BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **252,** 103–110 (1998)
ARTICLE NO. RC989546

Isolation of a Novel Human Canalicular Multispecific Organic Anion Transporter, cMOAT2/MRP3, and Its Expression in Cisplatin-Resistant Cancer Cells with Decreased ATP-Dependent Drug Transport

Takeshi Uchiumi,*,[1] Eiji Hinoshita,* Sei Haga,* Takanori Nakamura,* Toshiya Tanaka,* Satoshi Toh,* Manabu Furukawa,* Takeshi Kawabe,* Morimasa Wada,* Kazuhiro Kagotani,† Katsuzumi Okumura,† Kimitoshi Kohno,‡ Shin-ichi Akiyama,§ and Michihiko Kuwano*

**Department of Biochemistry, Kyushu University School of Medicine, Maidashi, Fukuoka 812-8582; ‡Department of Molecular Biology, University of Occupational and Environmental Health, Kita-Kyushu 807-0804; †Faculty of Bioresources, Mie University, Tsu, Mie 514-8507; and §Department of Cancer Chemotherapy, Institute for Cancer Research, Faculty of Medicine, Kagoshima University, Sakuraga-oka, Kagoshima 890-0075, Japan*

Received September 21, 1998

The human multidrug resistance protein (MRP) gene encodes a membrane protein involved in the ATP-dependent transport of hydrophobic compounds. We previously isolated a canalicular multispecific organic anion transporter, cMOAT1/MRP2, that belongs to the ATP binding cassette (ABC) superfamily, which is specifically expressed in liver, and cMOAT1/MRP2 is responsible for the defects in hyperbilirubinemia II/Dubin–Johnson syndrome. In this study, we isolated a new cDNA of the ABC superfamily designated cMOAT2/MRP3 that is homologous to human MRP1 and cMOAT1/MRP2: cMOAT2/MRP3 is 56% identical to MRP1 and 45% identical to cMOAT1/MRP2, respectively. Fluorescence *in situ* hybridization demonstrated the chromosomal locus of this gene on chromosome 17q22. The human cMOAT2 cDNA hybridized to a 6.5-kb mRNA that was mainly expressed in liver and to a lesser extent in colon, small intestine, and prostate. The cMOAT2/MRP3 gene was not overexpressed in cisplatin-resistant cell lines with increased ATP-dependent transport of cisplatin over their parental counterparts derived from human head and neck cancer and human prostatic cancer cell lines. The human cMOAT2/MRP3, a novel member of the ABC superfamily, may function as a membrane transporter in liver, colon, and prostate.

Key Words: **cMOAT; MRP; ABC transporters; drug resistance**

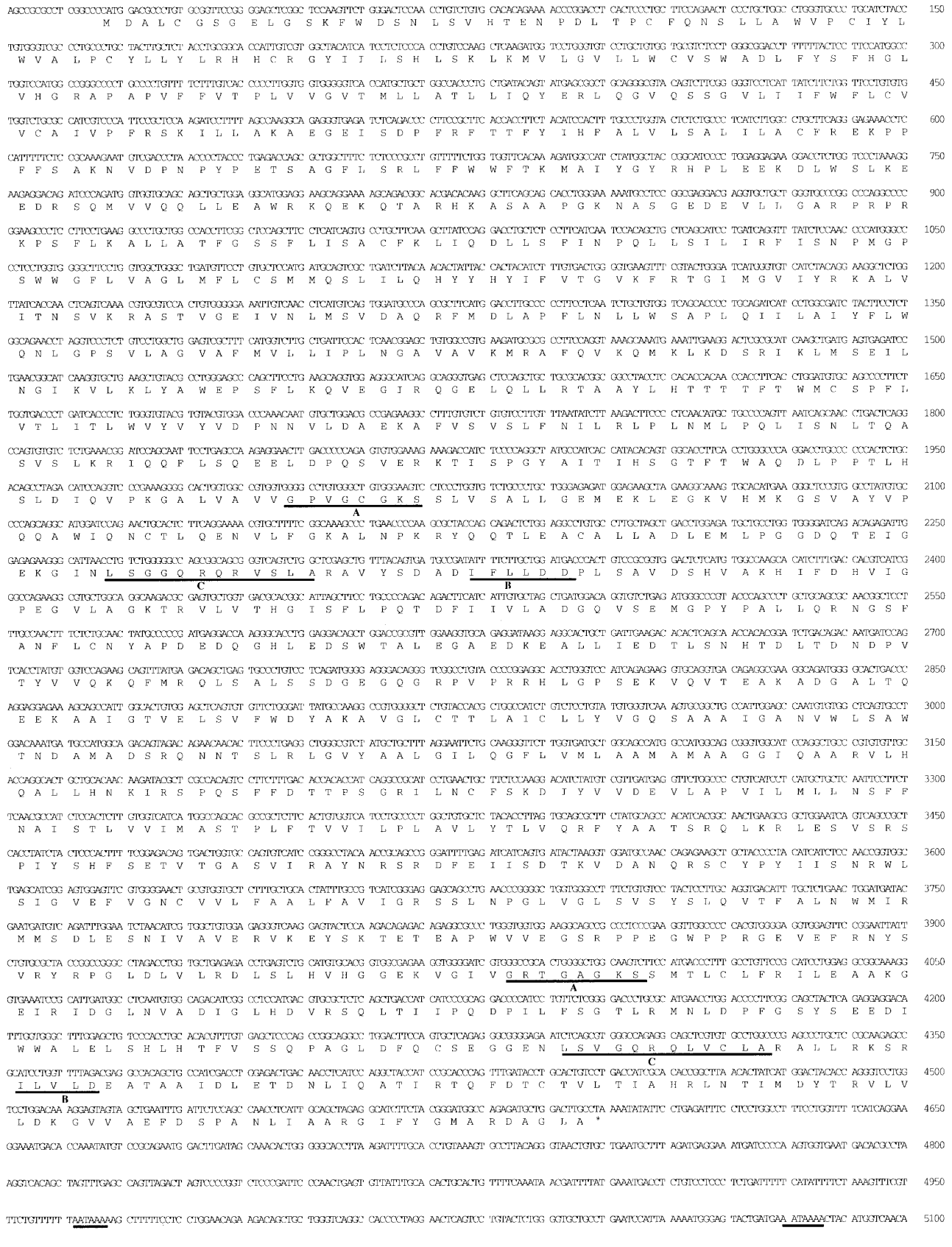
Two ATP binding cassette (ABC) transporter superfamily proteins, P-glycoprotein (P-gp) and multidrug resistance protein (MRP), are well known to confer multidrug resistance to cancer cells through enhanced drug efflux (1–4). Treatment of cancer cells with many of the natural product anticancer drugs including the vinca alkaloids (vincristine and vinblastine), anthracyclines (doxorubicin and daunomycin), colchicine, taxanes, and epipodophyllotoxins (etoposide and teniposide), often results in overexpression of Pgp and MRP1 (1, 2). Overexpression of human multidrug resistance (MDR)1 cDNA and MRP1 cDNA in cancer cells results in acquired resistance to anthracyclines, vinka alkaloids, epipodophyllotoxins, and heavy metal anions (3, 4), but does not result in any cross-resistance to platinum-containing compounds, alkylating agents, and antimetabolites (3, 5). These studies suggest the involvement of other ABC proteins in the ATP dependent efflux of cisplatin and other agents.

Cisplatin is a potent and representative platinum-containing anticancer agent that has been widely used to treat various malignant tumors. One can expect that decreased intracellular accumulation of cisplatin has a key role in limiting drug sensitivity to this potent anticancer agent (6, 7). The ATP-dependent active outward efflux of cisplatin is enhanced in some human cancer cell lines resistant to cytotoxic effects of cisplatin (8–10). Cisplatin forms glutathione(GSH)-conjugates in cancer cells, and a GS-X pump is expected to be involved in ATP-dependent efflux of GSH-cisplatin conjugates (11). MRP1 and yeast cadmium factor play an important role as active transporters for anticancer agents, and MRP1 can transport cysteinyl leukotriene C_4 (LTC_4) and other GSH-conjugates, suggesting that

[1] To whom correspondence should be addressed at Department of Biochemistry, Kyushu University School of Medicine, 3-1-1 Maidashi, Fukuoka 812-8582, Japan. Fax: 81-92-642-6203. E-mail: uchiumi@biochem1.med.kyushu-u.ac.jp.

0006-291X/98 $25.00

A



B

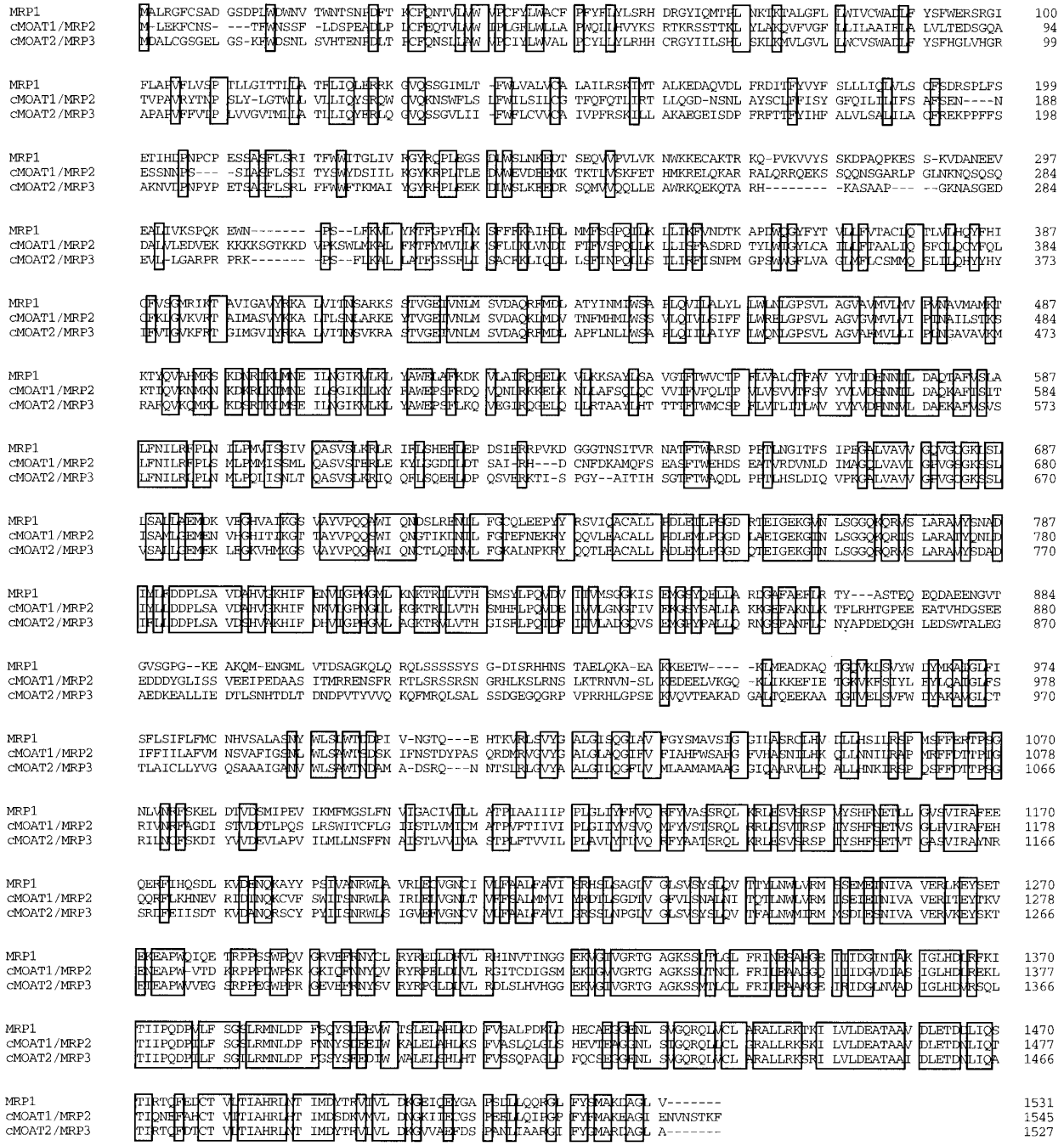


FIG. 1. (A) cDNA and deduced amino acid sequence of human cMOAT2/MRP3 genes. Walker A and B motifs and active transport family signature are indicated by single lines and denoted A, B, and C, respectively. A poly(A) additional signal is also underlined at 3′ noncoding region. (B) Comparison of amino acid sequences of human MRP1, cMOAT1/MRP2 and cMOAT2/MRP3. Boxes, amino acid identity. Amino acid differences are shown in their corresponding positions. Dashes, gaps that are introduced to maximize identity.

MRP1 may be a GS-X pump (11). However, there appears no apparent overexpression of MRP1 in cisplatin-resistant cancer cell lines which have decreased cisplatin accumulation (12). Moreover, transfection of MRP1 cDNA failed to confer resistance to cisplatin in cancer cells (13). It remains unclear whether any member of the ABC transporter superfamily could function as a GS-X pump for cisplatin.

We previously isolated the human canalicular multispecific organic anion transporter 1 (cMOAT1) gene belonging to the ABC superfamily by targeting the ATP binding domain conserved in MDR1, MRP1, and

cystic fibrosis transmembrane regulator (CFTR) genes (14). The human cMOAT1 gene is highly homologous to rat cMOAT, a homologue of the human MRP1 gene (14, 15). Mutations of cMOAT1/MRP2 are observed in Dubin–Johnson syndrome (16) and its model animals (17–19). Dubin–Johnson model animal is defective in ATP-dependent transport of glucuronic acid conjugates of bilirubin. The cMOAT1/MRP2 activity appears to mediate the ATP-dependent transport of various hydrophobic anionic compounds including the camptothecins and methotrexate in liver canalicular membranes and other tissues in the Dubin–Johnson model animals (20). The finding that cMOAT1/MRP2 can transport the cysteinyl leukotrienes (e.g., LTC_4) as well as other GSH conjugates (Kawabe *et al.*, unpublished data) suggests that cMOAT1/MRP2 may be a GS-X pump. The spectrum of hydrophobic anionic compounds transported by cMOAT1/MRP2 resembles that of MRP1 (21).

Expression of the cMOAT1/MRP2 gene is enhanced in cisplatin-resistant lines derived from various human cancer cell types (15). Koike *et al.* have reported that introduction of cMOAT1/MRP2 antisense cDNA into human hepatic cancer HepG2 cells results in increased sensitivity to cisplatin, vincristine, doxorubicin and the camptothecin derivatives (22). In these transfectants, cellular level of cisplatin and vincristine as well as GSH were increased, suggesting that cMOAT1/MRP2 and its related genes are involved in the membrane transport of the above drugs including cisplatin (22). However, these studies did not show if cMOAT1/MRP2 itself is directly involved in drug transport of cisplatin, or if other homologues of cMOAT1/MRP2 are involved in drug transport of cisplatin.

In this study, we isolated another clone which is homologous to MRP1 and cMOAT1/MRP2, and we designated this clone as the cMOAT2/MRP3. The cMOAT2/MRP3 gene is 56% identical to MRP1 and 45% identical to cMOAT1/MRP2, suggesting the cMOAT2/MRP3 gene is a member of MRP family. We also examined if expression of cMOAT2/MRP3 was altered in human cisplatin-resistant cancer cell lines with enhanced ATP-dependent efflux of cisplatin.

MATERIALS AND METHODS

Cell lines. We used cisplatin-resistant cell line, P/CDP5, and its cisplatin-sensitive revertant cell line, P/CDP5-R from human prostatic cancer PC-3 cells (8, 23). We also used another cisplatin resistant cell line, KB/KCP-4, and its cisplatin-sensitive revertant cell line, KB/KCP-4R, from human head and neck cancer KB cells (10, 24). These cell lines were cultured at 37°C under a humidified atmosphere of 5% CO_2 in Eagle's MEM (Nissui Seiyaku, Tokyo, Japan) containing 10% fetal bovine serum, glutamine, kanamycin and penicillin.

cDNA cloning. For the isolation of cMOAT2/MRP3, we used a low stringency hybridization method. cMOAT2/MRP3 cDNA clones were isolated by screening a pCMVSPORT human liver cDNA library (Life Technologies, Inc. Gaithersburg, MD) , using a 2-kb fragment of a human cMOAT1 clone as a probe under low stringency conditions. Several clones were isolated, subcloned into the pUC18 plasmid and sequenced. Except for MRP1 and cMOAT1/MRP2 clones, we obtained a novel cDNA clone homologous to MRP1 and cMOAT1/MRP2. For cloning of a full-length cDNA of the cMOAT2 gene, pCMVSPORT human liver cDNA library was screened with a cDNA fragment as a probe by standard procedures (25). Chain elongation and termination were performed with a DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Tokyo, Japan), and nucleotide sequencing was performed with a DNA sequencing system (Model 377; Applied Biosystems). Data were analyzed using GeneWorks software (IntelliGenetics, Mountain View, CA). Sequences have been deposited with GenBank (cMOAT2/MRP3: Accession No. AF083552)

Northern blot analysis. Northern blot analysis was performed with 20 μg of total RNA prepared with RNeasy spin columns (QIAGEN). After transfer to Hybond-N membrane (Amersham) and UV cross-linking, the blot was hybridized with a human cMOAT2/MRP3 cDNA probe generated by random primer labeling (Amersham). After hybridization, the blots were washed twice in washing buffer 1 (2 × SSC, 0.1% SDS) at 42°C and twice in washing buffer 2 (0.2 × SSC, 0.1% SDS) at 65°C. A human multiple tissue Northern blot was commercially obtained (Clontech). The amount of human cMOAT2/MRP3 encoding mRNA was quantified with a Fujix BAS 2000 image analyzer (Fuji, Tokyo, Japan).

Cisplatin accumulation. Cells were incubated overnight, then incubated with 20 μM cisplatin for 2 h at 37°C. Cells were then harvested, air-dried, and digested in nitric acid. After evaporation, the platinum content was measured by atomic absorption spectrophotometer (10, 24).

Colony formation assay. To assay colony formation, 300 cells were seeded in a 35-mm dish in the absence of any drug and incubated for an additional 7 days with various concentration of cisplatin.

FISH analysis. Probe labeling and *in situ* hybridization were performed as described previously (26).

RESULTS

Human MDR1, MRP1, and cystic fibrosis transmembrane regulator (CFTR) have been identified as members of the ABC transporter superfamily. The similarity of these three genes resides predominantly in two ATP binding domains. Based on their homology, we designed highly degenerate C-series primers and isolated the cDNA clone of cMOAT1/MRP2 gene (14). For isolating the full-length cDNA of cMOAT1/MRP2, we screened a human colon cDNA library using as a probe a PCR product, and obtained several clones. After sequencing these clones, we obtained a new clone (C51) that is homologous to MRP1 and cMOAT1/MRP2. Screening of human liver cDNA library by this clone, we isolated a 5.5-kb single clone designated cMOAT2/MRP3 (Fig. 1A). Sequencing of these clones revealed an open reading frame coding for 1527 amino acids that showed 56 and 45% similarity to human MRP1 and cMOAT1/MRP2, respectively (Fig. 1B and Table 1).

To determine the chromosome localization of the cMOAT2 gene, FISH analysis was performed. Hybridization of the cMOAT2 cDNA probe revealed positive signals at 17q22 (Fig. 2). Human MDR1, CFTR, MRP1 and cMOAT1 genes are thus localized on 7q2l.l, 7q31, 16p13.1, and 10q24 respectively (13, 14, 27, 28), suggest-

TABLE 1

Amino Acid Homology among Various ABC Transporter Superfamily Genes: MRP1, cMOAT/MRP2, cMOAT2/MRP3, MDR1, CFTR, EBCR, and SUR[a]

	MRP1 (%)	cMOAT1/MRP2 (%)	cMOAT2/MRP3 (%)	MDR 1 (%)	CFTR (%)	EBCR (%)	SUR (%)
MRP1	100						
cMOAT1/MRP2	47	100					
cMOAT2/MRP3	56	45	100				
MDR1	17	18	18	100			
CFTR	3	3	22	7	100		
EBCR	44	80	43	14	3	100	
SUR	30	2	25	12	19	25	100

Note. CFTR, cystic fibrosis transmembrane regulator; EBCR, epithelial basolateral conductance regulator; SUR, sulfonyl urea receptor.
[a] Percentages of identity and similarity were determined using the protein alignment program of Gene Works.

ing that the human cMOAT2/MRP3 gene is different from those relevant ABC transporter superfamily genes.

Multiple-tissue northern blot analyses with the cDNA probe revealed that human cMOAT2/MRP3 mRNA was highly expressed in liver, and slightly expressed in colon, small intestine, prostate and pancreas (Fig. 3). The tissue profile of human MRP1 and cMOAT1/MRP2 expression was different from that of cMOAT2/MRP3 (14).

To determine if cMOAT2/MRP3 is involved in the cisplatin-resistance phenotype mediated by ATP-dependent enhanced drug efflux, we examined expression of cMOAT2/MRP3 gene in cisplatin-resistant cancer cell lines with decreased ATP-dependent accumulation of cisplatin. ATP-dependent efflux of cisplatin was enhanced in a cisplatin-resistant line, KB/KCP-4, derived from human head and neck cancer KB cells (10, 24). Another cisplatin-resistant cell line, P/CDP-5, derived from human prostatic cancer PC-3 cells also showed decreased ATP-dependent efflux of cisplatin (8, 23, 24). In this study, we used cisplatin-sensitive revertants, KB/KCP-4R and P/CDP5-R, which were isolated after long-term culture of KB/KCP-4 and P/CDP5 in the absence of cisplatin (Table 2). Both cisplatin-sensitive revertants showed a partial restoration in their drug sensitivity to cisplatin and cellular accumulation of cisplatin in their respective drug-sensitive parental counterparts (Table 2).

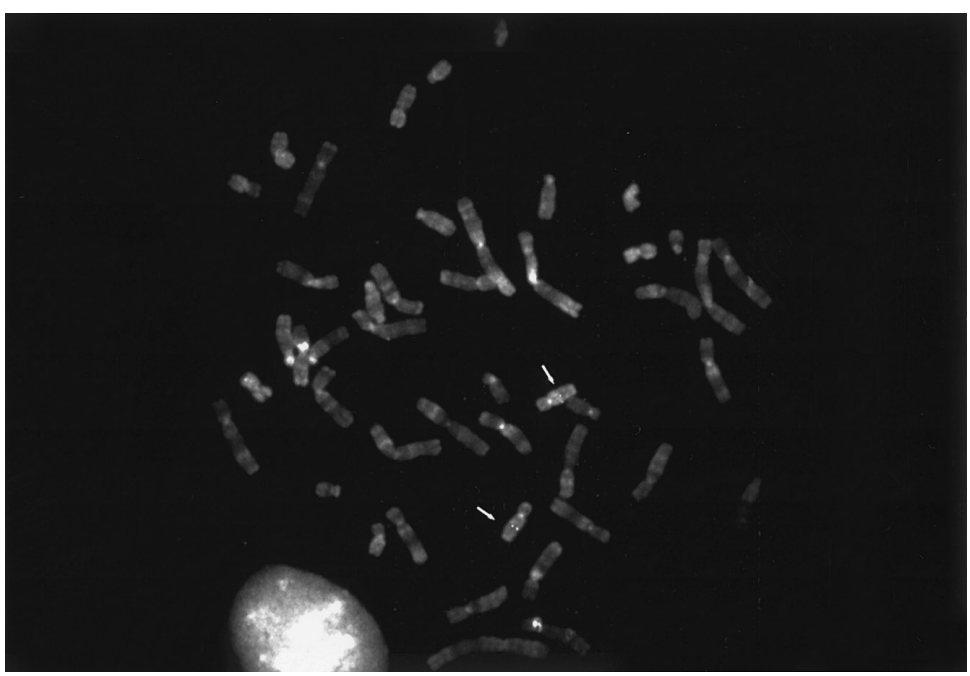


FIG. 2. Mapping of the gene region encoding human cMOAT2 by FISH. Human metaphase spreads were hybridized with cMOAT2 cDNA probe. Arrows, fluorescence signals on the R-banded metaphase chromosomes. Based on observations of more than 20 spreads, a band is identified at 17q22.

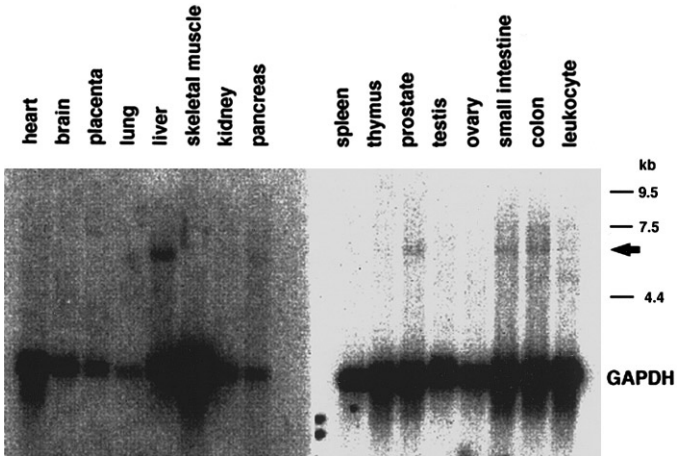


FIG. 3. Northern blot analysis. Human multiple tissue northern blots were probed with human cMOAT2/MRP3 and GAPDH cDNA probe. Arrow, the position of the cMOAT2/MRP3 transcript.

Northern blot analysis with specific cDNA probes of MRP1, cMOAT1/MRP2 and cMOAT2/MRP3 showed that the human cMOAT2/MRP3 gene was not overexpressed in both KB/KCP-4 and P/CDP5 compared to their parental drug-sensitive counterparts, KB and PC-3, and also that cellular mRNA levels of cMOAT2/MRP3 were not decreased in the revertant cell lines (Fig. 4). MRP1 mRNA levels were similar in all three cell lines derived from KB and PC-3. In contrast, the human cMOAT1/MRP2 gene was expressed in KB and PC-3, but markedly decreased in their cisplatin-resistant cell lines, KB/KCP-4 and P/CDP5 (Fig. 4). Although cellular mRNA levels of cMOAT2/MRP3 were similar between KB/KCP-4 and KB/KCP-4R, cMOAT2/MRP3 gene were overexpressed in P/CDP5-R cells in comparison with PC-3 and P/CDP5 (Fig. 4 and Table 2).

DISCUSSION

During characterization of the human ABC superfamily genes using the expressed sequence tags database, Allikmets *et al.* identified 21 new ABC genes including genes for transporters related to MRP1 (29). We observed an EST with an identical amino acid sequence on the C-terminal region of the cloned cMOAT2/MRP3 gene. In our present study, we isolated a full length human cMOAT2/MRP3 cDNA which was a homologue of the human MRP1 and human cMOAT1/MRP2 gene. cMOAT2/MRP3 is composed of 1527 amino acids containing two ATP-binding cassette regions with Walker motifs. The cMOAT2/MRP3 gene as well as MRP1 and cMOAT1/MRP2 genes had about 40% homology with the yeast cadmium factor gene, YCF1 (data not shown), suggesting that cMOAT2 gene belongs to the GS-X pump family genes including MRP1, cMOAT1/MRP2 and YCF1 genes within the ABC transporter superfamily.

The human MRP1 and human cMOAT1/MRP2 genes have been mapped to chromosome 16p13.1 and chromosome 10q24, respectively (13, 14). In contrast, the cMOAT2/MRP3 gene was located at chromosome band 17q22. These facts demonstrated that there is no cross hybridization among MRP1, cMOAT1/MRP2 and cMOAT2/MRP3 genes, suggesting that cMOAT2/MRP3 is a single gene on the human chromosome. Expression of the cMOAT2/MRP3 was high in liver, and to a less extent in colon, small intestine and prostate, consistent with the previous study (15). Immunohistochemical analysis and/or RNA *in situ* hybridization analysis should be required for determination of exact localization of cMOAT2/MRP3 in human tissues.

Kool *et al.* (1997) have reported expression of MRP family genes in cisplatin resistant cell lines from various human tumor types (15), but the underlying mech-

TABLE 2

Comparison of Cisplatin Accumulation, Drug Resistance, and Expression of MRP1, cMOAT1/MRP2, and cMOAT2/MRP3

Cell lines	Relative cisplatin resistance[a]	Cisplatin accumulation (%)[b]	Expression of ABC transporter[c]		
			MRP1	cMOAT1/MRP2	cMOAT2/MRP3
KB	1	100	1	1	1
KB/KCP-4	25	13	1.3	0.1	1
KB/KCP-4R	4	93	1.4	1	2.1
PC-3	1	100	1	1	1
P/CDP5	23	18	1.1	0.3	1
P/CDP5-R	4	71	1.1	0.9	3.0

[a] Relative resistance to cisplatin was presented when IC90 for the resistant line and its drug-sensitive revertant were normalized by that for each parental KB and PC-3. Cell survival curves for each cell line were determined by colony formation assay. The data were comparable to previous reports (23, 24).

[b] Cellular platinum content was determined by atomic absorption spectrophotometer. 100% corresponds to 120± pmol/10^6 cells in KB and 80± pmol/10^6 cells in PC-3 when incubated for 120 min with 20 μM cisplatin.

[c] mRNA levels: Northern blot analysis shows various levels of mRNA of three ABC transporter genes. Cellular mRNA levels of each ABC transporter in drug-resistant lines and these drug-sensitive revertants were normalized by those in their wild-type parental counterparts.

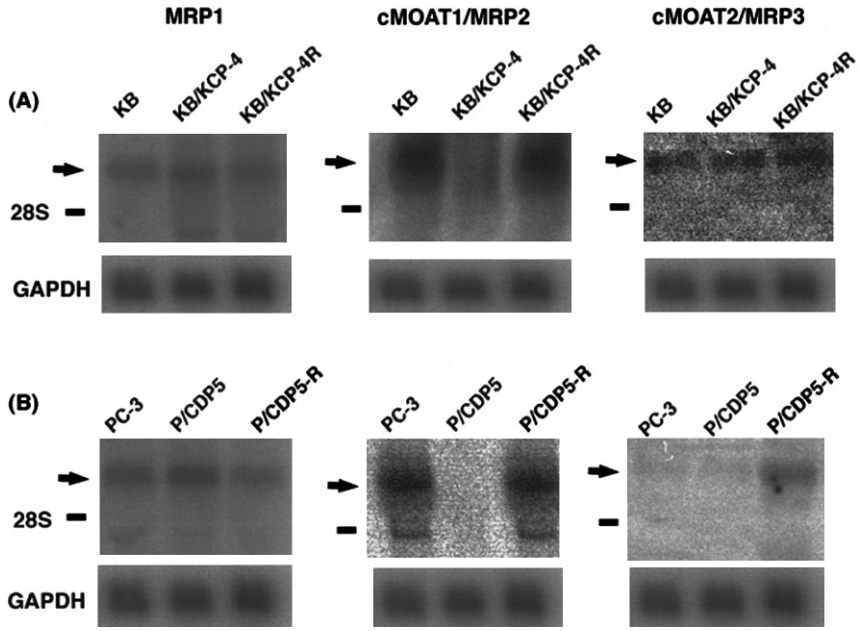


FIG. 4. Comparison of MRP1, cMOAT1/MRP2, and cMOAT2/MRP3 mRNA levels in drug-sensitive parental, cisplatin-resistant, and cisplatin-sensitive revertant cell lines from human head and neck cancer KB (A) and human prostatic cancer PC-3 (B) cells. Various parental, cisplatin-resistance and revertant cell lines were analyzed using the MRP1, cMOAT1/MRP2 and cMOAT2/MRP3 cDNA probes, respectively. GAPDH probe was used for control. Arrows indicates major transcription products.

anisms for cisplatin resistance in these cell lines are under pleiotropic controls including drug retention, detoxification through glutathione, DNA damage repair and ATP dependent efflux. In this study, we also examined expression of the cMOAT2/MRP3 gene in cisplatin resistant cell lines with decreased cisplatin accumulation, including human head/neck cancer KB and prostatic cancer PC-3 cell lines. KB/KCP-4 and P/CDP5 that do not overexpress Pgp or MRP1 show enhanced active ATP-dependent efflux of cisplatin. A cell-cell hybridization test indicated that both the drug resistance and the accumulation defect found in KB/KCP-4 cells are dominant traits (10), suggesting the existence of an active efflux system for cisplatin in KB/KCP-4 cells. Establishment of cisplatin-sensitive revertants thus appeared to be resulted in partial restoration of drug sensitive phenotype in both drug sensitivity and cellular drug accumulation (Table 2). In comparison with parental KB and PC-3 cells, the cellular levels of cMOAT2/MRP3 mRNA were not increased in KB/KCP-4 and P/CDP5. Kool *et al.* (1997) also reported no overexpression of cMOAT2/MRP3 gene in KB/KCP-4 cells(15). Moreover, cMOAT2/MRP3 gene was not decreased in their drug-sensitive revertants, KB/KCP-4R and P/CDP5-R with the partial restoration of both cisplatin sensitivity and cellular accumulation of cisplatin (Table 2). The presence or absence of cMOAT2/MRP3 might not be critical for limiting cisplatin resistance and cellular accumulation of cisplatin, possibly through its ATP-dependent efflux activity in these cisplatin-resistant cell lines.

Taniguchi *et al.* (1996) reported enhanced expression of cMOAT1/MRP2 in KB/KCP-4 cells (14), but the sequence of the probe employed in the previous study could be highly homologous to other ABC transporter superfamily genes. Human hepatic cancer cell lines transfected with antisense cMOAT1/MRP2 showed an increase in both sensitivity to cisplatin and drug accumulation (22), suggesting that cMOAT1/MRP2 and its related ABC transport family genes could be involved in sensitivity to cisplatin and its cellular accumulation. We observed that Chinese hamster ovary cell lines and pig kidney cell lines overexpressing human cMOAT1/MRP2 acquired a slightly increased drug resistance to cisplatin with a decrease in cisplatin accumulation (Kawabe *et al.,* unpublished data), suggesting that cMOAT1/MRP2 gene might have a partial role in limiting drug sensitivity to cisplatin. However, in our present study, we could not observe any specific change in cellular levels of the cMOAT2/MRP3 mRNA in cisplatin resistant cancer cell lines with decreasing drug accumulation. Further study should be required to determine which ATP dependent efflux pump has a critical role in the outward ATP transport of cisplatin in these cancer cell lines, and also if transfection of cMOAT2/MRP3 cDNA could change drug sensitivity to cisplatin and other anticancer agents.

ACKNOWLEDGMENTS

We thank Dr. Tito Fojo (NCI, NIH, U.S.A.) for critical reading of the manuscript. This study was supported by grants from the Min-

istry of Education, Science, Sports, and Culture of Japan; the Fukuoka Anti-Cancer Research Fund; Second-Term Comprehensive, Ten-Year Strategy for Cancer Control from the Ministry of Health and Welfare; Kaibara Morikazu Medical Science Promotion Foundation; and CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (J.S.T.).

REFERENCES

1. Gottesman, M. M., and Pastan, I. (1993) *Annu. Rev. Biochem.* **62,** 385–427.
2. Germann, U. A. (1996) *Eur. J. Cancer* **32A,** 927–944.
3. Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M., and Deeley, R. G. (1992) *Science* **258,** 1650–1654.
4. Loe, D. W., Deeley, R. G., and Cole, S. P. C. (1996) *Eur. J. Cancer* **32A,** 945–957.
5. Kruh, G. D., Chan, A., Myers, K., Gaughan, K., Miki, T., and Aaronson, S. A. (1994) *Cancer Res.* **54,** 1649–1652.
6. Tew, K. D. (1994) *Cancer Res.* **54,** 4313–4320.
7. Chu, X-Y., Kato, Y., Niinuma, K, Sudo, K., Hakusui, H., and Sugiyama, Y. (1997) *J. Pharmacol. Exp. Ther.* **281,** 304–314.
8. Nakagawa, M., Nomura, Y., Kohno, K., Ono, M., Mizoguchi, H., Ogata, J., and Kuwano, M. (1993) *J. Urol.* **150,** 1970–1973.
9. Kotoh, S., Naito, S., Yokomizo, A., Kumazawa, J., Asakuno, K., Kohno, K., and Kuwano, M. (1994) *Cancer Res.* **54,** 3248–3252.
10. Fujii, R., Match, M., Niwa, K., Yamada, K., Aiken, T., Nakagawa, M., Kuwano, M., and Akiyama, S. (1994) *Jpn. J. Cancer Res.* **85,** 426–433.
11. Ishikawa, T., Wright, C. D., and Ishizuka, H. (1994) *J. Biol. Chem.* **269,** 29085–29093.
12. Chuman, Y., Chen, Z. S., Sumizawa, T., Furukawa, T., Haraguchi, M., Takebayashi, Y., Niwa, K., Yamada, K., Aikou, T., and Akiyama, S. (1996) *Biochem. Biophys. Res. Commun.* **226,** 158–165.
13. Cole, S. P., Sparks, K. E., Fraser, K., Loc, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. (1994) *Cancer Res.* **54,** 5902–5910.
14. Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kagotani, K., Okumura, K., Akiyama, S., and Kuwano, M. (1996) *Cancer Res.* **56,** 4124–4129.
15. Kool, M., de Hass, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J. T., Juijn, J. A., Bass, F., and Borst, P. (1997) *Cancer Res.* **57,** 3537–3547.
16. Wada, M., Toh, S., Taniguchi, K., Nakamura, T., Takeshi, U., Kohno, K., Yoshida, I., Kimura, A., Sakisaka, S., Adachi, Y., and Kuwano, M. (1998) *Hum. Mol. Genet.* **7,** 203–207.
17. 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. (1996) *Science* **271,** 1126–1128.
18. Büchler, M., König, J., Brom, M., Kartenbeck, J., Spring, H., Horie, T., and Keppler, D. (1996) *J. Biol. Chem.* **271,** 15091–15098.
19. Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T., and Sugiyama, Y. (1997) *Am. J. Physiol.* **272,** G16–G22.
20. Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T., and Sugiyama, Y. (1998) *J. Biol. Chem.* **273,** 1684–1688.
21. Evers, R., Kool, M., van Deemter, L., Janssen, H., Calafat, J., Oomen, L. C. J. M., Paulusma, C. C., Oude Elferink, R. P. J., Bass, F., Schinkel, A. H., and Borst, P. (1998) *J. Clin. Invest.* **101,** 1310–1319.
22. Koike, K., Kawabe, T., Tanaka, T., Toh, S., Uchiumi, T., Wada, M., Akiyama, S., Ono, M., and Kuwano, M. (1997) *Cancer Res.* **57,** 5475–5479.
23. Saburi, Y., Nakagawa, M., Ono, M., Sakai, M., Muramatsu, M., Kohno, K., and Kuwano, M. (1989) *Cancer Res.* **49,** 7020–7025.
24. Fujii, R., Mutoh, M., Sumizawa, T., Chen, Z., Yoshimura, A., and Akiyama, S. (1994) *J. Natl. Cancer Inst.* **86,** 1781–1784.
25. Kohno, K., Sato, S., Uchiumi, T., Takano, H., Kato, S., and Kuwano, M. (1990) *J. Biol. Chem.* **265,** 19690–19696.
26. Kusaba, H., Kohno, K., Asakuno, K., Kuwano, M., Okumura, K., Green, E. D., Schlessinger, D., and Wada, M. (1996) *Genome Res.* **5,** 245–258.
27. White, R., Woodward, S., Leppert, M., O'Connell, P., Hoff, M., Herbst, J., Lalouel, J. M., Dean, M., and Vande, W. G. (1985) *Nature* **318,** 382–384.
28. Fojo, A., Lebo, R., Shimizu, N., Chin, J. E., Rononson, I. B., Merlino, G. T., Gottesman, M. M., and Pastan, I. (1986) *Somatic Cell Mol. Genet.* **12,** 415–420.
29. Allikmets, R., Gerrard, B., Hutchinson, A., and Dean, M. (1996) *Hum. Mol. Genet.* **5,** 1649–1655.