Increased Expression of an ATP-binding Cassette Superfamily Transporter, Multidrug Resistance Protein 2, in Human Colorectal Carcinomas[1]

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ABSTRACT

The expression of ATP-binding cassette superfamily transporter genes, such as P-glycoprotein/multidrug resistance (*MDR*) 1 and MDR protein (*MRP*) 1, is often up-regulated in various tumor types and is involved in responses to some anticancer chemotherapeutic agents. Five human *MRP* subfamily members (*MRP2–6*) with structural similarities to *MRP1* have been identified. The relationships between *MRP2–6* mRNA levels and drug resistance are not well understood. Data on 45 patients with colorectal cancer were analyzed. Of the ATP-binding cassette superfamily genes, we asked whether mRNA levels of *MDR1*, *MRP1*, *MRP2*, and *MRP3* correlated with drug resistance to anticancer agents. For this analysis, we used quantitative reverse transcription-PCR, and the sensitivity to anticancer agents in surgically resected colon carcinomas was determined using the *in vitro* succinate dehydrogenase inhibition test. *MDR1*, *MRP1*, and *MRP3* were highly expressed in normal colorectal mucosa, and the relative mRNA levels of *MDR1*, *MRP1*, and *MRP3* in cancerous tissues compared with noncancerous tissues were decreased or unchanged. By contrast, *MRP2* mRNA expression was low in normal colorectal mucosa and specifically increased in cancer regions compared with noncancerous regions. Of the anticancer agents prescribed for patients with colorectal cancers, including doxorubicin, mitomycin C, cisplatin, 5-fluorouracil, etoposide, and a camptothecin derivative, mRNA expression of *MRP2* was significantly associated with resistance to cis-

platin. *MRP2* **may be important for resistance to cisplatin treatment in colorectal cancer.**

INTRODUCTION

Two ABC[3] superfamily transporters, Pgp and MRP1, confer MDR on cancer cells through enhanced drug efflux (1–4). Treatment of cancer cells with many anticancer drugs, including *Vinca* alkaloids (vincristine and vinblastine), anthracyclines (doxorubicin and daunomycin), taxanes (Taxol and taxotere), and epipodophyllotoxins (etoposide and teniposide), can result in overexpression of Pgp and MRP1 (1, 2). Overexpression of human Pgp and MRP1 in cancer cells leads to drug resistance against anthracyclines, *Vinca* alkaloids, and epipodophyllotoxins (3, 4).

Patients with colorectal cancers have been treated primarily by surgical resection, and these cancers often have decreasing sensitivities to chemotherapeutic agents (5). Intrinsic drug resistance in untreated colon cancers is thought to be due in part to Pgp because normal colon tissues themselves express Pgp (5, 6). The expression of Pgp increases in colon cancers compared with findings in noncancerous regions (7), whereas no significant difference exists in Pgp levels between cancerous and noncancerous regions (8). Many other studies have noted a decreased expression of Pgp in cancerous regions compared with noncancerous regions in colon cancers (9–12). Thus, Pgp expression does not seem to be a prognostic marker for colorectal cancer (13, 14).

The *MRP1* gene is expressed in various tumor types, and MRP1 expression is associated with drug resistance in or prognosis of breast cancer, gastric cancer, neuroblastomas, retinoblastomas, and lung cancers (15–19). In a study of the expression of MRP1 in colorectal carcinomas, 7 of 30 cases showed strong MRP1 staining in tumors, whereas normal mucosal tissues showed weak MRP1 staining (12). MRP1 expression does not increase in cancerous regions compared with their noncancerous counterparts in colon cancers (20). Because expression of both Pgp and MRP1 occurs in normal colon mucosa, both ABC transporters may be involved in intrinsic drug resistance in colorectal cancers.

In addition to *MDR1* and *MRP1*, five human *MRP* subfamily members (*MRP2–6*) that show structural similarity to *MRP1* have been identified (21). The genes encoding *MRP1–6* are on different chromosomes, and *MRP1–6* mRNAs are expressed in a variety of normal tissues (21). Of these *MRP* family genes,

Characteristic	No.	%
Sex		
Male	25	55.6
Female	20	44.4
Age, (yrs)		
Median	61.7	
Range	25–87	
Site of primary tumor		
Colon	30	66.3
Rectum	15	33.3
TNM stage		
1	4	8.9
2	13	28.9
3a	12	26.7
3b	15	33.3
4	1	2.2
Differentiation of tumor		
Well-differentiated	31	68.9
Moderately differentiated	11	24.4
Poorly differentiated	1	2.2
Mucinous	1	2.2
Unknown	1	2.2
Recurrence	3	6.7

MRP2 appears to mediate ATP-dependent transport of various hydrophobic anionic compounds, including camptothecins and methotrexate in liver canalicular membranes and other tissues (22). *MRP1* and *MRP3*, but not *MRP2*, are expressed in normal colorectal mucosa (23, 24). Introducing *MRP2* antisense cDNA into human hepatic cancer cell lines results in increased sensitivity to cisplatin, vincristine, doxorubicin, and camptothecin derivatives (25).

The complete cDNA sequence of human *MRP3* (23) has a 56% amino acid identity to *MRP1* and a 45% identity to *MRP2*. *MRP3* was expressed mainly in the liver and was expressed to a lesser extent in the colon, small intestine, and prostate. Transfection of *MRP3* cDNA confers drug resistance against etoposide and methotrexate (26). *MRP3*, like *MRP1*, may contribute to drug resistance against etoposide, vincristine, and cisplatin in human lung cancer cells (27). In this study, we investigated which transporter of *MDR1*, *MRP1*, *MRP2*, and *MRP3* is specifically expressed and is responsible for the responses to the anticancer agents in colorectal carcinomas. We determined the mRNA levels of *MDR1*, *MRP1*, *MRP2*, and *MRP3* in tissues from 45 patients with colorectal cancer. The enhanced expression of *MRP2* in cancerous regions compared with noncancerous regions is discussed in association with resistance to anticancer agents.

PATIENTS AND METHODS

Patients. Between June 1994 and January 1999, 45 Japanese men and women with colorectal cancer underwent colorectal resection at the Department of Surgery II, Kyushu University Hospital (Fukuoka, Japan). Table 1 shows the clinicopathogenic characteristics of these 45 patients. No patient had received chemotherapy before surgery. Tumor and normal mucosal tissue samples were obtained after subjects provided informed consent, frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

Quantitative RT-PCR. For quantitative RT-PCR, we used real-time TaqMan technology and a Model 7700 sequence detector (Perkin-Elmer Applied Biosystems, Foster City, CA) as described previously (28). Four primer pairs and four TaqMan probes for *MDR1*, *MRP1*, *MRP2*, and *MRP3* were designed using the primer design software Primer Express (Perkin-Elmer Applied Biosystems). To avoid amplifying contaminated genomic DNA, primer pairs were placed in a different exon, and the probe was placed at the junction between two exons. Primers for *GAPDH* (TaqMan GAPDH control reagent kit) were purchased from Perkin-Elmer Applied Biosystems. Sequences for the TaqMan probes and primers were as follows: (*a*) *MDR1*, sense primer 5′-TGCTCAGACAGGATG TGAGTTG-3′, antisense primer 5′-TAGCCCCTTTAACTTGAGCAGC-3′, and probe 5′-AA AACACCACTGGAGCATTGACTACCAGGC-3′; (*b*) *MRP1*, sense primer 5′-TACCTCCTGT GGCTGAAT-CTGG-3′, antisense primer 5′-CCGATTGTCTTTGCTCTTC-ATG-3′, and probe 5′-ATGGCGATGAAGACCAAGACG-TATCAGGTG-3′; (*c*) *MRP2*, sense primer 5′-CAAACTC-TATCTTGCTAAGCAGG-3′, antisense primer 5′-TGAGTA-CAAGGGCCAGCTCTA-3′, and probe 5′-TTCGTTGGTTTT-CTTCTTATTCTAGCAGCC-3′; and (*d*) *MRP3*, sense primer 5′-CTTAAGACTTCCCCTCAACATGC-3′, antisense primer 5′-GGTCAAGTTCCTCTTGGCTC A-3′, and probe 5′-AGT-GTGTCTCTGAAACGGATCCAGCAATTC-3′. A hybridization probe specific for each PCR product was labeled with a reporter fluorescent dye (6-carboxyl-fluorescein or 2,7-dimethoxy-4,5-dichloro-6-carboxy-fluorescein) at the 5′ end and a quencher fluorescent dye (6-carboxy-tetramethyl-rhodamine) at the 3′ end. Reaction solutions (50 μl) contained 5 μl of 10× reverse transcription sample prepared as described previously (29), 5 μl of each 3 μM primer pair, 5 μl of 2 μl TaqMan probe, 5 μl of dH$_2$O, and 25 μl of 1× PCR mix (1.25 units of Ampli-Taq DNA polymerase, 1× PCR reaction buffer, and 0.5 unit of amperase; Perkin-Elmer Applied Biosystems). The C_T values corresponding to the cycle number at which the fluorescent emission monitored in real time reached a threshold of 10 SDs above the mean baseline from cycles 1–15 were measured. Serial 1:10 dilutions of plasmid DNA were analyzed for each target cDNA. These served as standard curves from which we determined the rate of change of threshold cycle values. Cycling parameters were as follows: 2 min at 50°C and 10 min at 95°C followed by 40 cycles of 30 s at 95°C and 2 min at 60°C.

Immunohistochemistry. Resected specimens of colorectal cancer were fixed in 10% formaldehyde, processed, and embedded in paraffin (30). Five-μm-thick sections were cut and stained immunohistochemically using the avidin-biotin-peroxidase complex method with mouse monoclonal antibodies for Pgp (C219) and MRP1 (QCRL-1) and rabbit polyclonal antibodies for MRP2 (25) as described previously.

SDI Test. The SDI test was performed using the methods described previously (31). In brief, tumor tissues were cut with scissors into fragments that were put into a sterile flask containing a mixture of pronase (protease type XXV; Sigma, St. Louis, MO) and collagenase (type 1; Sigma) in McCoy's 5A (Life Technologies, Inc.) solution with antibiotics. Enzymatic disaggregation was performed for 20 min at 37°C with gentle stirring

Fig. 1 mRNA expression indices. To determine the mRNA levels of four ABC transporters, we used a mRNA expression index, which is a mRNA expression level standardized by GAPDH. The mRNA expression indices of the four ABC transporters in the noncancerous (○) and cancerous regions (●) are shown. ∗, significant ($P < 0.05$) using ANOVA with Bonferroni's correction for multiple comparison.
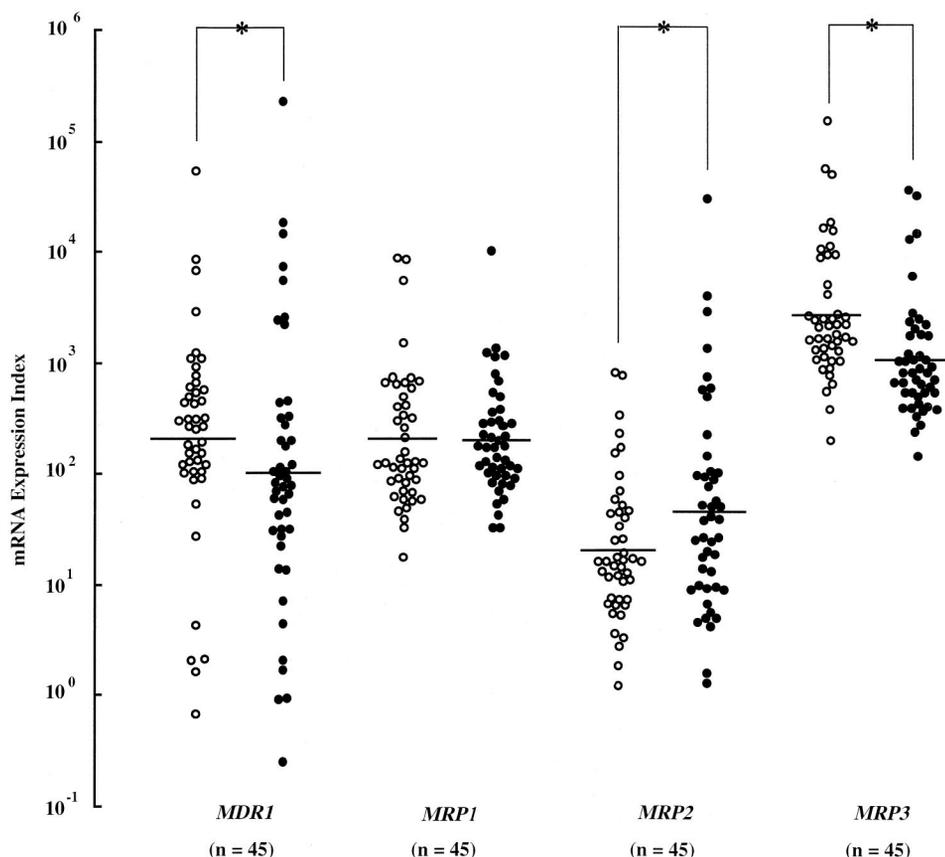
and was ended by adding sufficient amounts of MEM. Aliquots (100 ml) of this single cell suspension (3.0×10^5 cells/ml) were dispensed into 96-well microtiter plates and incubated at 37°C in a humidified 5% CO_2 atmosphere for 3 days in the presence of anticancer drugs. Each anticancer drug was tested at $10\times$ the peak plasma concentration. The chemosensitivity to a certain drug is given as a percentage of the succinate dehydrogenase activity in drug-treated cells compared with control cells. Chemosensitivity was defined as sensitive or resistant to the drug when the succinate dehydrogenase activity of the drug-treated cells decreased to less than 50% or more than 76% of the control, respectively.

Statistical Analysis. The correlations between groups were determined using Spearman's test. Spearman's test is usually used for nonparametric analysis when it is unclear whether the variables show normal distribution. Probability values of less than 0.05 were significant. The Spearman's correlation coefficient (*r*) and associated probability (*P*) were calculated for each combination of mRNA and SDI data sets. The relationships between the *MRP2* mRNA expression level and the drug sensitivity data sets were calculated using the same method. Four groups of T:N (tumor:normal) mRNA, the mRNA expression level of the cancerous region divided by the expression level of the noncancerous region, were compared using repeated-measures ANOVA with Bonferroni's correction for multiple comparison. Probability values of less than 0.05 were statistically significant. We determined that a strong correlation

Table 2 Spearman's correlation coefficients (*r*) and associated probabilities (*P*) for the correlations among mRNA levels in colorectal cancer tissues

We determined that a strong correlation would have a *r* value of 0.7 or above and that a weak correlation would have a *r* value of less than or equal to 0.5.

	Tumor	*MDR1*	*MRP1*	*MRP2*	*MRP3*
Normal tissue					
MDR1	*r*		0.4908	0.4853	0.4600
	P		0.0003[a]	0.0004[a]	0.0008[a]
MRP1	*r*	0.2331		0.4343	0.6812
	P	0.1032		0.0016[a]	0.0000[a]
MRP2	*r*	0.3403	0.5842		0.3585
	P	0.0156[a]	0.0000[a]		0.0106[a]
MRP3	*r*	0.4495	0.7065	0.5563	
	P	0.0011[a]	0.0000[a]	0.0000[a]	

[a] Significant ($P < 0.05$).

would have a *r* value of 0.7 or above and that a weak correlation would have a *r* value of less than or equal to 0.5.

RESULTS

To compare the mRNA levels of *MDR1*, *MRP1*, *MRP2*, and *MRP3* in human colorectal carcinomas and adjacent noncancerous tissues, we examined surgically removed colorectal samples from 45 patients using quantitative RT-PCR with spe-

Table 3 Relative expression (tumor: normal tissue) of four ABC transporters in the cancerous region compared with noncancerous region[a]

	MDR1	MRP1	MRP2	MRP3
>3-fold	7 (15.6%)	7 (15.6%)	19 (42.2%)	1 (2.2%)
1-3-fold	8 (17.8%)	18 (40.0%)	6 (13.3%)	9 (20.0%)
<1-fold	30 (66.7%)	20 (44.4%)	20 (44.4%)	35 (77.8%)
Fold increase	0.50 ± 11.14	0.98 ± 3.70	2.24 ± 13.37[b]	0.39 ± 3.35

[a] The data were derived from Fig. 1.

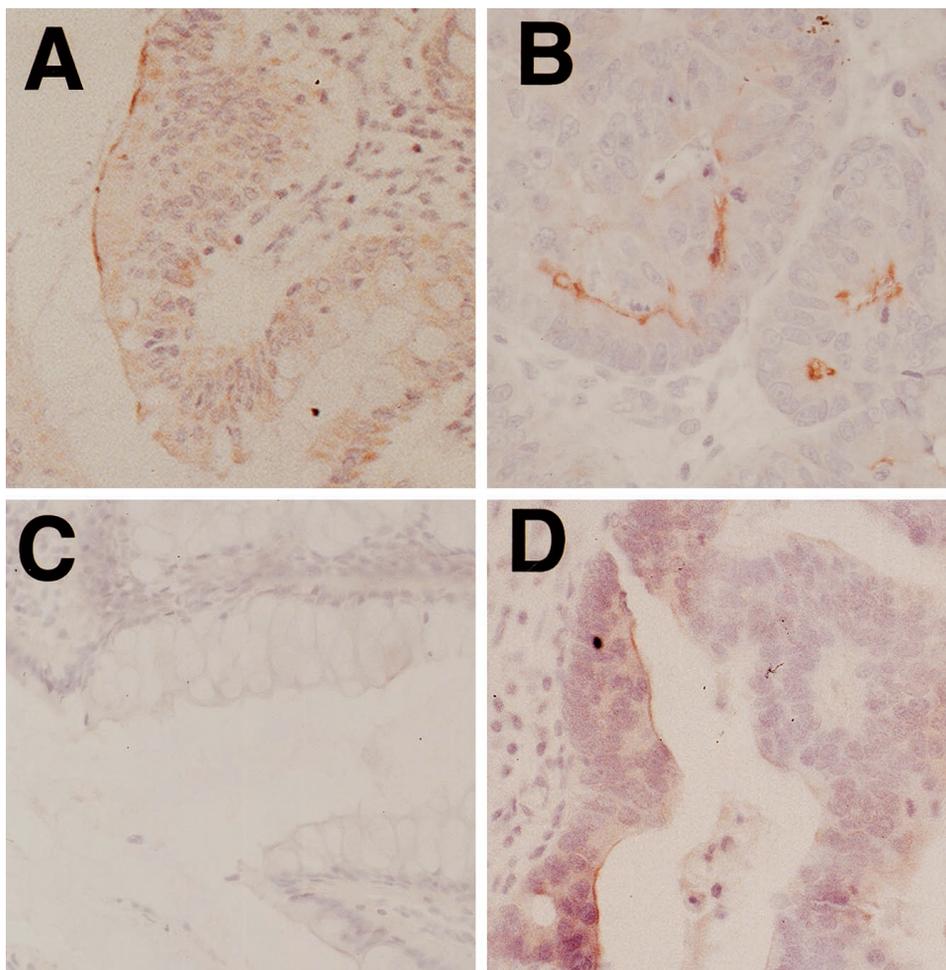[b] Significant ($P < 0.05$) compared with the other transporters.

Fig. 2 Immunologically stained colorectal cancer sections. Well-differentiated adenocarcinomas were stained with a monoclonal antibody against Pgp (C219; ×80; *A* and *B*). Noncancerous mucoepithelium (*A*) was stained with Pgp, and Pgp was detected in the noncancerous (*A*) and cancerous (*B*) regions. Well-differentiated adenocarcinomas were stained with a polyclonal antibody against MRP2 (×50; *C* and *D*). MRP2 was not evident in noncancerous regions (*C*) but was detected in cancerous regions (*D*).

cific primers and probes. The data were standardized against *GAPDH* mRNA levels of both cancerous and noncancerous regions. Fig. 1 shows the expression of ABC transporters *MDR1*, *MRP1*, *MRP2*, and *MRP3* in both noncancerous regions and cancerous regions. In noncancerous regions, *MRP3* mRNA expression was the highest, and *MDR1* and *MRP1* mRNA expressions were moderate among the four transporter genes. Very low or little expression of *MRP2* was seen in noncancerous regions (Fig. 1). The expression of *MDR1* and *MRP3* mRNA decreased, whereas *MRP1* mRNA expression was unchanged in cancerous regions compared with noncancerous regions. However, the expression of the *MRP2* gene was significantly in-creased in the cancerous tissues compared with noncancerous regions (Fig. 1).

In the noncancerous tissues, *MRP1*, *MRP2*, and *MRP3* mRNA expression showed a significant correlation with each other, and *MRP1* mRNA expression had the strongest correlation with *MRP3* mRNA expression (Table 2). In the cancerous tissues, all pairwise comparisons of mRNA expression showed significant correlations, and the *MRP1* mRNA level also showed the strongest correlation with the *MRP3* mRNA level (Table 2).

We next determined the relative expression of these four ABC transporter genes in cancerous tissue compared with noncancerous tissue. Table 3 summarizes the data of Fig. 1. Of the four ABC

Fig. 3 Correlation between *MRP2* mRNA expression and succinate dehydrogenase activity of cisplatin (*A*), 5-fluorouracil (*B*), mitomycin C (*C*), and doxorubicin (*D*). The Spearman's correlation coefficient (*r*) and associated probability (*P*) were calculated for each combination of *MRP2* mRNA expression and succinate dehydrogenase activity. *SD*, succinate dehydrogenase.
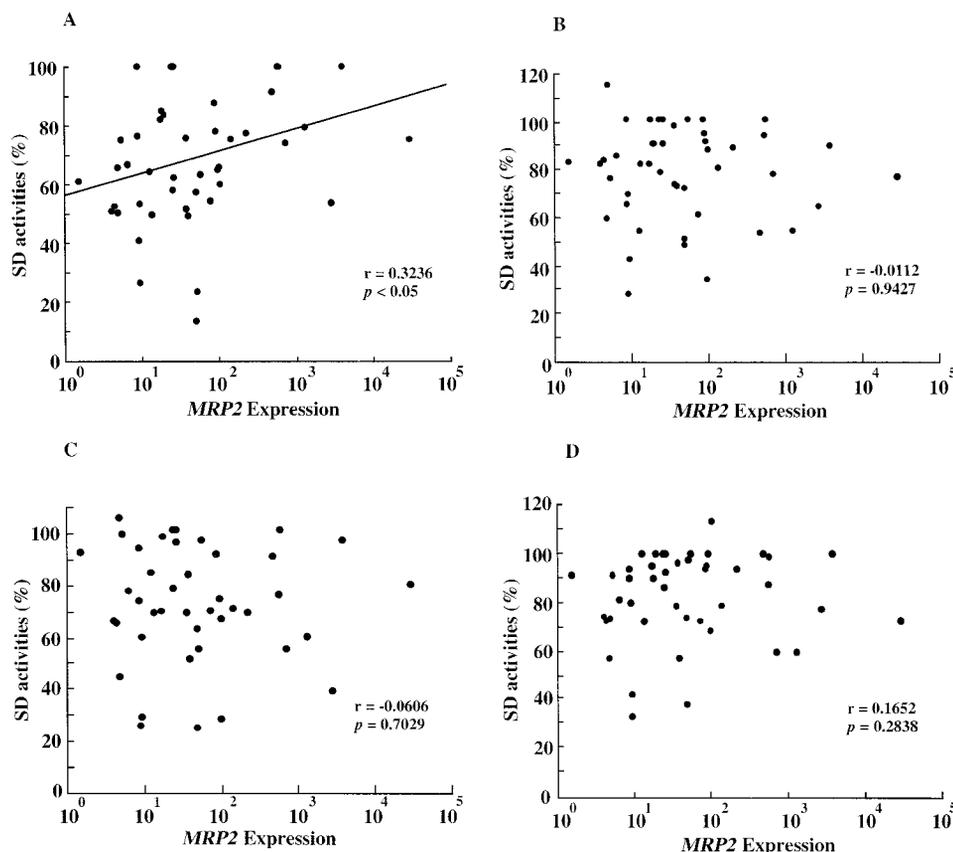
transporters, only *MRP2* showed a significant ($P < 0.05$) increase in the cancerous regions compared with their counterparts. Moreover, 19 of 45 patients (42.2%) showed a >3-fold increase in *MRP2* expression in cancerous regions compared with noncancerous regions. However, only 1–7 of 45 patients (2.2–15.6%) showed a >3-fold increase in expression of the other ABC transporters, *MDR1, MRP1,* and *MRP3* (Table 3).

Fig. 2 shows the results of immunostaining analysis with anti-Pgp and anti-*MRP2* antibodies. Positive staining for Pgp and *MRP2* was found primarily in the cytoplasm and cytoplasmic membrane of cancer cells. Each case also had increased expression of Pgp and *MRP2* in the cancerous region compared with adjacent noncancerous tissue (Fig. 2). Immunostaining with antibodies against Pgp and *MRP2* in the other five samples showed that *MDR1* and *MRP2* mRNA levels were comparable to Pgp and MRP2 protein levels in these cases (data not shown).

To investigate the effects of up-regulation of *MRP2* expression in response to anticancer agents, we searched for correlations between *MRP2* mRNA expression and drug response to various anticancer agents, including cisplatin, 5-fluorouracil, mitomycin C, doxorubicin, etoposide, and a camptothecin derivative (CPT-11). The averages of succinate dehydrogenase activities were 67.5 ± 20.8% for cisplatin, 77.2 ± 20.1% for 5-fluorouracil, 71.6 ± 22.4% for mitomycin C, 83.9 ± 18.2% for doxorubicin, 66.1 ± 30.0% for etoposide, and 88.0 ± 11.0% for CPT-11. We previously proposed that succinate dehydrogenase activities of <50% indicated drug sensitivity, whereas succinate dehydrogenase activities of >76% indicated drug resistance (31). According to these criteria, the colorectal carcinomas appeared to be partially sensitive to cisplatin, 5-fluorouracil, mitomycin C, and etoposide but was resistant to CPT-11 and doxorubicin. Spearman's correlation coefficients (*r*) and associated probabilities (*P*) were calculated to determine whether expression of these *MRP*-related genes was correlated with drug response of the 45 studied samples to cisplatin, 5-fluorouracil, mitomycin C, and doxorubicin (Fig. 3). The tissue samples showed a wide range of drug sensitivities. The relationships among *MRP2* mRNAs and the drug response to the six drugs were determined in six paired comparisons, and the probabilities were calculated to correct for multiple comparisons. Taking into account the number of comparisons and the variability inherent in PCR and SDI testing, we used a level of significance of 0.05 for each comparison, recognizing that any relationship identified as significant would require subsequent confirmation in independent studies. *MRP2* mRNA expression showed a significant correlation with drug response to cisplatin (Fig. 3). We could not recognize significant correlations among the mRNA expressions of the other three ABC transporters and the drug responses to the six anticancer agents (data not shown).

DISCUSSION

We first compared the mRNA levels of four ABC transporter family genes, *MDR1, MRP1, MRP2,* and *MRP3,* in car-

cinoma regions and adjacent noncancerous tissue in 45 patients with colon cancer, using a quantitative RT-PCR assay. Consistent with previous studies (23, 26, 32, 33), *MDR1*, *MRP1*, and *MRP3* were intrinsically expressed in normal colon mucosa tissue (Fig. 1). However, in colorectal cancers, either a decrease or no change in *MDR1* mRNA levels occurred (Table 3), findings consistent with previous studies (8, 34). We further observed a decreased expression of *MRP3* in colon cancer regions compared with noncancerous regions (Table 3). Decreased expression of both *MDR1* and *MRP3* in cancerous regions may perhaps lead to a sensitivity to anticancer agents targeted by both transporters.

MRP1 and *MDR1* are not coexpressed in colon cancers (12, 20), as we also observed (Table 2). In contrast, *MRP1* and *MRP3* were coexpressed, suggesting coordinate regulatory mechanisms for both ABC transporter genes in colorectal tissues and colorectal cancers. *MRP2* is not intrinsically expressed in normal colorectal mucosa (24). Unlike MDR1, *MRP1*, and *MRP3*, we observed up-regulation of *MRP2* in cancerous regions compared with noncancerous regions in colorectal carcinoma tissues (Table 3). *MRP2* might be involved in the membrane transport of endogenous substrates, such as glutathione and glutathione conjugates, in cancerous regions of colorectal cancers. The manner in which *MRP2* is specifically up-regulated in malignant colorectal tumors remains to be determined.

Of the six anticancer agents prescribed for treatment of colorectal carcinomas, we observed a significant correlation between MRP2 mRNA levels and drug response to cisplatin ($r = 0.3236$; $P = 0.0321$). *MRP2* levels thus appeared to be more closely correlated with response to cisplatin than with responses to 5-fluorouracil, mitomycin C, doxorubicin, etoposide, and CPT-11. Drug sensitivities to these agents were assayed using the SDI test. SDI tests can often predict the drug sensitivity of cancer cells in patients (35), but the fact can also be argued that SDI testing does not always reflect *in vivo* drug sensitivity (31). This study suggests that *MRP2* could be a sensitivity marker for cisplatin in patients with colorectal carcinoma. Expression of *MRP2* increased in cisplatin-resistant cell lines from various human tumor types, including colon cancer (21). Drug sensitivity to cisplatin, as well as vincristine and camptothecins, is also increased in stable transfectants of antisense *MRP2* cDNA in hepatic cancer cell lines (25). Sensitivity to vinblastine changes in *MRP2* cDNA-transfected canine kidney cell lines (36), transfection of *MRP2* cDNA confers drug resistance against methotrexate and camptothecin derivatives (37–39), and stable transfection of *MRP2* cDNA into both polarized and nonpolarized cells results in acquisition of drug resistance against cisplatin as well as vincristine, camptothecins, and methotrexate (36, 40, 41). However, it is unclear whether the expression of *MRP2* is directly associated with drug sensitivity to cisplatin, and whether *MRP2* can increase the transport of cisplatin itself, the cisplatin-glutathione conjugate, and both glutathione and cisplatin is unclear. Further study of the molecular basis of the GS-X pump for cisplatin, as originally proposed by Ishikawa *et al.* (42), is required.

In human lung cancer cell lines, *MRP* mRNA levels correlate with resistance to doxorubicin (43). A strong cor-

relation of *MRP3* mRNA levels with drug resistance against doxorubicin exists in lung cancer cell lines (27). Moreover, mRNA levels of both *MRP1* and *MRP3* correlate with resistance against vincristine, etoposide, and cisplatin in human lung cancer cell lines (27). In this study, *MDR1*, *MRP1*, and *MRP3* were not correlated with sensitivity to anticancer agents in colorectal cancers.

In conclusion, among *MDR1*, *MRP1*, *MRP2*, and *MRP3*, only *MRP2* was up-regulated in malignant colorectal tumors and correlated with resistance to cisplatin when surgically resected clinical samples from 45 patients were analyzed. Increased expression of *MRP2* might cause resistance to cisplatin in patients with human colorectal cancers.

REFERENCES

1. Gottesman, M. M., and Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. Annu. Rev. Biochem., *62:* 385–427, 1993.

2. Germann, U. A. P-glycoprotein: a mediator of multidrug resistance in tumour cells. Eur. J. Cancer, *32A:* 927–944, 1996.

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. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science (Washington DC), *258:* 1650–1654, 1992.

4. Loe, D. W., Deeley, R. G., and Cole, S. P. Biology of the multidrug resistance-associated protein, MRP. Eur. J. Cancer, *32A:* 945–957, 1996.

5. Beck, W. T. Circumvention of multidrug resistance with anti-P-glycoprotein antibodies: clinical potential or experimental artifact? J. Natl. Cancer Inst., *87:* 73–75, 1995.

6. Goldstein, L. J., Galski, H., Fojo, A., Willingham, M., Lai, S. L., Gazdar, A., Pirker, R., Green, A., Crist, W., Brodeur, G. M., Lieber, M., Cossman, J., Gottesman, M. M., and Pastan, I. Expression of a multidrug resistance gene in human cancers. J. Natl. Cancer Inst., *81:* 116–124, 1989.

7. Peters, W. H., Boon, C. E., Roelofs, H. M., Wobbes, T., Nagengast, F. M., and Kremers, P. G. Expression of drug-metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal mucosa. Gastroenterology, *103:* 448–455, 1992.

8. Mizoguchi, T., Yamada, K., Furukawa, T., Hidaka, K., Hisatsugu, T., Shimazu, H., Tsuruo, T., Sumizawa, T., and Akiyama, S. Expression of the *MDR1* gene in human gastric and colorectal carcinomas. J. Natl. Cancer Inst., *82:* 1679–1683, 1990.

9. Moscow, J. A., Fairchild, C. R., Madden, M. J., Ransom, D. T., Wieand, H. S., O'Brien, E. E., Poplack, D. G., Cossman, J., Myers, C. E., and Cowan, K. H. Expression of anionic glutathione *S*-transferase and P-glycoprotein genes in human tissues and tumors. Cancer Res., *49:* 1422–1428, 1989.

10. Kramer, R., Weber, T. K., Morse, B., Arceci, R., Staniunas, R., Steele, G., Jr., and Summerhayes, I. C. Constitutive expression of multidrug resistance in human colorectal tumours and cell lines. Br. J. Cancer, *67:* 959–968, 1993.

11. Caruso, M. L., Valentini, A. M., Armentano, R., and Pirrelli, M. P-170 glycoprotein expression in gastric and colorectal carcinomas and normal mucosa: an immunocytochemical study. In Vivo, *9:* 133–138, 1995.

12. Fillpits, M., Suchomel, R. W., Dekan, G., Stiglbauer, W., Haider, K., Depisch, D., and Pirker, R. Expression of the multidrug resistance-associated protein (*MRP*) gene in colorectal carcinomas. Br. J. Cancer, *75:* 208–212, 1997.

13. Mayer, A., Takimoto, M., Fritz, E., Schellander, G., Kofler, K., and Ludwig, H. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and *mdr* gene expression in colorectal cancer. Cancer (Phila.), *71:* 2454–2460, 1993.

14. Zochbauer, S., Wallner, J., Haider, K., Depisch, D., Huber, H., and Pirker, R. MDR1 RNA transcripts do not indicate long-term prognosis in colorectal carcinomas. Eur. J. Cancer, *33:* 1516–1518, 1997.

15. Nooter, K., Brutel de la Riviere, G., Look, M. P., van Wingerden, K. E., Henzen-Logmans, S. C., Scheper, R. J., Flens, M. J., Klijn, J. G., Stoter, G., and Foekens, J. A. The prognostic significance of expression of the multidrug resistance-associated protein (MRP) in primary breast cancer. Br. J. Cancer, *76:* 486–493, 1997.

16. Endo, K., Maehara, Y., Kusumoto, T., Ichiyoshi, Y., Kuwano, M., and Sugimachi, K. Expression of multidrug-resistance-associated protein (MRP) and chemosensitivity in human gastric cancer. Int. J. Cancer, *68:* 372–377, 1996.

17. Norris, M. D., Bordow, S. B., Marshall, G. M., Haber, P. S., Cohn, S. L., and Haber, M. Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. N. Engl. J. Med., *334:* 231–238, 1996.

18. Chan, H. S., Lu, Y., Grogan, T. M., Haddad, G., Hipfner, D. R., Cole, S. P., Deeley, R. G., Ling, V., and Gallie, B. L. Multidrug resistance protein (MRP) expression in retinoblastoma correlates with the rare failure of chemotherapy despite cyclosporine for reversal of P-glycoprotein. Cancer Res., *57:* 2325–2330, 1997.

19. Ota, E., Abe, Y., Oshika, Y., Ozeki, Y., Iwasaki, M., Inoue, H., Yamazaki, H., Ueyama, Y., Takagi, K., Ogata, T., Tamaoki, N., and Nakamura, M. Expression of the multidrug resistance-associated protein (MRP) gene in non-small-cell lung cancer. Br. J. Cancer, *72:* 550–554, 1995.

20. Reymann, A., Woermann, C., Froschle, G., Schneider, C., Brasen, J. H., Lage, H., and Dietel, M. Sensitive assessment of cytostatic drug resistance-mediating factors MDR1 and MRP in tumors of the gastrointestinal tract by RT-PCR. Int. J. Clin. Pharmacol. Ther., *36:* 55–57, 1998.

21. Kool, M., de Haas, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J., Juijn, J. A., Baas, 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.

22. Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T., and Sugiyama, Y. Functional analysis of a canalicular multispecific organic anion transporter cloned from rat liver. J. Biol. Chem., *273:* 1684–1688, 1998.

23. Uchiumi, T., Hinoshita, E., Haga, S., Nakamura, T., Tanaka, T., Toh, S., Furukawa, M., Kawabe, T., Wada, M., Kagotani, K., Okumura, K., Kohno, K., Akiyama, S., and Kuwano, M. 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. Biochem. Biophys. Res. Commun., *252:* 103–110, 1998.

24. 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.

25. Koike, K., Kawabe, T., Tanaka, T., Toh, S., Uchiumi, T., Wada, M., Akiyama, S., Ono, M., and Kuwano, M. A canalicular multispecific organic anion transporter (cMOAT) antisense cDNA enhances drug sensitivity in human hepatic cancer cells. Cancer Res., *57:* 5475–5479, 1997.

26. Kool, M., van der Linden, M., de Haas, M., Scheffer, G. L., de Vree, J. M., Smith, A. J., Jansen, G., Peters, G. J., Ponne, N., Scheper, R. J., Elferink, R. P., Baas, F., and Borst, P. MRP3, an organic anion transporter able to transport anti-cancer drugs. Proc. Natl. Acad. Sci. USA, *96:* 6914–6919, 1999.

27. Young, L. C., Campling, B. G., Voskoglou-Nomikos, T., Cole, S. P., Deeley, R. G., and Gerlach, J. H. Expression of multidrug resistance protein-related genes in lung cancer: correlation with drug response. Clin. Cancer Res., 5: 673–680, 1999.

28. Gibson, U. E., Heid, C. A., and Williams, P. M. A novel method for real time quantitative RT-PCR. Genome Res., *6:* 995–1001, 1996.

29. Nakayama, M., Wada, M., Harada, T., Nagayama, J., Kusaba, H., Ohshima, K., Kozuru, M., Komatsu, H., Ueda, R., and Kuwano, M.

Hypomethylation status of CpG sites at the promoter region and overexpression of the human *MDR1* gene in acute myeloid leukemias. Blood, *92:* 4296–4307, 1998.

30. Kawahara, N., Tanaka, T., Yokomizo, A., Nanri, H., Ono, M., Wada, M., Kohno, K., Takenaka, K., Sugimachi, K., and Kuwano, M. Enhanced coexpression of thioredoxin and high mobility group protein 1 genes in human hepatocellular carcinoma and the possible association with decreased sensitivity to cisplatin. Cancer Res., *56:* 5330–5333, 1996.

31. Okuyama, T., Maehara, Y., Endo, K., Baba, H., Adachi, Y., Kuwano, M., and Sugimachi, K. Expression of glutathione *S*-transferase-π and sensitivity of human gastric cancer cells to cisplatin. Cancer (Phila.), *74:* 1230–1236, 1994.

32. Fojo, A. T., Ueda, K., Slamon, D. J., Poplack, D. G., Gottesman, M. M., and Pastan, I. Expression of a multidrug-resistance gene in human tumors and tissues. Proc. Natl. Acad. Sci. USA, *84:* 265–269, 1987.

33. Cole, S. P., 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.

34. Chuman, Y., Sumizawa, T., Takebayashi, Y., Niwa, K., Yamada, K., Haraguchi, M., Furukawa, T., Akiyama, S., and Aikou, T. Expression of the multidrug-resistance-associated protein (*MRP*) gene in human colorectal, gastric and non-small-cell lung carcinomas. Int. J. Cancer, *66:* 274–279, 1996.

35. Mitsudomi, T., Kaneko, S., Tateishi, M., Yano, T., Ishida, T., Kohnoe, S., Maehara, Y., and Sugimachi, K. Chemosensitivity testing of human lung cancer tissues using the succinate dehydrogenase inhibition test. Anticancer Res., *10:* 987–990, 1990.

36. Evers, R., Kool, M., van Deemter, L., Janssen, H., Calafat, J., Oomen, L. C., Paulusma, C. C., Oude Elferink, R. P., Baas, F., Schinkel, A. H., and Borst, P. Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. J. Clin. Invest., *101:* 1310–1319, 1998.

37. Masuda, M., I'izuka, Y., Yamazaki, M., Nishigaki, R., Kato, Y., Ni'inuma, K., Suzuki, H., and Sugiyama, Y. Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats. Cancer Res., *57:* 3506–3510, 1997.

38. Sugiyama, Y., Kato, Y., and Chu, X. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. Cancer Chemother. Pharmacol., *42:* S44–S49, 1998.

39. Chu, X. Y., Kato, Y., Ueda, K., Suzuki, H., Niinuma, K., Tyson, C. A., Weizer, V., Dabbs, J. E., Froehlich, R., Green, C. E., and Sugiyama, Y. Biliary excretion mechanism of CPT-11 and its metabolites in humans: involvement of primary active transporters. Cancer Res., *58:* 5137–5143, 1998.

40. Cui, Y., Konig, J., Buchholz, J. K., Spring, H., Leier, I., and Keppler, D. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. Mol. Pharmacol., *55:* 929–937, 1999.

41. Kawabe, T., Chen, Z., 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:* 327–331, 1999.

42. Ishikawa, T., and Ali-Osman, F. Glutathione-associated *cis*-diamminedichloroplatinum (II) metabolism and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione-platinum complex and its biological significance. J. Biol. Chem., *268:* 20116–20125, 1993.

43. Campling, B. G., Young, L. C., Baer, K. A., Lam, Y. M., Deeley, R. G., Cole, S. P., and Gerlach, J. H. Expression of the *MRP* and *MDR1* multidrug resistance genes in small cell lung cancer. Clin. Cancer Res., *3:* 115–122, 1997.

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