Expression of Multidrug Resistance Protein-related Genes in Lung Cancer: Correlation with Drug Response

Leah C. Young, Barbara G. Campling, Theodora Voskoglou-Nomikos, Susan P. C. Cole, Roger G. Deeley, and James H. Gerlach


ABSTRACT

Recently, cDNAs have been identified that encode four human proteins (MRP2–5) with structural similarity to the multidrug resistance protein (MRP). Preliminary studies have shown that levels of mRNAs encoding MRP2, MRP3, and MRP5, are increased in some drug-selected cell lines, but the correlation of MRP2–5 mRNA levels with drug resistance has not been examined. Using a collection of small cell lung cancer (SCLC) and non-SCLC patient samples and unselected cell lines established from patients at various stages of treatment, we examined the expression of MRP2, MRP3, MRP4, and MRP5, as well as MDR1 and MRP, by PCR. The levels of individual mRNAs were correlated with the sensitivity of these cell lines to doxorubicin (DOX), vincristine, VP-16, and cis-diaminedichloroplatinum(II), as determined by a modified MTT assay. Using both SCLC and non-SCLC cell lines, we confirmed the previously observed correlation of MRP mRNA levels with resistance to DOX (B. G. Campling et al., Clin. Cancer Res., 3:115–122, 1997) and found a strong correlation of MRP3 mRNA levels with resistance of the cell lines to DOX. In addition, the mRNA levels of both MRP and MRP3 correlated with resistance of the cell lines to vincristine, VP-16, and cis-diaminedichloroplatinum(II). These findings are consistent with the suggestion that MRP3, like MRP, may contribute to the drug resistance phenotype of lung cancer cells.

INTRODUCTION

Members of the ABC superfamily of transport proteins have been implicated as major contributors to the multidrug resistance phenotypes observed in tumor cells. P-glycoprotein (encoded by MDR1; Ref. 1) and, more recently, MRP (encoded by MRP; Refs. 2–6) were the first human ABC superfamily members shown to confer resistance to multiple chemotherapeutic drugs. Like P-glycoprotein, MRP is overexpressed in numerous drug-selected cell lines and has been detected in a variety of tumor types (6–8). MRP expression has also been associated with drug resistance or poor patient outcomes in breast cancer (9, 10), gastric cancer (11), and neuroblastoma (12, 13) retinoblastoma (14), and lung cancer (15). Recognition of the potential importance of MRP-mediated multidrug resistance has led to a search for additional ABC superfamily members, and, recently, a number of MRP-related human gene products have been identified (16–22).

MRP2, also known as the canalicular multispecific organic anion transporter, is an ABC transporter more closely related to MRP than to P-glycoprotein, with respect to both its structure and substrate specificity (21, 23). MRP2 has been functionally characterized as a canalicular multispecific organic anion transporter by virtue of its absence from the hepatocanalicular of the TR rat model of Dubin-Johnson syndrome (20–22). Tissue distribution of MRP2 mRNA includes kidney, peripheral nerves, liver, ileum, and duodenum, and expression has also been detected in unselected lung, gastric, and colorectal tumor cell lines (19, 24–26). Increased MRP2 mRNA levels have been detected in some CDDP and DOX-resistant cell lines (19, 25). In addition, reduction of MRP2 mRNA and protein levels by antisense cDNA expression in hepatoma cells has been reported to increase sensitivity to VCR, CDDP, and, to a lesser extent, DOX, but did not increase sensitivity to VP-16 (27). However, the ability of MRP2 to confer multidrug resistance remains to be confirmed by transfection studies.

Three additional MRP-related ABC superfamily members, designated MRP3–5, have been identified from cDNA sequences in expressed sequence tag databases (18, 19). The genes encoding MRP and the related MRP3–5 are located on different chromosomes, and their cognate mRNAs are expressed in a variety of normal tissues (16–19). Recently, the complete cDNA sequences of MRP3 (17) and MRP4 (16) have been

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3 The abbreviations used are: ABC, ATP-binding cassette; SCLC, small cell lung cancer; NSCLC, non-SCLC; CDDP, cis-diaminedichloroplatinum(II); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TFR, transferrin receptor; AUC, area under the dose-response curve; DOX, doxorubicin; VCR, vincristine; MRP, multidrug resistance protein.

4 Unpublished results.

5 National Center for Biotechnology Information Entrez protein database accession numbers 3087794, 3132270, and 3132270.

6 National Center for Biotechnology Information Entrez protein database accession number 3335173.
determined. MRP3 is predicted to encode a 1527 amino acid protein that has 56% amino acid identity to MRP and 46% identity to MRP2 (17). In humans, MRP3 mRNA is present at high levels in the liver, colon, adrenal gland, and small intestine and at lower levels in the lung, spleen, bladder, and kidney (17, 19). MRP4 is the least similar to the other MRP-related gene products with 39% amino acid identity to MRP, and its mRNA is predicted to encode a 1325 amino acid polypeptide that apparently lacks the hydrophobic NH2-terminal extension that is a characteristic of the MRP branch of the ABC superfamily of transport proteins (16). A preliminary investigation has shown that MRP3 and MRP5 mRNA levels, but not MRP4, are increased in some drug-selected cell lines (19). However, correlations of MRP3–5 expression with drug resistance have not been established.

The amino acid sequence similarity of MRP2–5 to that of MRP, and the observation that the mRNA levels of MRP2 (19, 25), MRP3 (19), and MRP5 (19) are elevated in some drug-selected cell lines, suggest that expression of these MRP-related proteins may also contribute to drug resistance. Like MRP mRNA, the mRNAs from MRP3 (17, 19), MRP4 (16, 19), MRP5 (19) can be detected in normal lung, thus, increasing the likelihood that they may be expressed in tumors arising from this tissue. To investigate this possibility, we examined a panel of 23 unselected NSCLC and SCLC cell lines, as well as 15 patient samples, to determine possible correlations between the expression of these newly identified ABC proteins and resistance to four chemotherapeutic agents. We found that both MRP and MRP3 mRNA levels showed positive correlations with resistance of the unselected lung cancer cell lines to DOX, VCR, VP-16, and, unexpectedly, CDDP. In contrast, no significant, positive correlations were observed for MRP2, MRP4, or MRP5 mRNA levels with resistance to these four drugs. These data suggest that MRP and MRP3 may contribute to the drug resistance characteristics of unselected lung cancer cells in vitro and, consequently, they should be considered as possible components of the multifactorial mechanisms of clinical drug resistance in this disease.

MATERIALS AND METHODS

Cell Lines. The panel of cell lines used in these experiments was comprised of 10 SCLC cell lines and 13 NSCLC cell lines (4 adenocarcinoma, 4 large cell carcinoma, 4 squamous cell carcinoma, and 1 bronchoalveolar carcinoma), none of which had been selected for drug resistance in vitro. Of the 13 NSCLC cell lines, 3 were established from patients treated with chemotherapy, 2 were from patients treated with radiation, 7 were from untreated patients, and for 1 cell line the treatment history was not known. Of the 10 SCLC cell lines, 4 were established from chemotherapy-treated patients and 6 from untreated patients. The conditions for establishing and culturing these cell lines have been reported previously (28).

All of the SCLC cell lines have been described previously. SCLC cell lines established in this laboratory were AD-A, HG-E, JN-M, LD-T, SV-E, and LV-E (28, 29). SCLC cell lines established in other laboratories were NCI-H209 (30), MAR (31), SHP-77 (32), and RG-1 (28). With the exception of the LC-T adenocarcinoma cell line (28) and the QU-DB large cell carcinoma cell line (33), the NSCLC cell lines derived in this laboratory have not been described previously. The FR-E adenocarcinoma cell line was from a pericardial effusion from a patient treated with chemotherapy. Lastly, the BH-E squamous cell carcinoma cell line was from a pleural effusion from a patient treated with chemotherapy. The remaining NSCLC cell lines were established in other laboratories: SK-MES-1, A549, Calu-1, Calu-6 (obtained from J. Fogh, Memorial Sloan-Kettering Cancer Center, New York, NY), SW-900, SK-LU-1, SK-Luci-6, and SW-1573 (34–37).

Cryopreserved Patient Samples. Patient samples were collected between May 1988 and July 1992 from individuals treated at the Kingston Regional Cancer Center (Kingston, Ontario, Canada). With the patient’s informed consent, a sample was sent for research purposes as part of a study approved by the Research Ethics Board of Queen’s University. Solid tumor samples were mechanically disaggregated, and cells from effusion samples were collected by centrifugation. RBCs and nonviable cells were separated from tumor cells by Ficoll-Hypaque density gradient centrifugation, and only samples composed of >90% tumor cells were cryopreserved and included in this study (29). For each sample, the source, histology, and treatment history are listed in Table 1. For seven patient samples, a continuously growing cell line has been established (Table 1).

Drug Sensitivity Testing. The sensitivity of each cell line to DOX, VCR, VP-16, and CDDP was measured using an MT T assay (38). For both the SCLC and NSCLC cell lines, cells were exposed to the chemotherapeutic agent for 48 h. Sensitivity was expressed as the AUC, as calculated by the trapezoidal rule. The sensitivity of each cell line was determined.

A summary of the histology, origin, and prior treatment of the cryopreserved patient samples is listed in Table 1. If a patient sample was also used to establish a cell line, the associated cell line is listed.

- **Table 1** Cryopreserved patient samples

<table>
<thead>
<tr>
<th>Histology</th>
<th>Origin</th>
<th>Rx(^a)</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC</td>
<td>PE</td>
<td>C</td>
<td>JN-M(^b)</td>
</tr>
<tr>
<td>SCLC</td>
<td>PE</td>
<td>C</td>
<td>LD-T</td>
</tr>
<tr>
<td>SCLC</td>
<td>PT</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>SCLC</td>
<td>PT</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>PCE</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>SCLC</td>
<td>PE</td>
<td>C</td>
<td>LV-E</td>
</tr>
<tr>
<td>SCLC</td>
<td>PE</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Adeno</td>
<td>PT</td>
<td>U</td>
<td>LC-T</td>
</tr>
<tr>
<td>Adeno</td>
<td>PE</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>L</td>
<td>U</td>
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<td>FR-E</td>
</tr>
<tr>
<td>Adeno</td>
<td>PE</td>
<td>U</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Rx, treatment history before sample; adeno, adenocarcinoma; large, large cell carcinoma; squamous, squamous cell carcinoma; PE, pleural effusion; PT, primary tumor; L, lymph node; PCE, pericardial effusion; C, chemotherapy; U, untreated.

\(^b\) Patient sample 4 was obtained after the commencement of chemotherapy, and the associated cell line was established from a sample obtained before chemotherapy.
response curve than the value at which 50% of the cells are nonviable, commonly referred to as the IC50 (39).

**mRNA Isolation and cDNA Synthesis.** Poly(A+) mRNA was extracted from the cell lines and patient samples using the QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech Inc., Baie d’Urfé, Québec, Canada). For each cell line and patient sample, 0.3 μg of mRNA and 50 ng of random hexanucleotide primers (Pharmacia Biotech Inc.) were heated at 70°C for 10 min and then chilled on ice for 10 min. The remainder of the reaction components were added to a final volume of 10 μl at a concentration of 1 mM dNTP (Pharmacia Biotech Inc.), 1.35 units/μL RNAguard (Pharmacia Biotech Inc.), and 0.4 units/μL AMV reverse transcriptase (Life Sciences Inc., St. Petersburg, FL) in the provided buffer concentrate (1/10 reaction volume). The samples were incubated at 42°C for 40 min, 95°C for 10 min, and 24°C for 10 min. Before use, the cDNA was diluted 20-fold in double-distilled water (not treated with diethyl-pyrocarbonate).

**PCR.** The cDNA was amplified using the Expand Long Template PCR System (Boehringer Mannheim Corp., Laval, Québec, Canada) according to the manufacturer’s instructions, with the addition of 1.25 μCi of [35S]dATP (1250 Ci/mmol; DuPont NEN, Boston, MA) to each 10-μl reaction. Using a PCT-100 thermocycler (MJ Research Inc., Incline Village, NV), the samples underwent second strand synthesis (a 1-min denaturation at 94°C, 1 min at the primer-specific annealing temperature, and a 3-min elongation at 68°C) and then were amplified (a 35-s denaturation, 1-min annealing, and a 1-min elongation), followed by a final elongation of 7 min. For each primer set, in both the cell lines and patient cDNA samples, the number of amplification cycles was selected such that all reactions were maintained within the exponential range of amplification. This ranged from 23–28 cycles for the cell lines and 28–33 cycles for the patient samples. The greater number of cycles for the patient samples was required because of the lower mRNA yields.

The PCR primers, annealing temperature, and expected product size were as follows: for MDR1, a 623-bp product was generated using 5'-ACACCGCCTTACAGATGTAGTCTC-3' (forward primer) and 5'-CGAGATGGGAATCTGGAAATGAC-3' (reverse primer) at an annealing temperature of 58°C; for MRP1, 657-bp product was generated using 5'-AGTGACCTGTGGCTCTTAAACCAAGG-3' (forward primer) and 5'-AGGTGAGAGAAGTGTATCTTCG-3' (reverse primer) at an annealing temperature of 56°C; for MRP2, a 322-bp product was generated using 5'-AGTAGAAGAGTGTATCTTCG-3' (forward primer; Ref. 19) and 5'-CTACTCCATCAATGTAATCTGACC-3' (reverse primer) at an annealing temperature of 52°C; for MRP3, a 262-bp product was generated using 5'-CAGTTGGCCTGATGTTGCTG-3' (reverse primer; Ref. 19) at an annealing temperature of 50°C; for MRP4, a 239-bp product was generated using 5'-CACATTGAAGATCTCGATCGG-3' (forward primer; Ref. 19) and 5'-GGGTGTTCAATGCTGTGAC-3' (reverse primer; Ref. 19) at an annealing temperature of 50°C; for MRP5, a 381-bp product was generated using 5'-GGATAAACTCTGGATCAGG-3' (forward primer; Ref. 19) and 5'-GTAGAAGAGTGTATCTTCG-3' (reverse primer) at an annealing temperature of 50°C; and for TFFR, a 512-bp product was generated using 5'-GGATAAAGCGGTCTCTGGTACCAGC-3' (forward primer) and 5'-TGCGTACGAGAAGTGTATCTTCG-3' (reverse primer) at an annealing temperature of 58°C. All primers were synthesized by Cortec DNA Service Laboratories Inc. (Kingston, Ontario, Canada).

PCR-amplified samples were separated on a 2% agarose gel and transferred to a positively charged nylon membrane (Zetaprobe; Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) by downward alkaline transfer for 2–3 h (29).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>MDR1</th>
<th>MRP1</th>
<th>MRP2</th>
<th>MRP3</th>
<th>MRP4</th>
<th>MRP5</th>
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<tr>
<td><strong>Cell lines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SCLC (n = 10)</td>
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<td>100%</td>
<td>40%</td>
<td>40%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>NSCLC (n = 13)</td>
<td>54%</td>
<td>100%</td>
<td>69%</td>
<td>77%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong> (n = 23)</td>
<td>39%</td>
<td>100%</td>
<td>56%</td>
<td>61%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Patient samples</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SCLC (n = 8)</td>
<td>25%</td>
<td>88%</td>
<td>75%</td>
<td>12%</td>
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<tr>
<td>NSCLC (n = 7)</td>
<td>43%</td>
<td>100%</td>
<td>57%</td>
<td>86%</td>
<td>71%</td>
<td>100%</td>
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<tr>
<td><strong>Total</strong> (n = 15)</td>
<td>33%</td>
<td>93%</td>
<td>67%</td>
<td>47%</td>
<td>87%</td>
<td>100%</td>
</tr>
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</table>

References:

(38, 39)
[^35]SdATP incorporated into the PCR product was measured by exposure (24–48 h) to a Storage Phosphor Screen GP (Kodak, Rochester, NY) and analyzed with a STORM 820 Phosphor-Imager (Molecular Dynamics, Sunnyvale, CA) using ImageQuantNT Software, version 4.2a (Molecular Dynamics, Sunnyvale, CA). The linear range of detection of the Phosphor Screen is much greater than that of X-ray film and, therefore, allows for accurate quantitation of both low- and high-intensity radiolabeled PCR products on a single exposure. To account for variability in mRNA extraction, mRNA quantitation, and cDNA synthesis, the measurement of each mRNA of interest was normalized to the expression of the TFRR.

### Statistical Analysis
Statistical analyses were performed using the Systat software package, version 7.0 (SPSS Inc., Chicago, IL). The distribution of mRNA levels (normalized to TFRR) was skewed toward low values and consequently required transformation (natural-logarithmic) to more closely approximate a normal distribution. The ln-transformed mRNA values were used in all subsequent calculations and figures. The AUC data were normally distributed and, thus, were not transformed. The Pearson correlation coefficient (r) and associated probability (P) were calculated for each combination of mRNA and AUC data sets. Relationships within the drug sensitivity data sets and within the mRNA data sets were calculated by the same method.

### RESULTS
#### Drug Sensitivity
The resistance of the 23 unselected SCLC and NSCLC cell lines to DOX, VCR, VP-16, and CDDP was measured using a modified MTT assay (38). The cell lines exhibited a wide range of sensitivities, and the median level of resistance to all drugs tested was higher for the NSCLC cell lines than the SCLC cell lines (Fig. 1). Also, with all four drugs, the range of sensitivities for the SCLC and NSCLC cell lines overlapped and, within these two subtypes of lung cancer cell lines, the drug sensitivity data were normally distributed (Fig. 1).

**PCR.** Poly(A+) mRNA was extracted from cell lines and patient samples and reverse-transcribed into cDNA by priming with random hexanucleotides. The cDNAs corresponding to MDR1, MRP, and MRP2–5 were amplified using specific primers, under conditions determined to be in the exponential range of amplification for these samples. MDR1 mRNA was not detected in the majority of cell lines and patient samples (Table 2; Fig. 2F). MRP3 and MRP2 mRNAs were detected in about half of the cell lines and patient samples, and MRP, MRP4, and MRP5 mRNAs were detected in most cell lines and patient samples (Table 2 and Fig. 2). For seven cell lines, the corresponding original patient samples were analyzed (indicated in Table 1). When these seven patient samples and corresponding cell lines were compared for expression of each of these six mRNAs, they were in accordance 35 of a possible 42 times (data not shown).

We observed differences in the patterns of expression of these genes between SCLC and NSCLC in both the cell lines and patient samples. Median MRP3 mRNA levels were higher in the NSCLC cell lines and patient samples than in the SCLC cell lines and patient samples (Fig. 2A). This difference was particularly pronounced in the patient samples where seven of eight SCLC samples did not express detectable levels of MRP3.
mRNA, whereas six of seven NSCLC samples were \textit{MRP3} positive (Fig. 2A). In the case of \textit{MRP}, median mRNA levels were higher in the NSCLC cell lines than in the SCLC cell lines (Fig. 2B). However, in the patient samples, median \textit{MRP} mRNA levels were higher in SCLC samples than in the NSCLC samples (Fig. 2B). For both \textit{MRP4} and \textit{MRP5}, the median mRNA levels were higher in the SCLC cell lines and patient samples than in the NSCLC cell lines and patient samples. The mRNA levels of both \textit{MRP2} and \textit{MDR1} tended to be low or nondetectable with only a few samples having significant levels of expression (Fig. 2, C and F).

\textbf{Correlation Analyses.} Pearson correlation coefficients (\(r\)) and associated probabilities (\(P\)) were calculated to determine whether the expression of these \textit{MRP}-related genes correlated with the resistance of the 23 unselected cell lines to DOX, VCR, VP-16, or CDDP (Table 3). Examining the relationships between these six mRNAs and resistance to four drugs results in 24 pair-wise comparisons. As the number of comparisons increases, so does the possibility that correlations that are due solely to chance will seem statistically significant (“false-positive”). Probabilities can be calculated to correct for multiple comparisons (e.g., Bonferroni adjustment). However, using corrected probabilities increase the possibility that meaningful correlations will be discarded (“false-negative”). Because this is an exploratory investigation, the use of corrected probabilities could mask associations that should be explored further. Taking into account the number of comparisons and the experimental variability inherent in PCR and the MTT assay, we chose a level of significance of 0.05 for each comparison with the recognition that any relationships identified as significant would require subsequent confirmation in independent studies. The terms used to describe the strength of the correlations are as follows: (a) strong, \(|r| \geq 0.7\); (b) moderate, \(|r| \geq 0.5\); (c) weak, \(|r| \geq 0.3\); and (d) no appreciable correlation, \(|r| < 0.3\).

\textit{MRP3} mRNA levels in the cell lines demonstrated a strong, significant correlation with DOX resistance (\(r = 0.782; P < 0.001\)) and moderate, significant correlations with resistance to VCR, VP-16, and CDDP (Table 3). \textit{MRP} mRNA levels demonstrated moderate, significant correlations with resistance to DOX, VCR, and VP-16 and a weak, but significant, correlation with resistance to CDDP. \textit{MRP4} and \textit{MRP5} mRNA values with resistance of the cell lines to VCR and CDDP (Table 3). No combination of \textit{MRP4} mRNA values with resistance of the cell lines to any of these four drugs produced significant correlations (Table 3). Interestingly, \textit{MRP5} mRNA levels in the cell lines showed a moderate, negative correlation with VP-16 resistance that was statistically significant and a weak, negative correlation with VCR resistance that was not statistically significant.

All pair-wise comparisons of the drug sensitivity data displayed correlations that were statistically significant. Within the natural products, there was a strong correlation between resistance to VP-16 and VCR and moderate correlations between DOX resistance and resistance to VCR and VP-16 (Table 4). Resistance of the cell lines to CDDP correlated strongly with their resistance to DOX and moderately with their resistance to VCR and VP-16 (Table 4).

Within the cell lines, \textit{MRP} mRNA levels demonstrated a moderate, significant correlation with \textit{MRP3} mRNA levels (Table 5). \textit{MRP2} showed correlations with \textit{MRP} and \textit{MRP3} mRNA levels that were not as strong (Table 5). No other combinations of the mRNA data sets generated significant correlations. Because of the small number of patient samples, correlations between their mRNA levels were not examined.

\textbf{DISCUSSION}

Our data support the possibility that among the four \textit{MRP}-related proteins identified recently, \textit{MRP3}, like \textit{MRP}, is important in the multidrug resistance phenotype of human lung cancer cells. We found a striking correlation between \textit{MRP3} mRNA levels and resistance to DOX (\(r = 0.782\)), which was even stronger than that observed with \textit{MRP} (\(r = 0.519\); Table 3). Additionally, both \textit{MRP3} and \textit{MRP} mRNA levels showed moderate correlations with resistance of the cell lines to VCR and VP-16 (Table 3). We observed correlations with CDDP resistance that were moderate for \textit{MRP3} mRNA levels and weak for \textit{MRP} mRNA levels (Table 3). Considering the \textit{MRP}-mediated drug resistance profile (2, 3), the weak correlation between \textit{MRP} mRNA levels and CDDP resistance was unexpected. The strength of the correlations between CDDP resistance and resistance to the other drugs (Table 4) may have contributed, in part, to this correlation of \textit{MRP} with CDDP resistance (Table 3; \(r = 0.428; P = 0.041\)), which was noticeably weaker than those with the other drugs (Table 3; \(r = 0.519–0.562; P < 0.011\)).

Although significant correlations due to chance could be expected in this type of exploratory investigation using multiple...
MRP-related Gene Expression in Lung Cancer

Table 5  Pearson correlation coefficients (r) and associated probabilities (P) for the correlation of mRNA levels measured in the unselected lung cancer cell lines

<table>
<thead>
<tr>
<th></th>
<th>MRP</th>
<th>MRP2</th>
<th>MRP4</th>
<th>MRP5</th>
<th>MDR1</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.570</td>
<td>0.495</td>
<td>-0.011</td>
<td>0.310</td>
<td>0.031</td>
</tr>
<tr>
<td>P</td>
<td>0.004</td>
<td>0.016</td>
<td>0.959</td>
<td>0.150</td>
<td>0.890</td>
</tr>
<tr>
<td>r</td>
<td>0.502</td>
<td>-0.140</td>
<td>-0.265</td>
<td>-0.219</td>
<td>0.315</td>
</tr>
<tr>
<td>P</td>
<td>0.015</td>
<td>0.524</td>
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<tr>
<td>P</td>
<td></td>
<td>0.524</td>
<td>0.406</td>
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<tr>
<td>r</td>
<td></td>
<td>0.206</td>
<td>0.227</td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.344</td>
<td>0.297</td>
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<td></td>
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<td>0.362</td>
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<td>P</td>
<td></td>
<td>0.089</td>
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</table>

comparisons, all of the Ps for the correlations with MRP3 mRNA are low (P < 0.01; Table 3), and it is extremely unlikely that the MRP3 mRNA levels would correlate with the resistance of the cell lines to all four drugs by chance alone. Moreover, despite the fact that significant correlations were observed within MRP3, MRP, and MRP2 mRNA levels (r = 0.570–0.495) and within the drug sensitivity data of the four drugs (r = 0.600–0.781), MRP3 demonstrated correlations with resistance that were at least as strong as those for MRP and stronger than those for MRP2 (Tables 3–5). This indicates that the MRP-like pattern of correlation observed between MRP mRNA and drug resistance is not simply a consequence of the correlation between MRP and MRP3 mRNA levels.

The mRNA levels of both MRP and MRP3 tended to be higher in the NSCLC cell lines than in the SCLC cell lines (Fig. 2, A and B). Immunohistochemical studies have shown that, in untreated lung cancer tumors, MRP is more prevalent in NSCLC than SCLC (40). In the patient samples, we found that the median mRNA level of MRP3, but not MRP, was higher in the NSCLC than in the SCLC samples (Fig. 2, A and B). The unexpectedly high median level of MRP mRNA in the SCLC patient samples, as compared with the NSCLC samples, may be due to the differences in patient treatment history. The majority of the NSCLC samples were from untreated patients (five of seven), whereas six of eight SCLC samples were obtained from patients who were failing chemotherapy treatment (Table 1). Unlike MRP mRNA, which was detected in all cell lines and almost all patient samples, MRP3 mRNA was not detected in the majority of SCLC cell lines and patient samples (Table 2; Fig. 2, A and B). In the patient samples, the relative lack of detectable MRP3 mRNA in the SCLC samples (one of eight), as compared with NSCLC (six of seven), is interesting in view of the differences in patient treatment histories and suggests that MRP3 may be more significant in the intrinsic resistance of NSCLC than in the acquired resistance of SCLC (Tables 1 and 2; Fig. 2, A and B). Although the correlation between MRP3 and MRP mRNA levels in the cell lines suggests some degree of coordinate expression (Table 5), it appears that expression of MRP3 may not be related to chemotherapy treatment and that the coordinate expression of MRP3 and MRP may be particular to NSCLC.

In comparing these results with those of previous studies (29, 41, 42), there is a consensus that MRP mRNA levels correlate with resistance of unselected lung cancer cell lines to chemotherapeutic agents included in the drug resistance phenotype mediated by MRP. In a panel of 10 SCLC and 6 NSCLC unselected lung cancer cell lines, Giaccone et al. (41) found that MRP mRNA levels correlated with resistance to DOX, but not with resistance to CDDP or VP-16. In a panel of 14 unselected NSCLC cell lines, Berger et al. (42) observed that MRP mRNA levels correlated with resistance to DOX, daunomycin, VP-16, and vinblastine, but did not correlate with resistance to CDDP or bleomycin. Previously, we noted a moderate correlation of MRP mRNA levels with resistance of 23 unselected SCLC cell lines to DOX, but that significant correlations were not found with VCR, VP-16, or CDDP (29). The correlations of MRP mRNA levels with resistance in the present study are stronger than those found in our previous study with only SCLC cell lines (29). These stronger correlations are due to the inclusion of the NSCLC cell lines, which resulted in a wider range of MRP mRNA levels and drug sensitivity profiles (Fig. 2B).

We detected MRP2 mRNA in 56% of the unselected lung cancer cell lines and 67% of the lung cancer patient samples (Table 2) and observed no difference in the MRP2 mRNA levels between SCLCs and NSCLCs (Fig. 2C). Despite the significant correlations between the mRNA levels of MRP2 with both MRP3 (r = 0.495) and MRP (r = 0.502), only weak and statistically nonsignificant correlations were observed between MRP2 mRNA levels in the lung cancer cell lines and resistance to the four drugs. MRP2 is located primarily in apical membranes of polarized epithelial cells (22, 23, 43), and it is not known to what extent the level of MRP2 protein correlates with MRP2 mRNA levels in cells lacking an apical membrane. At the moment, there is little evidence to support or refute a role for MRP2 in the drug resistance of lung cancer cells.

We found no evidence to support the involvement of MRP4 or MRP5 in the drug resistance characteristics of either NSCLC or SCLC cell lines. We detected MRP4 mRNA in most and MRP5 mRNA in all cell lines and patient samples (Table 2; Fig. 2, D and E). However, all Pearson correlation coefficient values for MRP4 and MRP5 mRNA levels were negative, suggesting, if anything, an inverse association with the resistance of the lung cancer cell lines to all four drugs tested (Table 3). We found that MRP5 mRNA levels in the cell lines showed a weak, negative correlation with VCR resistance and a moderate, negative correlation with VP-16 resistance (Table 3). Although small mRNA elevations are observed in some drug-selected cell lines (19),7 MRP5 mRNA levels have not been found to be increased in drug-selected SCLC or NSCLC cell lines (19). Moreover, in the limited number of cell lines examined, there has been no reported increase in MRP4 mRNA levels in drug-selected bladder, colon, epidermoid carcinoma, leukemia, adenocarcinoma, or SCLC or NSCLC cell lines (19). Our data do not support a role for either MRP4 or MRP5 in increased drug resistance in lung cancer cells.

Of the MRP-related proteins examined in the present study, MRP3 is most closely related to MRP, and we have shown that

7 Unpublished results.
both MRP and MRP3 mRNA levels correlated with resistance of 23 unselected lung cancer cell lines to the chemotherapeutic drugs tested, including a strong correlation of MRP3 with DOX.

Moreover, like MRP, MRP3 mRNA levels were higher in the NSCLC cell lines than in the SCLC cell lines. This difference in MRP3 expression between the two major subclasses of lung cancer was also reflected in the tumor samples. In contrast, we found, no evidence to support an association of MDR1, MRP2, MRP4, or MRP5 expression with the drug resistance of lung cancer cell lines. These data are consistent with the idea that expression of both MRP and MRP3 contribute to the multifactorial, multidrug resistance phenotype of lung cancer cells, particularly that of NSCLC.

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Expression of Multidrug Resistance Protein-related Genes in Lung Cancer: Correlation with Drug Response

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