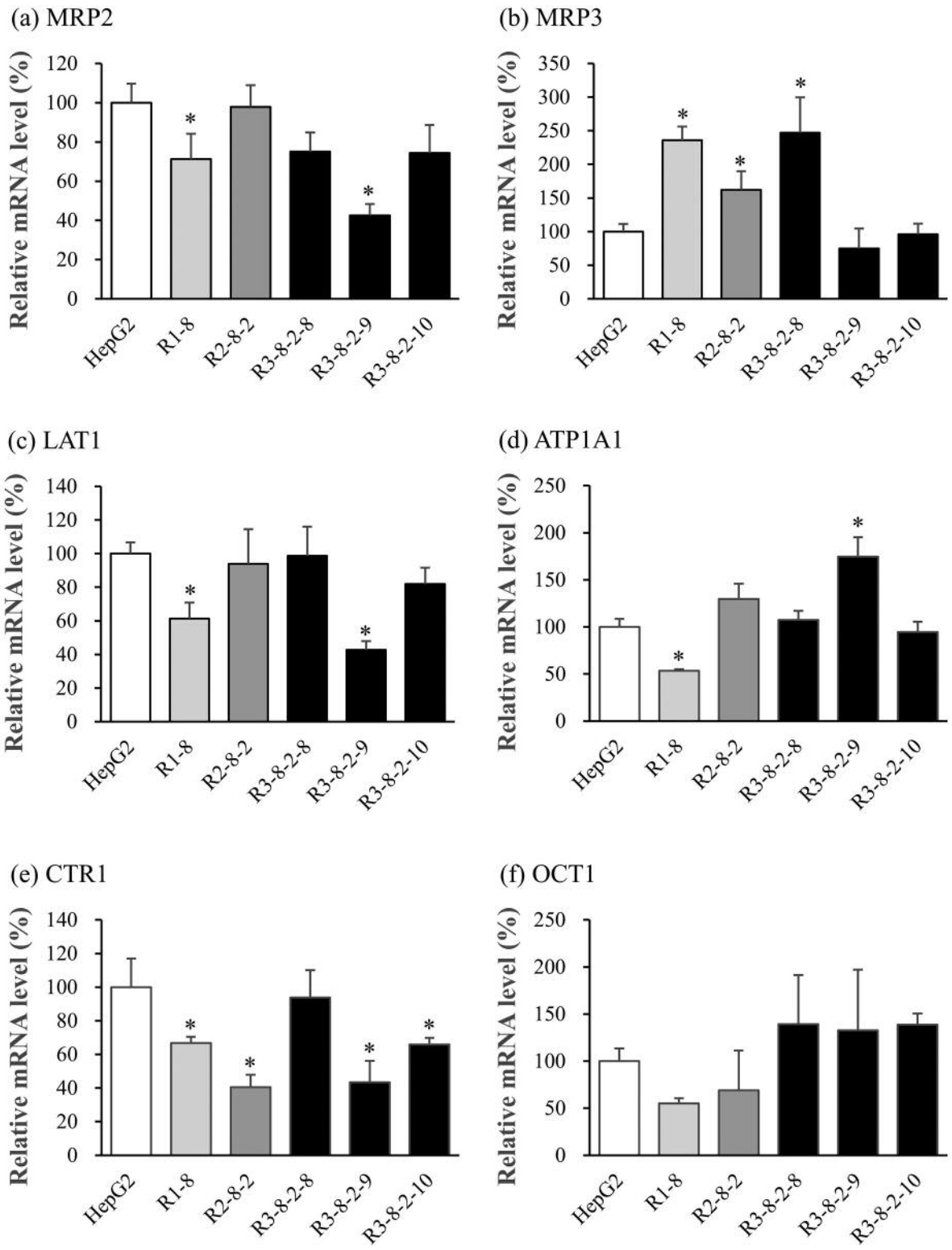

Figure 5. *Relative mRNA levels of transporter genes in CDDP-resistant HepG2-R1-8 cell lines. mRNA levels of MRP2 (a), MRP3 (b), LAT1 (c), ATP1A1 (d), CTR1 (e), and OCT1 (f) were normalized to GAPDH mRNA. The relative mRNA level is expressed as the fold increase in each normalized mRNA level in CDDP-resistant HepG2-R1-8 cell lines compared to that in HepG2 cells. Data are presented as means±SD for 3-5 individual experiments. *p<0.05 vs. HepG2 cells.*

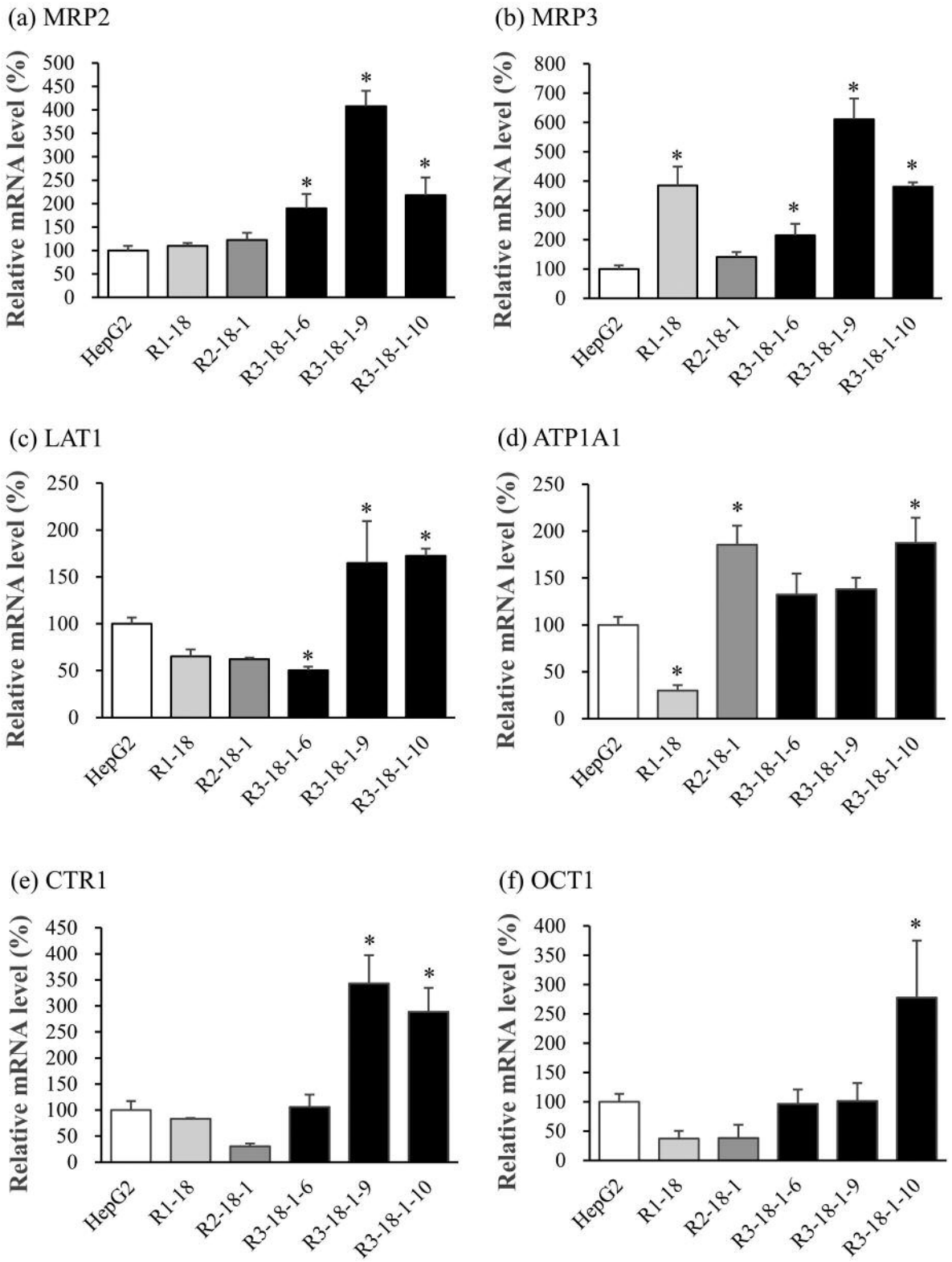

Figure 6. *Relative mRNA levels of transporter genes in CDDP-resistant HepG2-R1-18 cell lines. mRNA levels of MRP2 (a), MRP3 (b), LAT1 (c), ATP1A1 (d), CTR1 (e), and OCT1 (f) were normalized to GAPDH mRNA. The relative mRNA level is expressed as the fold increase in each normalized mRNA levels in CDDP-resistant HepG2-R1-18 cell lines compared to that in HepG2 cells. Data are presented as means±SD for 3-5 individual experiments. *p<0.05 vs. HepG2 cells.*

However, no significant changes in the sensitivity to CDDP and amounts of cellular platinum were observed in R1-8 and R1-18 cells with changed expression of these three transporters. Furthermore, the secondly generation R2-8-2 and R2-18-1 cells showed a significant decrease in CDDP sensitivity, but no significant decrease in intracellular platinum levels.

R2-8-2 cells maintained the changes in R1-8 cells for increased expression of MRP3 and decreased expression of CTR1. Zisowsky *et al*. reported that HeLaCK cells had 2-times lower CTR1 expression and 2-times lower platinum accumulation compared to the parental HeLa cells (9). In R2-18-1 cells, a decrease in OCT1 expression maintained a change seen in R1-18 cells. All six cell lines at the third selection showed a significant decrease in CDDP sensitivity. A significant decrease in the intracellular platinum level was observed in three of these cell lines: R3-8-2-10, R3-18-1-9, and R3-18-1-10. Among the four cell lines isolated from R1-8, only R3-8-2-8 cells showed an intracellular platinum content almost equal to that of HepG2 cells and exhibited an expression level of CTR1 equal to that of HepG2 cells.

Decreased expression of CTR1 was the only factor among the transporters examined that appeared to be involved in the decreased amount of intracellular platinum observed in these cell lines. On the other hand, the expression levels of two efflux transporters, MRP2 and MRP3, were increased significantly in R3-19-1-9 and R3-18-1-10 cells. Kawabe *et al*. reported that polarized Chinese hamster ovary LLC-PK1 cells transfected with MRP2 accumulated about 30% less platinum than the control (4). Thus, MRP 2 and MRP 3 were factors causing a decrease in intracellular platinum content in these cell lines. However, enhanced CTR1 expression was a negative factor for CDDP resistance.

Increased expression of the LAT1 was also observed in the two cell lines. Amino acids that are transported into cells through LAT1 stimulate the mammalian target of rapamycin (mTOR) signaling pathway. mTOR acts as a resistance factor against anticancer drugs through its anti-apoptotic action. Yamauchi *et al*. reported that a LAT1 inhibitor enhanced the antitumor activity of CDDP against the Hep-2 head and neck cancer cells (20). Thus, LAT1 also plays a role in CDDP resistance observed in R3-19-1-9 and R3-18-1-10 cells.

In this study, 10 cell lines originating from R1-8 and R1-18 cells were isolated as CDDP-resistant strains. Although CDDP sensitivity was significantly decreased in all cell lines after the second generation, only three cell lines of the third generation showed a significant decrease in the accumulation of platinum. Furthermore, there was one resistant cell line with no difference in platinum accumulation compared with the parental HepG2 cells. Thus, in these CDDP-resistant strains, there were some in which the decrease in the accumulated platinum was caused by changes in transporter expression, and others in which a change in platinum accumulation was not observed.

Expression of individual transporters was not necessarily maintained in a constant direction within the populations originating from R1-8 and R1-18 cells. Among these cell lines, it is suggested that CTR1, MRP2, and MRP3 contribute to a decrease in the amount of intracellular platinum. However, there were cell lines that lacked any contribution of these transporters. *In vitro* studies involving CDDP resistance have found various factors, including transporters, that transport CDDP, but none were commonly present in all CDDP-resistant strains. Thus, strains with the similar resistance characteristics are not necessarily isolated from a single cell line. Based on these findings, treatment strategies targeting a single transporter are unlikely to overcome CDDP resistance because transporter expression shows heterozygous changes.

In the future, as reported by Cruz *et al*. (21), proteins associated with resistance are identified and the development of therapeutic agents targeting those proteins will be promoted. Proteins such as apoptosis regulators and proteasome pathways that can induce cell death in all cells by combining with anticancer agents should receive attention as targets rather than transporters that exhibit heterozygous expression fluctuations.

References

1 Choi MK and Kim DD: Platinum transporters and drug resistance. Arch Pharm Res *29(12)*: 1067-1073, 2006.

2 Burger H, Loos WJ, Eechoute K, Verweij J, Mathijssen RH and Wiemer EA: Drug transporters of platinum-based anticancer agents and their clinical significance. Drug Resist Updat *14(1)*: 22-34, 2011.

3 Liu JJ, Lu J and McKeage MJ: Membrane transporters as determinants of the pharmacology of platinum anticancer drugs. Curr Cancer Drug Targets *12(8)*: 962-986, 2012.

4 Kawabe T, Chen ZS, Wada M, Uchiumi T, Ono M, Akiyama S and Kuwano M: Enhanced transport of anticancer agents and leukotriene C4 by the human canalicular multispecific organic anion transporter (cMOAT/MRP2). FEBS Lett *456(2)*: 327-331, 1999.

5 Liedert B, Materna V, Schadendorf D, Thomale J and Lage H: Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. J Invest Dermatol *121(1)*: 172-176, 2003.

6 Haga S, Hinoshita E, Ikezaki K, Fukui M, Scheffer GL, Uchiumi T and Kuwano M: Involvement of the multidrug resistance protein 3 in drug sensitivity and its expression in human glioma. Jpn J Cancer Res *92(2)*: 211-219, 2001.

7 Ishida S, Lee J, Thiele DJ and Herskowitz I: Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad Sci USA *99(22)*: 14298-14302, 2002.

8 Song IS, Savaraj N, Siddik ZH, Liu P, Wei Y, Wu CJ and Kuo MT: Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. Mol Cancer Ther *3(12)*: 1543-1549, 2004.

9 Zisowsky J, Koegel S, Leyers S, Devarakonda K, Kassack MU, Osmak M and Jaehde U: Relevance of drug uptake and efflux for cisplatin sensitivity of tumor cells. Biochem Pharmacol *73(2)*: 298-307, 2007.
10 Yonezawa A, Masuda S, Nishihara K, Yano I, Katsura T and Inui K: Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat. Biochem Pharmacol *70(12)*: 1823-1831, 2005.
11 Yonezawa A, Masuda S, Yokoo S, Katsura T and Inui K: Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). J Pharmacol Exp Ther *319(2)*: 879-886, 2006.
12 Kishimoto S, Kawazoe Y, Ikeno M, Saitoh M, Nakano Y, Nishi Y, Fukushima S and Takeuchi Y: Role of Na^+, K^+-ATPase alpha1 subunit in the intracellular accumulation of cisplatin. Cancer Chemother Pharmacol *57(1)*: 84-90, 2006.
13 Andrews PA, Mann SC, Huynh HH and Albright KD: Role of the Na^+, K^+-adenosine triphosphatase in the accumulation of cis-diamminedichloroplatinum(II) in human ovarian carcinoma cells. Cancer Res *51(14)*: 3677-3681, 1991.
14 Hayashi K and Anzai N: Novel therapeutic approaches targeting L-type amino acid transporters for cancer treatment. World J Gastrointest Oncol *9(1)*: 21-29, 2017.
15 Kaira K, Takahashi T, Murakami H, Shukuya T, Kenmotsu H, Naito T, Oriuchi N, Kanai Y, Endo M, Kondo H, Nakajima T and Yamamoto N: Relationship between LAT1 expression and response to platinum-based chemotherapy in non-small cell lung cancer patients with postoperative recurrence. Anticancer Res *31(11)*: 3775-3782, 2011.
16 Nakanishi T: Drug transporters as targets for cancer chemotherapy. Cancer Genomics Proteomics *4(3)*: 241-254, 2007.
17 Konkimalla VB, Kaina B and Efferth T: Role of transporter genes in cisplatin resistance. In Vivo *22(3)*: 279-283, 2008.
18 Denison TA and Bae YH: Tumor heterogeneity and its implication for drug delivery. J Control Release *164(2)*: 187-191, 2012.
19 Somasundaram R, Villanueva J and Herlyn M: Intratumoral heterogeneity as a therapy resistance mechanism: role of melanoma subpopulations. Adv Pharmacol *65*: 335-359, 2012.
20 Yamauchi K, Sakurai H, Kimura T, Wiriyasermkul P, Nagamori S, Kanai Y and Kohno N: System L amino acid transporter inhibitor enhances anti-tumor activity of cisplatin in a head and neck squamous cell carcinoma cell line. Cancer Lett *276(1)*: 95-101, 2009.
21 Cruz IN, Coley HM, Kramer HB, Madhuri TK, Safuwan NA, Angelino AR and Yang M: Proteomics analysis of ovarian cancer cell lines and tissues reveals drug resistance-associated proteins. Cancer Genomics Proteomics *14(1)*: 35-51, 2017.

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