# ATP-binding Cassette Superfamily Transporter Gene Expression in Human Primary Ovarian Carcinoma[1]

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## ABSTRACT

***Purpose*: The purpose of this study is to attempt to characterize patients with unfavorable clinical outcome by the relative mRNA levels of ABC transporter expression in their tumor samples and to examine whether relative mRNA levels of each of the ABC transporters can be a useful predictor of progression-free survival in advanced ovarian carcinoma.**

***Experimental Design*: We examined tumor samples taken from 30 patients with primary serous papillary adenocarcinoma of the ovary for the expression of *MDR1* and *MRP1*, *MRP2*, and *MRP3* mRNA by using real-time reverse transcription-PCR, and we evaluated its correlation with clinical outcome. All 30 patients were divided into three groups according to clinical outcome after debulking surgery and platinum-based chemotherapy: 8 patients were classified into the unfavorable group; 11 were classified into the favorable group; and 11 were classified into intermediate group.**

***Results*: The relative mRNA levels of *MRP1* and *MRP3* were significantly different among the three groups, and the mRNA levels of *MRP1* and *MRP3* in the unfavorable group were significantly higher than those in the favorable group by multiple comparison. The relative mRNA levels of *MRP1* expression were significantly correlated with those of *MRP3* expression. In the 30 patients with serous papillary adenocarcinoma, univariate and multivariate analysis demonstrated that the high relative mRNA levels of *MRP1* expression were significantly correlated with a short period of progression-free survival.**

***Conclusions*: In patients with advanced ovarian serous papillary adenocarcinoma, these results suggest that patients with an unfavorable clinical outcome are characterized by increased levels of coordinated *MRP1* and *MRP3* mRNA expression in their tumor samples. Furthermore, a higher level of *MRP1* mRNA expression can be a candidate for a useful predictor of a shorter period of progression-free survival.**

## INTRODUCTION

Patients with advanced ovarian carcinoma have been treated by debulking surgery followed by platinum-based chemotherapy. Platinum-based combination chemotherapy plays a major role in the treatment of ovarian carcinoma. Although the majority of advanced ovarian carcinomas will initially respond to chemotherapy, most will relapse, and less than 15% of patients will be long-term survivors (1). Resistance to anticancer drugs presents a major obstacle in attempts to improve clinical outcome.

One of the important mechanisms of drug resistance is a decrease in the accumulation of the drug, caused by enhanced drug efflux mediated by transporters such as the ABC superfamily. Some members of the ABC superfamily transporters have been shown to confer drug resistance *in vitro* (2). P-gp[3]/*MDR1* was the first human ABC transporter shown to confer multidrug resistance. MRP1 cloned from the doxorubicin-resistant H69AR small cell lung cancer cell line (3) has been shown to transport various substrates with or without glutathione conjugation (4, 5) and is associated with drug resistance or poor prognosis in a variety of malignant tumors (6–15). Recently, five human MRP superfamily members (MRP2–6) were identified (16). MRP3 and MRP2 are the ABC transporters that are most closely related to MRP1 with 58% and 49% amino acid identity, respectively (17). *MRP2* and *MRP3* mRNA and protein levels are increased in some drug-selected cell lines (16, 18, 19), and several studies have demonstrated that MRP3 and MRP2, like MRP1, can transport various substrates, including anticancer drugs (20, 21).

Several studies have demonstrated that the expression of MRP1 and MRP3 contributes to drug resistance against platinum agents (2, 22–24). MRP2 has been found to be overexpressed in a number of cisplatin-resistant cell lines (16, 18, 25). The increased sensitivity to cisplatin was reported to be displayed by transfection of *MRP2* antisense cDNA in hepatocellular carcinoma cells (26). These studies suggest that the resist-

Received 3/4/02; revised 7/19/02; accepted 7/29/02.
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[1] Supported by the Foundation for the Promotion of Cancer Research in Japan and Grant-in-Aid for General Scientific Research 12670167 from the Ministry of Education, Science, Sports and Culture, Tokyo, Japan.
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[3] The abbreviations used are: P-gp, P-glycoprotein; RT-PCR, reverse transcription-PCR; EI, expression index; AU, arbitrary unit(s); CBDCA, carboplatin.

ance against platinum agents is, at least in part, explained by the expression of ABC transporters such as MRP1, MRP2, and MRP3. In 1996, the efficacy of the paclitaxel-containing regimen in the treatment of primary ovarian carcinoma was reported by McGuire *et al.* (27). *MDR1*/P-gp is known to confer drug resistance against paclitaxel (28), whereas *in vitro* studies have shown that MRP1 expression contributes to minimal resistance against paclitaxel (29), although conflicting data also exist (30). As for MRP2 and MRP3, their role in resistance against paclitaxel is unknown. ABC transporters seem to contribute to drug resistance against platinum agents and/or paclitaxel, which are the key drugs in the treatment of advanced ovarian carcinoma, and the mRNA levels of the ABC transporter are reported to be correlated with protein levels *in vitro* (2). Although it is important to evaluate the expression of these transporter genes in ovarian carcinoma, this has yet to be elucidated. In the present study, we assayed relative mRNA levels of ABC transporters in specimens of ovarian carcinoma obtained from patients before chemotherapy and compared these with the clinical outcome.

## MATERIALS AND METHODS

**Patients and Specimens.** A total of 50 patients with primary untreated ovarian carcinoma (30 patients with serous papillary adenocarcinoma and 20 patients with clear cell adenocarcinoma), who had undergone debulking surgery before chemotherapy at Kyushu University Hospital between 1994 and 2000, were examined. The performance status of all of the patients was below grade 1. Patients with borderline malignancy, mixed epithelial carcinoma, and peritoneal carcinoma were excluded from the study. Informed consent was obtained from all patients. All of the patients were staged according to the International Federation of Obstetrics and Gynecology classification (FIGO; Ref. 31) and were treated with chemotherapy containing at least cisplatin or carboplatin. Of the 30 patients with serous papillary adenocarcinoma, 24 were treated with cisplatin (70 mg/m$^2$ body surface/day 1), epirubicin (50 mg/m$^2$ body surface/day 1), and cyclophosphamide (500 mg/m$^2$ body surface/day 1), whereas 6 were treated with paclitaxel (180 mg/m$^2$ body surface/day 1) and carboplatin (CBDCA/day 1). The dose of CBDCA was calculated by Calvert's formula (32): dose (mg/body) = target area under curve × (glomerular filtration rate + 25). We set the target area under curve at 5 mg/ml × min (32). All 20 patients with clear cell adenocarcinoma were treated with cisplatin (60 mg/m$^2$ body surface/day 1) and irinotecan hydrochloride (CPT-11; 60 mg/m$^2$ body surface/day 1, 8, and 15). Each course comprised 28 days. For patients with advanced-stage disease, chemotherapy was repeated for a maximum of six courses, provided the treatment continued to be effective. The effect of chemotherapy was evaluated 3–4 weeks after each administration of chemotherapy by ultrasonography or computed tomography. After chemotherapy, all patients were followed up every 2 months for the first year, every 3 months for the next year, every 4 months for the next year, every 6 months for the next 2 years, and every year thereafter. Specimens were collected from non-necrotic cancer tissue during surgery and immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction. Specimens were confirmed to be composed of more than 90% cancer cells arranged in solid sheets, tubules, or papillae with thin mesenchymal cores. For routine histological studies, adequate numbers of 3-μm sections of tissue specimens fixed with 10% formalin and embedded in paraffin were stained with H&E. Tumors were classified according to the WHO classification (33) and graded as grade 1, 2, or 3 according to Silverberg's proposal (34).

All 30 patients with serous papillary adenocarcinoma were stage III or IV, and we divided these 30 patients into three groups according to clinical outcome. Clinical outcome was measured by progression-free survival defined as the interval from the date at first laparotomy to the date at diagnosis of progression. The first, unfavorable group was defined as patients with progressive disease during treatment. The second, favorable group was defined as patients with a progression-free survival period of more than 2 years. The third, intermediate group was defined as patients with relapse within 2 years after complete remission. Although the conventional method is to use a 6-month or 1-year cutoff, the correct definition of a significant recurrence-free interval is not clear, and it is believed that the shorter the interval, the more likely it is for the tumor to be resistant. This is why we divided patients into three groups and compared the two extremities. We did not apply these criteria for 20 patients with clear cell adenocarcinoma because 14 patients were stage I, and all of them remain progression-free at present. It is impossible to determine whether a favorable clinical outcome is dependent upon lack of aggressive biological behavior, excellent drug response, or excellent surgical removal in the case of stage I patients. Therefore we used the data of clear cell phenotype only for evaluating the differences between serous and clear cell phenotype.

In patients with advanced ovarian carcinoma treated by platinum-based chemotherapy, the median progression-free survival has been reported to be about 1 year (27, 35). Hence, we designated the patients with a progression-free survival period of more than 2 years as relatively favorable.

Five samples of normal ovarian tissue were obtained from the five patients who underwent hysterectomy and oophorectomy as indicated by early-stage uterine carcinoma. Histologically, the ovarian samples were determined to be free of malignant cells and to be composed mainly of stromal cells with a tiny amount of surface epithelium lining on the ovarian surface or inclusion cysts.

**Real-time RT-PCR.** Real-time RT-PCR (TaqMan PCR) using an ABI PRISM 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA) was performed as described previously (36). The sequences of oligonucleotide primer pairs and TaqMan probes for *MDR1*, *MRP1*, *MRP2*, and *MRP3* are summarized in Table 1 (36). Serial 1:10 dilutions of plasmid DNA containing each target cDNA ($10^7$ to $10^1$ copies/μl) were analyzed and served as standard curves, from which we determined the rate of change of threshold cycle values. Correlation coefficients of standard curves above 0.95 assure the accuracy of our data. Plasmid DNA also played the role of positive control for each reaction. We confirmed the sensitivity of the quantitation by demonstrating amplification curves using low-level positive control cells comprising MNNG/HOS cells, MNNG/HOS/DXR 1000 cells, and MG 63/DOX 10 cells (37). Copy numbers of the target cDNA were estimated by standard curves. All of the reactions for standard samples and samples of

*Table 1* Sequences of each pair of primers and probe for real-time RT-PCR

| | |
|---|---|
| TaqMan PCR | |
| *MDR1* cDNA | 5′-TGCTCAGACAGGATGTGAGTTG-3′ |
| | 5′-TAGCCCCTTTAACTTGAGCAGC-3′ |
| TaqMan probe | 5′-AAAACACCACTGGAGCATTGACTACCAGGC-3′ |
| *MRP1* cDNA | 5′-TACCTCCTGTGGCTGAATCTGG-3′ |
| | 5′-CCGATTGTCTTTGCTCTTCATG-3′ |
| TaqMan probe | 5′-ATGGCGATGAAGACCAAGACGTATCAGGTG-3′ |
| *MRP2* cDNA | 5′-CAAACTCTATCTTGCTAAGCAGG-3′ |
| | 5′-TGAGTACAAGGGCCAGCTCTA-3′ |
| TaqMan probe | 5′-TTCGTTGGTTTTCTTCTTATTCTAGCAGCC-3′ |
| *MRP3* cDNA | 5′-CTTAAGACTTCCCCTCAACATGC-3′ |
| | 5′-GGTCAAGTTCCTCTTGGCTC-3′ |
| TaqMan probe | 5′-AGTGTGTCTCTGAAACGGATCCAGCAATTC-3′ |

patients were performed in triplicate. The data were averaged from the values obtained in each reaction. To determine the mRNA levels of four ABC transporters, we used a mRNA EI, which is a relative mRNA expression level standardized by glyceraldehyde-3-phosphate dehydrogenase. The mRNA EI was calculated as follows (in AU): mRNA EI = (copy numbers of ABC transporter mRNA/copy numbers of glyceraldehyde-3-phosphate dehydrogenase mRNA) × 1000 AU. A high (low) level of each transporter gene expression was defined as an mRNA EI above (below) the median value of the 30 patients with serous papillary adenocarcinoma.

**Immunohistochemistry.** The following monoclonal antibodies were used as the primary antibody: (*a*) anti-P-gp (JSB-1; 1:80; Sanbio, Uden, the Netherlands); (*b*) anti-MRP1 (MRPr1; 1:50; Nichirei, Tokyo, Japan); (*c*) anti-MRP2 (M2 III-6; 1:80; Sanbio); and (*d*) anti-MRP3 (M3II-9; 1:80; Kamiya Biomedical, Seattle, WA). Sections of tissue samples fixed with 10% formalin and embedded in paraffin were cut at 4 μm. Sections were stained immunohistochemically using the avidin-biotin-peroxidase complex method. For staining with all of the antibodies except the anti-MRP1 antibody, sections were pretreated with microwave irradiation for the purpose of antigen retrieval. Positive and negative controls played an appropriate role in each of the antibodies.

**Statistical Analysis.** We used the statistical software StatView version 4.5 (Abacus Concepts Inc., Berkeley, CA) and StatXact version 3 (CYTEL Software Corp., Cambridge, MA). Differences among/between groups were analyzed by Kruskal-Wallis analysis, Bonferroni test, or Mann-Whitney U test. The Spearman's correlation coefficient (ρ) and associated probability (*P*) were calculated for each combination of mRNA EI data sets. Clinicopathological variables were analyzed by $\chi^2$ statistics or Fisher's exact test. Differences in progression-free survival were analyzed using log-rank statistics. The influences of possible confounding factors for progression-free survival were analyzed by Cox proportional hazards regression model, using a non-stepwise method. Only *P*s $< 0.05$ were considered significant.

## RESULTS

**Patients.** Of the 30 patients with ovarian serous papillary adenocarcinoma, 8 were classified into the unfavorable group, 11 were classified into the favorable group, and 11 were classified into the intermediate group. The median progression-free survival of all 30 patients was 432 days (range, 0–1939 days), whereas the median survival was 839 days (range, 86–1939 days). The median follow-up of those patients who are currently progression free is 1101 days (range, 847–1939 days). The clinical and pathological characteristics of both serous and clear cell phenotype are summarized in Table 2.

**mRNA EI of ABC Transporters.** In the 30 patients with serous papillary adenocarcinoma, the *MRP1* mRNA EI was significantly correlated with *MRP3* ($\rho = 0.82$; $P < 0.0001$), but not with *MRP2* ($\rho = 0.23$; $P = 0.25$; Table 3A). In the 20 patients with clear cell adenocarcinoma, there was no correlation in any of the data sets (Table 3B).

In the 30 patients with serous papillary adenocarcinoma, the mRNA EIs of *MRP1* and *MRP3* were significantly different among the unfavorable group, intermediate group, and favorable group ($P = 0.012$ and $P = 0.025$; Kruskal-Wallis analysis, respectively), and the mRNA EIs in the unfavorable group (median, 29.0 and 90.9 AU, respectively) were both significantly higher than those in the favorable group (median, 9.5 and 7.3 AU, respectively; $P = 0.0064$ and $P = 0.013$, Bonferroni test, respectively; Fig. 1). For the *MRP2* mRNA EI, such a significant difference was not observed ($P = 0.72$, Kruskal-Wallis analysis).

The interval of progression-free survival for the patients with high levels of *MRP1* mRNA EI (>15.3 AU) was significantly shorter than that for those with low levels by log-rank statistics ($P = 0.037$; Fig. 2*A*). High levels of *MRP1* mRNA EI were independently correlated with short-term progression-free survival, with adjustments for possible confounding factors such as chemotherapy regimen and the size of residual lesions ($P = 0.022$; hazard ratio, 8.70; 95% confidence interval, 1.38–55.03; Table 4). No significant correlation was observed between expression levels of *MRP2* or *MRP3* mRNA EI and progression-free survival.

As for the comparison between serous and clear cell phenotype, only *MRP3* mRNA EI in clear cell adenocarcinoma (median, 84.5 AU) was significantly higher than that in serous papillary adenocarcinoma (median, 22.6 AU; $P = 0.018$, Mann-Whitney U test; Fig. 3). No significant differences regarding other transporters were observed between serous and clear cell phenotype.

From the five samples of normal ovaries, various levels of

*Table 2* Clinical and pathological characteristics in 50 patients with ovarian carcinoma

| | Serous papillary adenocarcinoma | | Clear cell adenocarcinoma |
|---|---|---|---|
| | F/I/U[a] | *MRP1* high/low | |
| | ($n$ = 11/11/8) | ($n$ = 15/15) | ($n$ = 20) |
| Age (yrs) | $P$ = 0.34 | $P$ = 0.71 | |
| ≤53 | 6/3/5 | 8/7 | 4 |
| >53 | 5/8/3 | 7/8 | 16 |
| FIGO stage[b] | $P$ = 0.34 | $P$ = 0.68 | |
| I | 0/0/0 | 0/0 | 14 |
| II | 0/0/0 | 0/0 | 1 |
| III | 10/7/5 | 12/10 | 5 |
| IV | 1/4/3 | 3/5 | 0 |
| Grade | $P$ = 0.52 | $P$ = 0.71 | |
| G1 | 0/0/0 | 0/0 | 16 |
| G2 | 6/3/3 | 5/7 | 3 |
| G3 | 5/8/5 | 10/8 | 1 |
| Residual lesion | $P$ = 1.00 | $P$ = 0.71 | |
| ≤2 cm | 5/4/3 | 5/7 | 19 |
| >2 cm | 6/7/5 | 10/8 | 1 |
| Chemo-regimen[c] | $P$ = 0.18 | $P$ = 0.17 | |
| CDDP/Epi/Cyclo | 7/9/8 | 14/10 | 0 |
| CBDCA/paclitaxel | 4/2/0 | 1/5 | 0 |
| CDDP/CPT-11 | 0/0/0 | 0/0 | 20 |

[a] F/I/U, favorable/intermediate/unfavorable.
[b] FIGO, International Federation of Obstetrics and Gynecology.
[c] CDDP, cisplatin; Epi, epirubicin; Cyclo, cyclophosphamide.

*Table 3* Spearman's correlation coefficient (ρ) and associated probability ($P$) for the correlations among mRNA levels of each of the ABC transporters

| A. Serous papillary adenocarcinoma | | | |
|---|---|---|---|
| | | *MRP2* | *MRP3* |
| *MRP1* | ρ | 0.23 | 0.82[a] |
| | $P$ | 0.25 | <0.0001[a] |
| *MRP2* | ρ | | −0.079 |
| | $P$ | | 0.58 |
| B. Clear cell adenocarcinoma | | | |
| | | *MRP2* | *MRP3* |
| *MRP1* | ρ | 0.37 | 0.083 |
| | $P$ | 0.14 | 0.72 |
| *MRP2* | ρ | | 0.34 |
| | $P$ | | 0.12 |

[a] Significant.

*MRP1*, *MRP2*, and *MRP3* mRNA EI were detected (median levels of 33.4, 1.1, and 14.1, respectively).

*MDR1* gene expression was at an undetectable level in all 50 patients and in the 5 normal ovarian samples.

**Immunohistochemistry.** Immunohistochemically, MRP1, MRP2, and MRP3 proteins were localized on cancer cells and the surface epithelium of normal ovaries. Fig. 4 shows the results of immunostaining analysis of control tissues and representative tissues using anti-MRP1 and -MRP3 antibodies. Both of these antigens were localized on the plasma membrane or the cytoplasm of cancer cells and control tissue. In polarized cells, the basolateral membrane was intensely stained (Fig. 4). P-gp was not detected in any of the 50 tumor samples or in the surface epithelium of normal ovaries. Mesenchymal cells were not immunoreactive for the antibodies.

## DISCUSSION

In this study, we focused on the relative mRNA levels of ABC transporter expression and the localization of the transporters in ovarian carcinoma. The expression of the ABC transporter genes or proteins has been examined previously, and the correlation with clinical outcome or drug response in epithelial ovarian carcinoma has been evaluated; however, the conclusions are conflicting (38–41). We attempted, as far as possible, to select patients for this study under similar clinical conditions to perform a more accurate evaluation. Accordingly, we selected 30 patients with primary, untreated and advanced, moderate- to high-grade serous papillary adenocarcinoma who had been treated by debulking surgery and subsequent platinum-based chemotherapy.

We divided these 30 patients into three groups (favorable, unfavorable, and intermediate groups) according to clinical outcome and compared the relative mRNA levels of the ABC transporters among the three groups. To the best of our knowledge, this is the first analysis of *MRP2* and *MRP3* mRNA expression in clinical specimens of ovarian carcinoma. Our data demonstrated that relative mRNA levels of *MRP1* and *MRP3* expression in the unfavorable group were significantly higher than those in the favorable group by multiple comparison. Young *et al.* (2) demonstrated that the mRNA levels of *MRP1* and *MRP3* expression were correlated with protein levels and drug response for cisplatin, doxorubicin, etoposide, and vincristine in human non-small cell lung cancer cells. Our data were consistent with their hypothesis that MRP3 and MRP1 expressions are both components of the multifactorial resistant pheno-

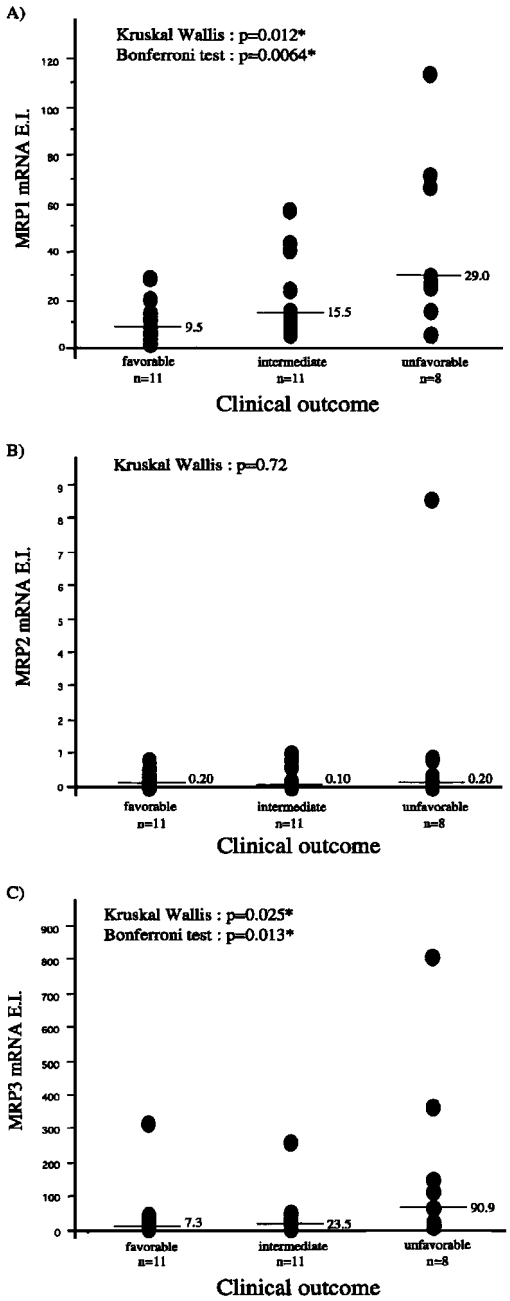


*Fig. 1* Comparison of relative mRNA levels of (*A*) *MRP1*, (*B*) *MRP2*, and (*C*) *MRP3* among 30 patients with serous papillary adenocarcinoma.

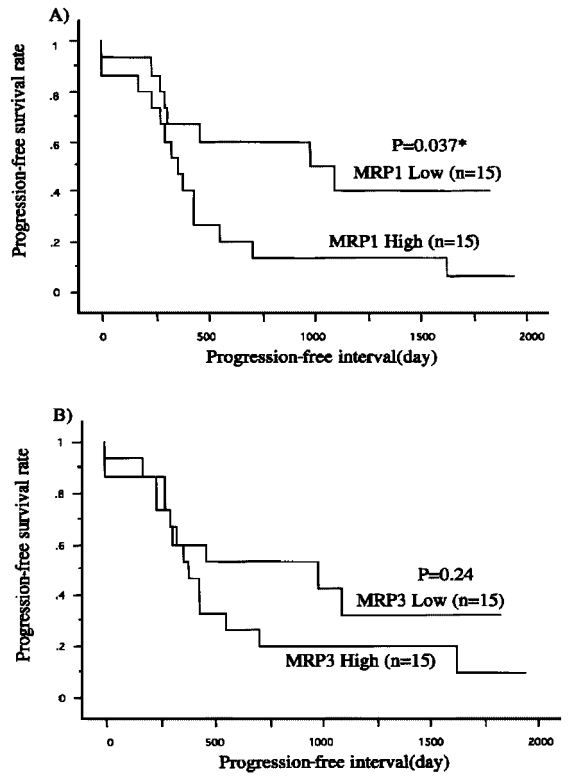


*Fig. 2* Comparison of progression-free survival curves in patients whose tumors showed high and low relative mRNA levels of (*A*) *MRP1* and (*B*) *MRP3*.

type of carcinoma cells against platinum agents (2). The coordinated expression of MRP1 and MRP3 is well established (2, 23, 36, 42), and we also demonstrated significant correlation between *MRP1* and *MRP3* mRNA expression in serous phenotype. Our data suggest that the patients with unfavorable clinical outcome are characterized by increased levels of coordinated *MRP1* and *MRP3* mRNA expression in their tumor samples of serous papillary adenocarcinoma. In our data, only high levels of *MRP1* mRNA EI were significantly correlated with a shorter interval of progression-free survival in patients with serous papillary adenocarcinoma under similar clinical conditions. Kigawa *et al.* (43) demonstrated that the levels of MRP1 mRNA expression in nonresponders were about 2-fold higher than those in responders using clinical specimens of patients with advanced ovarian carcinoma. Although the materials and methods used in their study differ from ours, the results of their study share a certain similarity with ours in that increased levels of intrinsic or acquired *MRP1* mRNA expression seem to be associated with unfavorable clinical outcome or drug response for platinum-based chemotherapy in advanced ovarian carcinoma. In the present study, however, patients who received paclitaxel as the initial therapy were more likely to be in the favorable or intermediate group. The fact that this would seem to confound the clinical correlation cannot be refuted. On the other hand, Kavallaris *et al.* (39) did not detect any association between mRNA levels of *MRP1* expression and progression-free survival in various phenotypes of primary ovarian carcinoma, although they found various levels of *MRP1* gene expression in 100% of 53 patients by using competitive RT-PCR assay. The difference in the findings could possibly be explained by the differences in the techniques used and the clinical features of patients examined in each study. The influences of possible confounding factors were analyzed by the Cox proportional hazards regression model, and a high level of *MRP1* mRNA EI as estimated by real-time RT-PCR assay was the candidate for an independent predictor of progression-free survival in advanced, moderate- to high-grade, serous papillary adenocarcinoma of the ovary. De-

*Table 4* Progression-free survival analysis in 30 patients with serous papillary adenocarcinoma

| Confounding factors | Univariate (p) | Multivariate (*P*) | Hazard ratio | 95% CI[a] |
|---|---|---|---|---|
| Age (≤53 yrs) | 0.94 | 0.97 | 0.98 | 0.33–2.86 |
| FIGO stage (stage III)[b] | 0.17 | 0.098 | 0.40 | 0.14–1.18 |
| Histological grade (grade 2) | 0.35 | 0.93 | 0.95 | 0.34–2.68 |
| Residual lesion (>2 cm) | 0.28 | 0.73 | 0.79 | 0.22–2.90 |
| Chemo-regimen (CEP)[c] | 0.27 | 0.77 | 1.28 | 0.25–6.39 |
| High MRP1 EI (>15.3) | 0.037[d] | 0.022[d] | 8.70 | 1.38–55.03 |
| High MRP2 EI (>0.15) | 0.30 | 0.20 | 0.44 | 0.12–1.56 |
| High MRP3 EI (>22.6) | 0.24 | 0.12 | 0.22 | 0.031–1.52 |

[a] CI, confidence interval.
[b] FIGO, International Federation of Obstetrics and Gynecology.
[c] CEP, cisplatin, epirubicin, and cyclophosphamide.
[d] Significant.

spite the correlated expression of *MRP1* and *3* mRNA, only the high *MRP1* mRNA category showed clinical significance in progression-free survival analyses. This discrepancy may be explained by the conversion of consecutive variables of *MRP1* and *MRP3* data into categorical ones. Furthermore, we suppose that the contribution of *MRP3* expression to prognosis may be less important than that of *MRP1*, and the sample number of this study may be too small to demonstrate a significant predictive value of *MRP3* for clinical outcome, although this is a matter for speculation.

MRP2 expression is more widely accepted as contributing to resistance against platinum agents (16, 18, 25); however, we demonstrated much lower levels of *MRP2* mRNA expression than *MRP1* or *MRP3*, and *MRP2* mRNA levels were not associated with clinical outcome after platinum-based chemotherapy in our 30 patients with serous papillary adenocarcinoma. Arts *et al.* (40) investigated the prognostic value of MRP2 protein expression in ovarian carcinoma by immunohistochemistry of frozen tissue sections of 115 ovarian carcinoma patients, and they found that MRP2 expression did not predict response to chemotherapy and was not related to progression-free survival. Preliminary studies, including ours, demonstrated that the intrinsic expression of MRP2 does not seem to be associated with unfavorable clinical outcome or drug resistance in ovarian carcinoma.

Concerning the comparison between clear and serous phenotype, we demonstrated that only relative mRNA levels of *MRP3* expression in clear cell phenotype were significantly higher than those in serous phenotype. Clear cell adenocarcinoma has been known to be a chemoresistant phenotype (44), and our data suggest that the chemoresistant nature of the clear cell phenotype may be explained, at least in part, by increased relative mRNA levels of *MRP3* expression.

We demonstrated that all 50 patients with ovarian carcinoma expressed undetectable levels of the *MDR1* gene and P-gp. Although a result similar to ours was reported previously (38), several studies have demonstrated at least low levels of the *MDR1* gene in untreated ovarian carcinoma (39, 45, 46). Further analysis is needed to resolve this contradiction. The clinical significance of *MDR1*/P-gp expression in ovarian carcinoma is still uncertain (39, 40), and our data have failed to offer further clarification.

In the present study, all of the patients were treated with

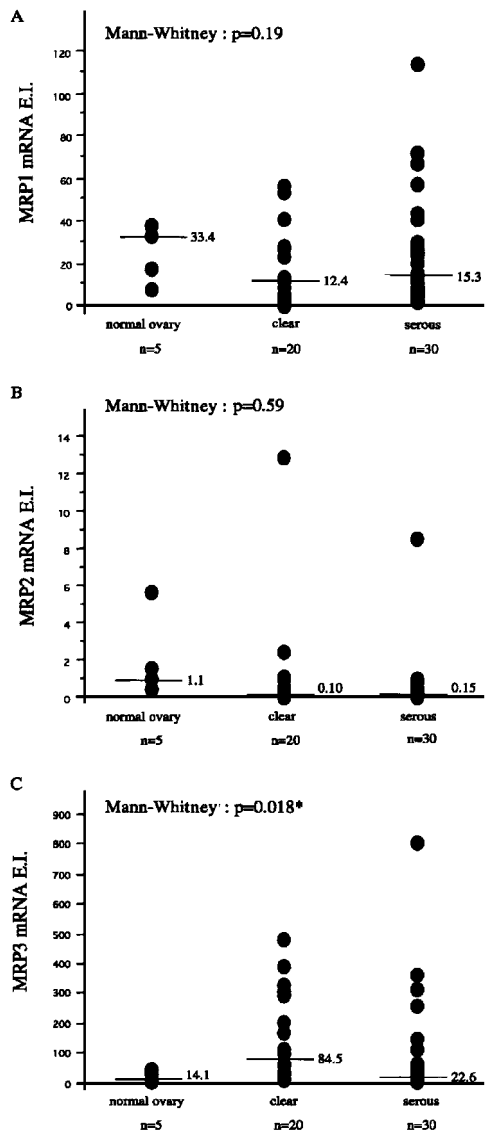


*Fig. 3* Comparison of relative mRNA levels of (*A*) *MRP1*, (*B*) *MRP2* and (*C*) *MRP3* between serous and clear cell phenotype. Data for normal ovaries are also shown.

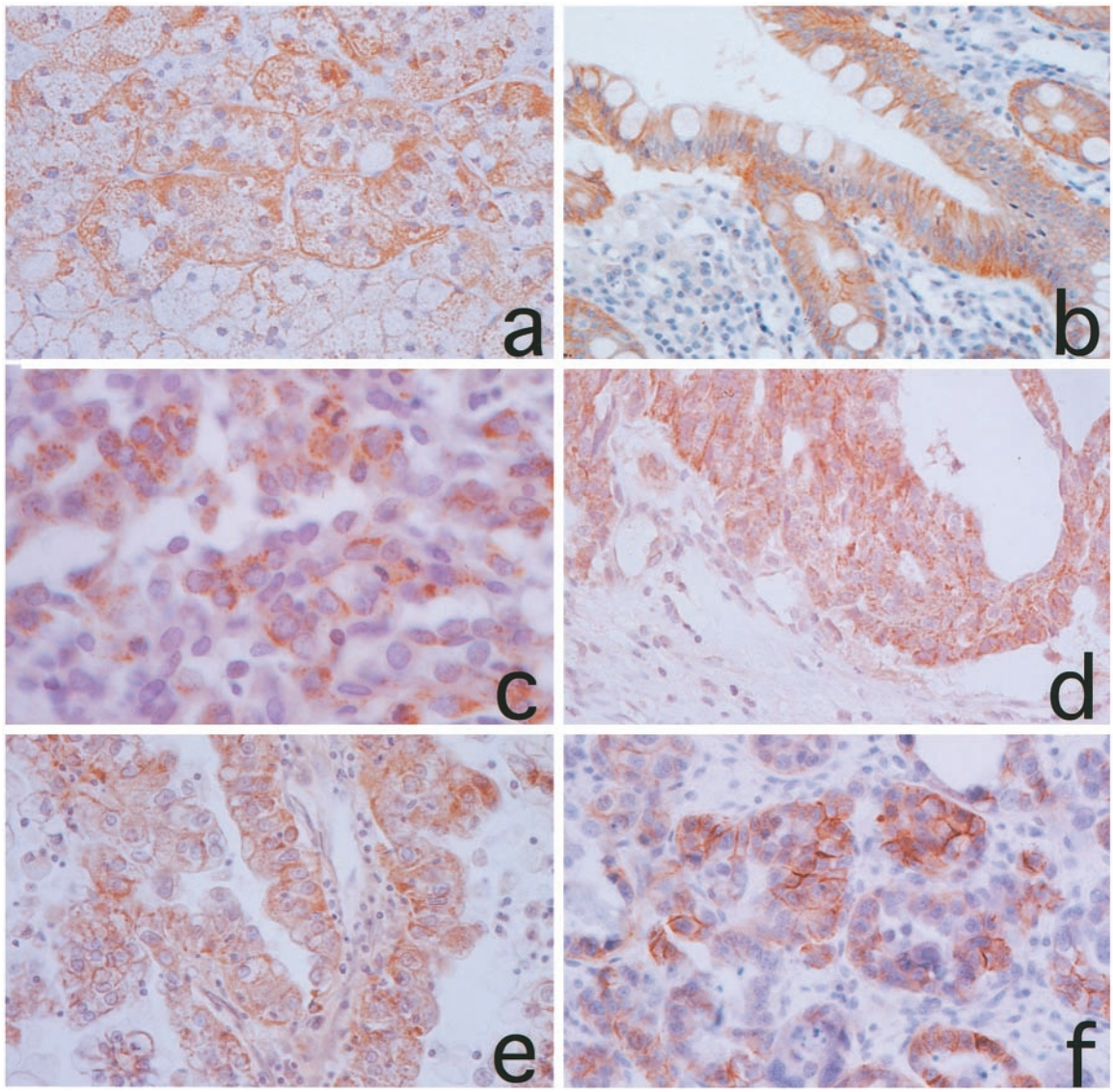


*Fig. 4* Immunohistochemically stained ovarian carcinoma sections and positive control tissue sections. MRP1 is localized on the plasma membrane or cytoplasm of (*a*) adrenal gland (positive control), (*c*) serous papillary adenocarcinoma, and (*e*) clear cell adenocarcinoma. MRP3 is localized on the plasma membrane or cytoplasm of (*b*) colonic mucosa (positive control), (*d*) serous papillary adenocarcinoma, and (*f*) clear cell adenocarcinoma. In polarized cells, the basolateral membrane is intensely stained.

a chemotherapy regimen containing epirubicin (24 patients) or paclitaxel (6 patients), both of which are P-gp substrates. The negative results with *MDR1* are consistent with the general perception regarding the efficacy of epirubicin/paclitaxel as an anticancer agent against ovarian cancer. Epirubicin, which was used in most patients, is also generally known as a MRP1 substrate, and this may be part of the reason why the relative mRNA levels of *MRP1* are correlated with the clinical outcome.

In conclusion, patients with unfavorable clinical outcome may be characterized by increased levels of coordinated expression of *MRP1* and *MRP3* mRNA in their tumor samples, both of which are basolateral targeted membrane transporter genes, and a high relative mRNA level of *MRP1* is a candidate for a useful predictor of short-term progression-free survival in advanced and moderate- to high-grade serous papillary adenocarcinoma of the ovary treated by debulking surgery followed by platinum-based chemotherapy. Additionally, the chemoresistant nature of clear cell adenocarcinoma may be explained by increased relative mRNA levels of *MRP3* expression.

To confirm and generalize our conclusion, further analysis is necessary within a systematically treated, larger group of patients with absolutely the same chemotherapy regimen for several phenotypes of ovarian carcinoma. Other mechanisms responsible for drug resistance, such as the conjugation system or increased DNA repair activity, should be examined and compared with the clinical outcome or ABC transporter expression to fully clarify the mechanisms involved in advanced ovarian carcinoma.

## ACKNOWLEDGMENTS

Part of this study was carried out at the Morphology Core, Graduate School of Medical Sciences, Kyushu University. The English used in the manuscript was revised by K. Miller (Royal English Language Centre, Fukuoka, Japan).

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*Clin Cancer Res* 2002;8:3767-3775.