[CANCER RESEARCH 57, 5475–5479, December 15, 1997]

Advances in Brief

A Canalicular Multispecific Organic Anion Transporter (*cMOAT*) Antisense cDNA Enhances Drug Sensitivity in Human Hepatic Cancer Cells[1]

Koji Koike,[2] Takeshi Kawabe, Toshiya Tanaka, Satoshi Toh, Takeshi Uchiumi, Morimasa Wada, Shin-ichi Akiyama, Mayumi Ono, and Michihiko Kuwano

Department of Biochemistry, Kyushu University School of Medicine, 3-1-1 Maidashi, Fukuoka 812-82 [K. K., T. K., T. T., S. T., T. U., M. W., M. O., M. K.], and Department of Cancer Chemotherapy, Institute for Cancer Research, Faculty of Medicine Kagoshima University, Sakuraga-oka 890 [S. A.], Japan

Abstract

The human *cMOAT* gene encodes a membrane protein involved in the ATP-dependent transport of hydrophobic compounds. To determine whether cMOAT is associated with drug sensitivity, we transfected an expression vector containing *cMOAT* antisense cDNA into the HepG2 human hepatic cancer cell line. We observed a reduction in cMOAT protein, as well as an enhanced level of glutathione, in the antisense transfectants. The transfectants displayed an increased sensitivity to cisplatin, vincristine, doxorubicin, and the camptothecin derivatives, (4*S*)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino)carbonyloxy]dione hydrochloride triethydrate and 7-ethyl-10-hydroxycamptothecin, but not to etoposide, 3-[4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea, 5-fluorouracil, and mitomycin C. Results suggest that cMOAT levels are inversely correlated with those of glutathione, and that *cMOAT* and its related genes may be involved in the sensitivity of cells to certain anticancer agents.

Introduction

The acquisition of MDR[3] by cancer cells is thought to play a critical role in the resistance of tumors to multiple anticancer agents (1–3). Many of the natural anticancer drugs, including the *Vinca* alkaloids (vincristine and vinblastine), anthracyclines (doxorubicin and daunomycin), colchicine, taxols, and epipodophyllotoxins (etoposide and teniposide), induce the overexpression of a membrane glycoprotein, Pgp, encoded by the *MDR1* gene. This results in a decreased accumulation of drug and the acquisition of the MDR phenotype. Pgp is an integral membrane glycoprotein of M_r 170,000 that binds to anticancer agents, effluxes them from the cells, and is distributed in the colon, small intestine, adrenal gland, kidney, and liver (1, 2). MRP is another well-characterized M_r 190,000 integral membrane protein associated with the acquisition of the MDR phenotype (3, 4). Introduction of *MRP* cDNA into cells induces enhanced resistance to etoposide, anthracyclines, and *Vinca* alkaloids (5). MRP and Pgp confers similar patterns of sensitivity against various naturally occurring anticancer agents. However, the tissue-specific expression and intracellular distribution of both MRP and Pgp differ (3, 4). Consistent with these findings, *MRP* and *MDR1* cDNAs exhibit a relatively low homology (3, 4).

It has been shown that cMOAT activity mediates the ATP-dependent transport of various hydrophobic anionic compounds in liver canalicular membranes and other tissues (6). The finding that MRP can transport the cysteinyl leukotrienes (*e.g.*, LTC_4), as well as other GSH conjugates (7–9), suggests that this protein may be a GSH conjugate/organic anion transporter. MRP confers resistance to heavy metals that interact with GSH (5) and transports oxidized GSH, steroid glucuronides, and bile salt derivatives (3). The spectrum of hydrophobic anionic compounds transported by the cMOAT resembles that of MRP (6). The expression of *cMOAT* is decreased in Dubin-Johnson syndrome model rats (10–12), which are defective in the transport of a number of conjugates including LTC_4 and the glucuronides of bilirubin and thyroxine (13). These findings suggest that MRP may be one of several transporters that contribute to cMOAT activity. Both rat and human *cMOAT* cDNAs have been shown to be highly homologous to human *MRP* cDNA (10–12, 14). Similar pharmacological features have been reported for the MRP- and cMOAT-mediated transport of oxidized GSH, steroid glucuronides, and leukotrienes (3, 6). The recent cloning of the rat (10–12) and human (14) *cMOAT* genes has demonstrated that *cMOAT* and *MRP* are different genes that are located on different chromosomes. Both are expressed in the liver (10–12, 14), with cMOAT being localized to hepatocanalicular membranes and MRP found in the basolateral sides of liver cells (15).

Although we had previously observed that an increased expression of *cMOAT* in cisplatin-resistant lines derived from human cancer cells that showed decrease in drug accumulation (14), it is not clear whether *cMOAT* is specifically associated with a sensitivity to cisplatin or other anticancer agents. We now tested this possibility by introducing *cMOAT* antisense cDNA into cancer cell lines to determine the effect on drug resistance.

Materials and Methods

Drugs and Chemicals. Cisplatin was donated by Bristol-Myers Squibb K.K. (Kanagawa, Japan). Doxorubicin, vincristine, and colchicine were from Sigma Chemical Co. (St. Louis, MO). Etoposide, 5-FU, and mitomycin C were obtained from Nippon Kayaku Co. (Tokyo, Japan) and Kyowa Co. (Tokyo, Japan), respectively. CPT-11 and SN-38 were donated by Yakult (Tokyo, Japan), and ACNU was obtained from Sankyo (Tokyo, Japan). Lipofectin and geneticin (G418) were purchased from Life Technologies, Inc. (Bethesda, MD).

Cell Culture and Cell Lines. The human hepatic cancer cell line, HepG2 and its cMOAT antisense transfectants and the human epidermoid cancer cell line, KB, and its vincristine-resistant (KB/VJ300) and etoposide-resistant (KB/VP-4) subclones were cultured as described previously (16).

Construction of Antisense cMOAT Expression Vector. Lambda phage clone L3 was digested with *Not*I and *Pst*I to isolate an 805-bp fragment containing 770 bp of coding sequence and 35 bp of the 5′ noncoding fragment sequence. After ligation of *Sal*I linkers, the fragment was subcloned into the *Sal*I sites of the pBact-S′ vector (17), which was used to transform competent

Received 8/29/97; accepted 10/28/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science, Sports and Culture of Japan, and by the Fukuoka Anticancer Research Fund.

[2] To whom requests for reprints should be addressed. Phone: 81-92-642-6100; Fax: 81-92-642-6203; E-mail: xtckk@biochem1.med.kyushu-u.ac.jp.

[3] The abbreviations used are: MDR, multidrug resistance; Pgp, P-glycoprotein; MRP, multidrug resistance protein; cMOAT, canalicular multispecific organic anion transporter; GSH, glutathione; LTC_4, leukotriene C_4; CPT-11, (4*S*)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino)carbonyloxy]dione hydrochloride triethydrate; SN-38, 7-ethyl-10-hydroxycamptothecin; ACNU, 3-[4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea; 5-FU, 5-fluorouracil.

Escherichia coli. Minipreparations of ampicillin-resistant clones were analyzed for the orientation of their inserts by restriction site mapping.

Transfection with cMOAT Antisense Expression Vector. Exponentially growing HepG2 cells (5×10^6) were washed with PBS and placed in serum-free medium. A mixture of 50 μg of Lipofectin, 10 μg of *cMOAT* antisense expression vector, and 1 μg of pSV2-neo DNA was added for 12 h, after which fresh medium was added. The cells were incubated in selection medium (*i.e.*, containing 400 μg/ml G418) for 3–4 weeks. Two stable transfectants, which also showed reduced cellular cMOAT levels, were selected among the G418-resistant clones. As a control, we used a G418-resistant mock transfectant that had cellular cMOAT levels similar to those in the parental HepG2 cells.

Production of GST-cMOAT Fusion Proteins. A full-length human *cMOAT* cDNA was digested with *Mun*I and *Eco*RI. The 351-bp fragment was subcloned into the *Eco*RI site of pGEX-4T expression vector (Pharmacia, Uppsala, Sweden). The resulting GST-cMOAT fusion proteins were expressed in *E. coli* and purified with glutathione-Sepharose beads (Pharmacia) according to the manufacturer's protocol.

Production of Human cMOAT Antibodies. Antibodies directed against human cMOAT were elicited by synthetic peptides that corresponded to unique sequences at the linker region. The linker region peptide, DYGLISSVEEIPEDAAS, was cross-linked to BSA, and the conjugated peptides were used for multiple immunization of a New Zealand White rabbit (18). Antibodies to cMOAT were affinity purified with the GST-cMOAT fusion proteins. A 1:1000 dilution of antibody was able to detect 1 ng of GST-cMOAT by dot-blot analysis. This antibody did not cross-react with Pgp or MRP.

Western Blot Analysis. Immunoblot analysis of YB-1, Pgp, MRP, and cMOAT was performed as described (16). Aliquots of whole-cell lysates (150 μg) were electrophoresed on 7.5% SDS-polyacrylamide gels and transferred onto Immobilon-P membranes. The membranes were incubated with antiserum against YB-1 (YBC; Ref. 18), human Pgp (MC6–4), MRP (QCRL-1), or cMOAT for 1 h at room temperature and then with horseradish peroxidase-linked second antibody for 1 h at room temperature. Membranes were developed by chemiluminescence following the ECL protocol (Amersham International, Buckinghamshire, United Kingdom).

Drug Resistance of Antisense Transfectants. About 10^3 cells were plated in 35-mm dishes in the absence of drugs, and various drugs, freshly prepared in physiological saline or DMSO, were added 24 h later. After incubation for 7 days at 37°C, the number of Giemsa-stained colonies was counted. Control experiments were performed by adding equivalent volumes of saline or DMSO. The 90% lethal dose (IC_{90}) for each cell line was calculated from the dose-response curve. Relative resistance was determined in two separate experiments with three dishes each (16).

Determination of GSH Levels. Intracellular total GSH was determined by an enzymatic recycling assay based on the glutathione reductase method (19).

Cellular Accumulation of Vincristine, Etoposide, and Cisplatin. The accumulation of vincristine and etoposide was determined as described (16). Cells (2×10^5) were plated in 24-well plates and incubated for 48 h at 37°C. The plates were chilled for 15 min, washed twice with ice-cold PBS, and incubated for 37°C in 200 μl of buffer (serum-free DMEM and 20 mM HEPES, pH 7.5) containing [^{3}H]vincristine (20 μM and 0.13 μCi/ml) and [^{3}H]etoposide (1 μM and 1 μCi/ml). The cells were then washed three times with ice-cold PBS and resuspended in 400 μl of 0.25 M NaOH at 37°C for 30 min. The cell lysates were mixed thoroughly with 4 ml of Scintisol EX-H (Waco Chemicals, Osaka, Japan), and the radioactivity was determined. All results were normalized to cellular protein.

The intracellular concentration of cisplatin was measured as described (20). Exponentially growing cells were incubated for 2 h with 100 μM cisplatin, washed twice with ice-cold PBS, harvested, and sonicated. Platinum concentration was determined by flameless atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer 180–70; Hitachi, Tokyo, Japan). All results were normalized to cellular protein.

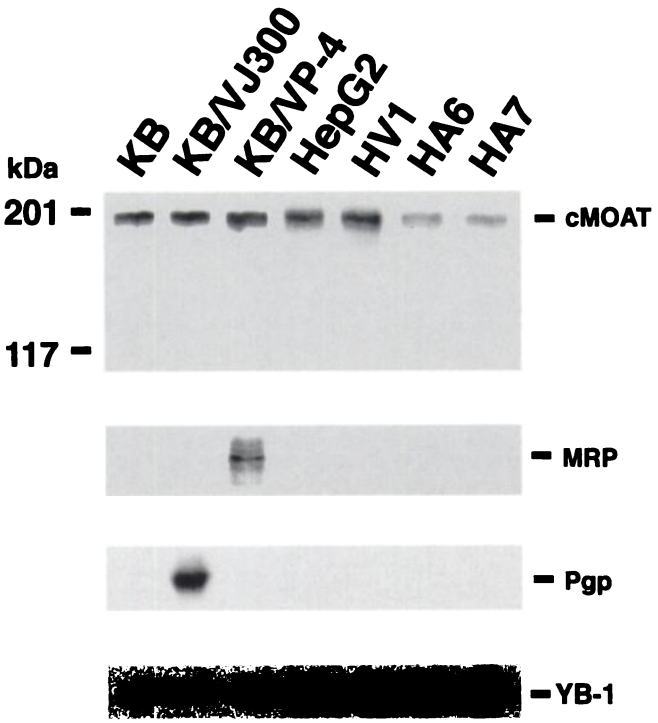

Fig. 1. Western blot analysis for cMOAT, MRP, Pgp, and YB-1 in KB and HepG2 and their derivatives.

Results

By Western blot analysis with polyclonal antibody directed against human cMOAT, we demonstrated the presence of a M_r 190,000–200,000 cMOAT protein in the human KB epidermoid cancer cell line and its vincristine-resistant (KB/VJ300) and etoposide-resistant (KB/

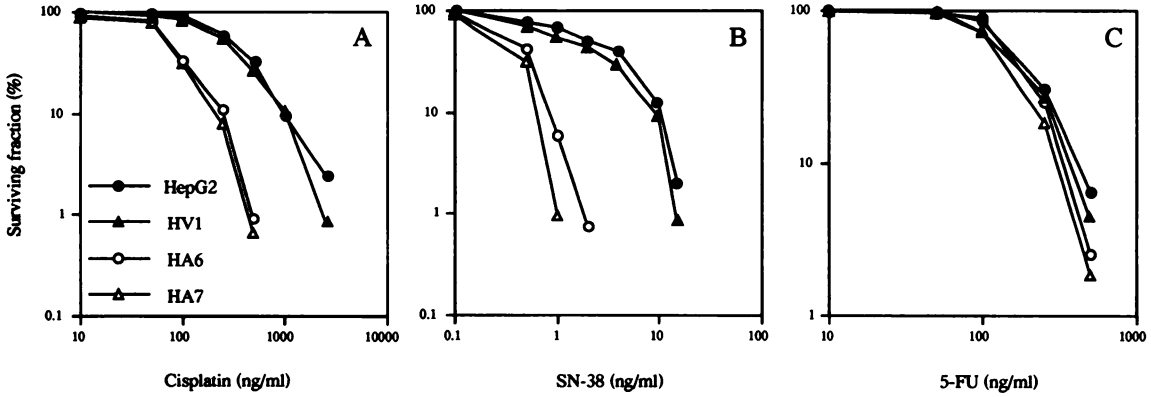

Fig. 2. Dose-response curve to cisplatin (*A*), SN-38 (*B*), and 5-FU (*C*) of HepG2 and its sublines. Each point indicates the average value for two separate experiments with triplicate dishes. 100% corresponds to the number of colonies in the absence of any drug.

VP-4) sublines, as well as in the human HepG2 hepatic cancer cell line (Fig. 1). To determine whether cellular sensitivity to anticancer agents was closely associated with cellular levels of cMOAT, we established cell lines with reduced levels of cMOAT by introducing the *cMOAT* antisense expression plasmid into HepG2 cells. Compared with HepG2 cells, two G418-resistant clones, HA6 and HA7, had decreased levels of cMOAT; in contrast, cells transfected with pSV2-neo plasmid alone (HV1) had the same level of cMOAT as HepG2 cells (Fig. 1). Pgp and MRP were not expressed in HepG2, HV1, HA6, and HA7 cells (Fig. 1). KB cell lines and HepG2 cell lines had the same levels of YB-1, human Y-box binding protein, respectively (Fig. 1).

When we assayed the sensitivity of the HepG2, HV1, HA6, and HA7 cells to cisplatin and to the camptothecin derivative, SN-38, we found that HA6 and HA7 were 5–15-fold more sensitive to the cytotoxic effect of both drugs than were HepG2 and HV1 (Fig. 2, *A* and *B*). In contrast, all four cell lines showed similar sensitivities to the cytotoxic effect of 5-FU (Fig. 2*C*). HA6 and HA7 were also 3–10-fold more sensitive to doxorubicin, vincristine, and CPT-11 than were HV1 and HepG2, whereas both antisense transfectants showed either similar, or only slightly enhanced, sensitivity to etoposide, ACNU, and mitomycin C (Table 1).

Because the altered cellular MRP levels, a protein homologous to cMOAT, often alters the intracellular level of GSH (21–23), we examined whether GSH levels were also influenced by decreased cMOAT levels in HepG2 cells. Compared with HV1 cells, cellular GSH levels in the HA6 and HA7 cells were increased about 4-fold (Fig. 3). In contrast, HepG2 and its three sublines had the same level of mRNA encoding γ-glutamylcysteine synthetase, a rate-limiting enzyme in the synthesis of GSH (data not shown).

We also determined whether the cellular accumulation of drugs was affected by the reduced cellular levels of cMOAT. Compared with the HV1 cells, cellular levels of vincristine and cisplatin were increased 1.5–1.8-fold in HA6 and HA7 cells, whereas levels of etoposide were the same in the three cell lines (Fig. 4).

Discussion

We have shown that the stable transfection of HepG2 cells with a *cMOAT* antisense construct leads to a marked increase in cellular GSH, which suggests a close relationship between intracellular GSH levels and cMOAT. GSH has also been implicated in ATP-dependent transport by MRP, a protein highly homologous to cMOAT (7–9, 21–23). For example, ATP-dependent vincristine transport was found to be markedly enhanced in the presence of increasing concentrations of GSH (9), and ATP-dependent transport of GSH-conjugates (*e.g.*, LTC_4) is mediated via MRP (7–9). GSH levels were shown to be altered in *MRP*-overproducing cells that were selected by drug resistance (21). Cells transfected with *MRP* cDNA had significantly lower GSH levels than untransfected cells (23). Increased intracellular concentration of GSH has been observed in the *MRP* double-knockout cell line and in RBCs isolated from *MRP* double-knockout mouse (31, 32).

Table 1 *Sensitivity of HepG2 and sublines to various drugs*[a]

	HepG2	Relative resistance		
Drugs	IC_{90} (μg/ml)	HV1	HA6	HA7
Cisplatin	1.0	1.1	0.2	0.2
CPT-11	2.0	1.0	0.3	0.3
SN-38	0.0080	0.8	0.07	0.06
Vincristine	0.10	0.9	0.1	0.1
Doxorubicin	0.12	0.9	0.2	0.2
Etoposide	0.38	1.0	0.7	0.7
ACNU	1.5	0.9	1.0	0.9
5-FU	0.41	0.9	0.8	0.7
Mitomycin C	0.020	0.8	0.8	0.7

[a] The sensitivity of each cell line was assayed by colony formation as described in "Materials and Methods." The IC_{90} for each drug was obtained from two separate experiments with triplicated dishes. Relative resistance was obtained by dividing the mean IC_{90} of each subline by that of the HepG2 cells.

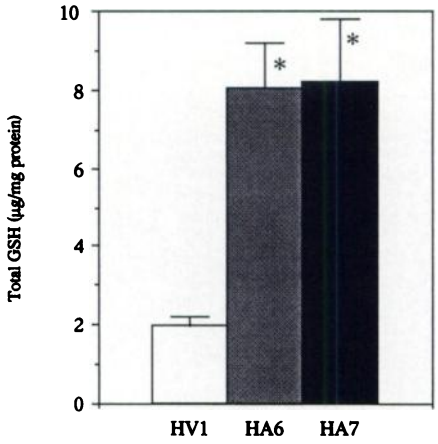

Fig. 3. Total intracellular GSH levels in stably transfected HepG2 cell lines. Each point is the average of three separate experiments; *bars*, SD. *, $P < 0.01$ compared to HV1.

Overexpression of *MRP*, which confers resistance to daunorubicin, vincristine, and other drugs, has been demonstrated to reduce ATP-dependent accumulation of drugs and to enhance drug efflux (5), whereas buthionine sulfoximine, an inhibitor of GSH synthesis, induced a decrease in cellular GSH levels and overcame resistance to vincristine or daunorubicin (22). We have shown here that reduced cellular levels of cMOAT markedly enhanced cell sensitivity to vincristine and reduced the cellular accumulation of this drug. Because GSH conjugates of vincristine were not detected in the culture medium of cells overexpressing *MRP* (22), however, it is unclear whether MRP and cMOAT can cotransport GSH and drugs without the formation of conjugates.

We also found that hepatic cancer cells transfected with *cMOAT* antisense cDNA displayed an enhanced sensitivity to cisplatin, CPT-11, SN-38, vincristine, and doxorubicin but not to etoposide, ACNU, mitomycin C, and 5-FU. Human epidermoid carcinoma KB cell lines transfected with *cMOAT* antisense cDNA also enhanced sensitivity to cisplatin and CPT-11.[4] It has also been shown that cells transfected with *MRP* cDNA as well as drug-selected *MRP*-overproducing cells acquired resistance to etoposide, doxorubicin, and vincristine but not to CPT-11, SN-38, and cisplatin (5, 16). Thus, cMOAT appears to modulate the sensitivity to certain anticancer agents as MRP.

ATP-dependent MRP has been reported to efficiently transport glucuronidated etoposide (24). Etoposide, however, may not be a specific substrate for ATP-dependent cMOAT. In contrast, cellular sensitivity to the topoisomerase I-targeting derivatives of the naturally occurring camptothecins, CPT-11 and SN-38, appeared to be rather specifically modulated by cMOAT, not by MRP. CPT-11 and its active form, SN-38, are glucuronidated, and their secretion into bile acid is blocked in the Dubin-Johnson syndrome model rat in which cMOAT activity is decreased (25). Although it is not known whether CPT-11 or SN-38 forms GSH conjugates (25), ATP-dependent cMOAT transporter may be involved in the efflux of these camptothecin derivatives.

Of the pleiotropic mechanisms involved in drug resistance, a decrease in drug accumulation is believed to play a major role in limiting the sensitivity to cisplatin (26). Goto *et al.* (27) have reported that

[4] Unpublished data.

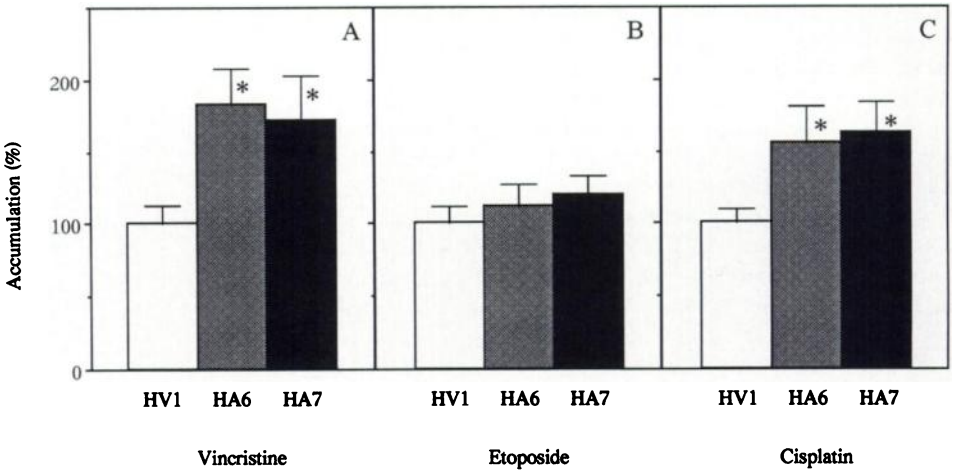

Fig. 4. Cellular accumulation of vincristine (*A*), etoposide (*B*), and cisplatin (*C*) in stably transfected HepG2 cell lines. All results were normalized to cellular protein, and relative accumulation was calculated by dividing the radioactivity (*A* and *B*) or the amount of drug (*C*) in HA6 and HA7 cells by that in the HV1 cells. Each value represents the average of two separate experiments with triplicate dishes; *bars*, SD. *, $P < 0.05$ compared to HV1.

cisplatin rapidly interacts with GSH. Thus, MRP may be a GS-X pump that promotes the ATP-dependent efflux of GSH-conjugated cisplatin (28). However, cells transfected with sense *MRP* cDNA and drug-selected *MRP*-overproducing cells did not show any altered sensitivity to cisplatin (3, 5, 16). In contrast, elevated levels of *cMOAT* mRNA were observed in cisplatin-resistant cell lines derived from various human tumors. Kool *et al.* (29) recently examined the expression of message encoded by the *MRP* family, including *cMOAT*, in a large number of cisplatin- and doxorubicin-resistant cell lines and showed a correlation between *cMOAT* transcript levels and sensitivity to cisplatin but not to doxorubicin. These findings are in agreement with those shown here in that sensitivity to cisplatin was enhanced in *cMOAT* antisense transfectants of HepG2 cells.

Our results also indicate that *cMOAT* antisense cDNA did not increase the expression of *MRP* and *MDR1*, although cellular levels of these proteins in HepG2 cells were not high enough to determine whether their expression was decreased in the transfectants. The effect of *cMOAT* antisense cDNA on the expression of other *cMOAT* family genes is not yet known. Our results support the hypothesis that *cMOAT* and its related genes may constitute a novel ATP-binding cassette superfamily of genes that are associated with cellular drug sensitivity and/or ATP-dependent drug transport. Transfection experiment with total *cMOAT* cDNA should be required to understand whether cMOAT itself could specifically alter drug sensitivity, which is now in progress in our laboratory.

Acknowledgments

We thank T. Nakamura for fruitful discussions as well as K. Yamada and T. Matsuguma in our laboratory for experimental and editorial help.

Note Added in Proof

We identified mutations in the cMOAT gene in patients with Dubin-Johnson syndrome (30), strongly implicating the human cMOAT transport glucuronide conjugates, including bilirubin.

References

1. Endicott, J. A., and Ling, V. The biochemistry of P-glycoprotein-mediated multidrug resistance. Annu. Rev. Biochem., *58:* 137–171, 1989.
2. Gottesman, M. M., and Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu. Rev. Biochem., *62:* 385–427, 1993.
3. Loe, D. W., Deeley, R. G., and Cole, S. P. C. Biology of multidrug resistance-associated protein, MRP. Eur. J. Cancer, *32A:* 945–957, 1996.
4. Cole, S. P. C., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M. V., and Deeley, R. G. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science (Washington DC), *258:* 1650–1654, 1992.
5. Cole, S. P. C., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. Pharmacological characterization of multidrug resistant MRP-transfected human tumor cells. Cancer Res., *54:* 5902–5910, 1994.
6. Keppler, D., and Kartenbeck, J. The canalicular conjugate export pump encoded by the *cmrp/cmoat* gene. Prog. Liver Dis., *14:* 55–67, 1996.
7. Leier, I., Jedlitschky, G., Buchholz, U., Cole, S. P. C., Deeley, R. G., and Keppler, D. The *MRP* gene encodes an ATP-dependent export pump for leukotriene C_4 and structurally related conjugates. J. Biol. Chem., *296:* 27807–27810, 1994.
8. Müller, M., Meijer, C., Zaman, G. J. R., Borst, P., Scheper, R. J., Mulder, N. H., de Vries, E. G. E., and Jansen, P. L. M. Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATP-dependent glutathione S-conjugate transport. Proc. Natl. Acad. Sci. USA, *91:* 13033–13037, 1994.
9. Loe, D. W., Almquist, K. C., Deeley, R. G., and Cole, S. P. C. Multidrug resistance protein (MRP)-mediated transport of leukotriene C_4 and chemotherapeutic agents in membrane vesicles: demonstration of glutathione-dependent vincristine transport. J. Biol. Chem., *271:* 9675–9682, 1996.
10. Paulusma, C. C., Bosma, P. J., Zaman, G. J. R., Bakker, C. T. M., Otter, M., Scheffer, G. L., Scheper, R. J., Borst, P., and Oude Elferink, R. P. J. Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science (Washington DC), *271:* 1126–1128, 1996.
11. Büchler, M., König, J., Brom, M., Kartenbeck, J., Spring, H., Horie, T., and Keppler, D. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein cMrp reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. J. Biol. Chem., *271:* 15091–15098, 1996.
12. Ito, K., Suzuki, H., Hirohashi, T. Kume, K. Shimizu, T., and Sugiyama, Y. Molecular cloning of canalicular multispecific organic anion transporter defective in EHBR. Am. J. Physiol., *272:* G16–G22, 1997.
13. Nishida, T., Gatmaitan, Z., Roy-Chowdhry, J., and Arias, I. M. Two distinct mechanisms for bilirubin glucuronide transport by rat bile canalicular membrane vesicles: demonstration of defective ATP-dependent transport in rats (TR^-) with inherited conjugated hyperbilirubinemia. J. Clin. Invest., *90:* 2130–2135, 1992.
14. Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kagotani, K., Okumura, K., Akiyama, S., and Kuwano, M. A human canalicular multispecific organic anion transporter (*cMOAT*) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation. Cancer Res., *56:* 4124–4129, 1996.
15. Mayer, R., Kartenbeck, J., Büchler, M., Jedlitschky, G., Leier, I., and Keppler, D. Expression of the *MRP* gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. J. Cell Biol., *131:* 137–150, 1995.
16. Koike, K., Abe, T., Hisano, T., Kubo, T., Wada, M., Kohno, K., and Kuwano, M. Overexpression of multidrug resistance protein gene in human cancer cell lines selected for drug resistance to epipodophyllotoxins. Jpn. J. Cancer Res., *87:* 765–772, 1996.
17. Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda, N., and Mikoshiba, K. Primary structure and functional expression of the inositol 1,4,5,-triphosphate-binding protein P_{400}. Nature (Lond.), *342:* 32–38, 1989.
18. Ohga, T., Koike, K., Ono, M., Makino, Y., Itagaki, Y., Tanimoto, M., Kuwano, M., and Kohno, K. Role of the human Y-box binding protein YB-1 in cellular sensitivity to the DNA-damaging agents cisplatin, mitomycin C, and ultraviolet light. Cancer Res., *56:* 4224–4228, 1996.
19. Yokomizo, A., Kohno, K., Wada, M., Ono, M., Morrow, C. S., Cowan, K. H., and Kuwano, M. Markedly decreased expression of glutathione *S*-transferase-pi gene in human cancer cell lines resistant to buthionine sulfoximine, an inhibitor of cellular glutathione synthesis. J. Biol. Chem., *270:* 19451–19457, 1995.
20. Kotoh, S., Naito, S., Yokomizo, A., Kumazawa, J., Asakuno, K., Kohno, K., and Kuwano, M. Increased expression of DNA topoisomerase I gene and collateral sensitivity to camptothecin in human cisplatin-resistant bladder cancer cells. Cancer Res., *54:* 3248–3252, 1994.
21. Cole, S. P. C., Downes, H. F., Mirski, S. E. L., and Clements D. J. Alterations in glutathione and glutathione-related enzymes in a multidrug resistant small cell lung cancer cell line. Mol. Pharmacol., *37:* 192–197, 1990.

22. Zaman, G. J. R., Lankelma, J., van Tellingen, O., Beijnen, J., Dekker, H., Paulusma, C., Oude Elferink, R. P. J., Baas, F., and Borst, P. Role of glutathione in the export of compounds from cells by the multidrug-resistance-associated protein. Proc. Natl. Acad. Sci. USA, *92:* 7690–7694, 1995.
23. Lautier, D., Canitrot, Y., Deeley, R. G., and Cole, S. P. C. Multidrug resistance mediated by the multidrug resistance protein (*MRP*) gene. Biol. Pharmacol., *52:* 967–977, 1996.
24. Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G., and Keppler, D. Transport of glutathione, glucuronate, and sulfate conjugates by the *MRP* gene-encoded conjugate export pump. Cancer Res., *56:* 988–994, 1996.
25. Chu, X-Y., Kato, Y., Niinuma, K., Sudo, K., Hakusui, H., and Sugiyama, Y. Multispecific organic anion transporter (cMOAT) is responsible for the biliary excretion of the camptothecin derivative irinotecan, CPT-11, and its metabolites in rats. J. Pharmacol. Exp. Ther., *281:* 304–314, 1997.
26. Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res., *54:* 4313–4320, 1994.
27. Goto, S., Yoshida, K., Morikawa, T., Urata, Y., Suzuki, K., and Kondo, T. Augmentation of transport for cisplatin-glutathione adduct in cisplatin-resistant cancer cells. Cancer Res., *55:* 4297–4301, 1995.
28. Ishikawa, T., Wright, T., and Ishizuka, H. Coordinated induction of *MRP/GS-X* pump and γ-glutamylcysteine synthetase by heavy metals in human leukemia cells. J. Biol. Chem., *271:* 14981–14988, 1996.
29. Kool, M., de Hass, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J. T., Juijn, J. A., Bass, F., and Borst, P. Analysis of expression of *cMOAT (MRP2), MRP3, MRP4,* and *MRP5* homologues of the multidrug resistance-associated protein gene (*MRP1*) in human cancer cell lines. Cancer Res., *57:* 3537–3547, 1997.
30. Wada, M., Toh, S., Taniguchi, K., Nakamura, T., Takeshi, U., Kohno, K., Yoshida, I., Kimura, A., Sakisaka, S., Adachi, Y., and Kuwano, M. Mutations in the canalicular multispecific organic anion transporter (*cMOAT*) gene, a novel ABC transporter, in patients with hyperbilirubinemia II/Dubin-Johnson syndrome. Hum. Mol. Genet., in press, 1998.
31. Rappa, G., Lorico, A., and Sartorelli, A. C. Development of MRP double knockout cell lines: rule of MRP in the export of glutathione. Proc. Am. Assoc. Cancer Res., *38:* 252(Abstract 1691), 1997.
32. Lorico, A., Rappa, G., Finch, R. A., Flavell, R. A., and Sartorelli, A. C. Generation of mice with a homozygous disruption of the MRP gene. Proc. Am. Assoc. Cancer Res., *38:* 252(Abstract 1692), 1997.

A Canalicular Multispecific Organic Anion Transporter (*cMOAT*) Antisense cDNA Enhances Drug Sensitivity in Human Hepatic Cancer Cells

Koji Koike, Takeshi Kawabe, Toshiya Tanaka, et al.

Cancer Res 1997;57:5475-5479.