Alterations in expression of the multidrug resistance-associated protein (MRP) gene in high-grade transitional cell carcinoma of the bladder

SC Clifford1, DE Neal2 and J Lunec1

1Cancer Research Unit and 2Department of Surgery, Medical School, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE2 4HH, UK.

Summary Expression of the MRP gene has been demonstrated in vitro to be a causal factor in non-P-glycoprotein-mediated multidrug resistance, and is implicated in resistance to a number of the chemotherapeutic agents currently used in the treatment of high-grade tumours (TCC) of the bladder (doxorubicin, epirubicin and vinblastine). Using a sensitive RT–PCR-based technique, we have quantified MRP mRNA levels in a series of untreated TCC (n=24), normal bladder (n=5) and control tissue and cell line samples. MRP mRNA was expressed and detectable in all samples analysed, with considerable (up to 190-fold) variation observed between individual tumour samples. MRP mRNA levels found in TCC samples were lower than those determined for normal peripheral (4.1-fold) and transitional (2.3-fold) and testis (4.0-fold) samples, previously reported to be high-expressing tissues, and varied over a similar range to that observed in normal bladder samples. Results indicate that MRP mRNA levels in a greater proportion of high-grade (G3) bladder tumours (55%, 6/11) are significantly reduced (P<0.018) compared with low- and moderate-grade (G1/2) bladder tumours (8%, 1/13), and suggest that MRP mRNA levels frequently become reduced as a consequence of tumour progression to advanced, poorly differentiated disease. No correlation was apparent between MRP and MDR1 mRNA levels, thus providing no evidence to suggest common regulation of the two genes. In a limited number of patients, no evidence was found to support a role for MRP mRNA levels as a determinant of response to chemotherapy in patients being uniformly treated with either cisplatin–methotrexate–vinblastine, or cisplatin–methotrexate–vinblastine–epirubicin–mercaptopurine. Similarly, no overall pattern of altered MRP mRNA expression was observed following chemotherapy in four patients from whom post-chemotherapy biopsies were taken. This study provides a useful pilot investigation regarding the level, variation and pattern of MRP mRNA expression in TCC of the bladder, and suggests that further studies to establish the clinical significance of these variations are required.

Keywords: multidrug resistance-associated protein (MRP); bladder cancer; chemotherapy; polymerase chain reaction (PCR)

Transitional cell carcinoma (TCC) of the bladder represents the fifth most prevalent malignancy in Western populations, with peak incidence found in males of the 60- to 70-year-old age group (Davies, 1982). TCCs arise from the specialised urinary epithelium (urothelium) and comprise >90% of all bladder tumours. TCCs are clinically classified by their degree of bladder wall invasion (Stage; Ta to T4; UICC, 1978) and their degree of de-differentiation (Grade; G1 to G3) (reviewed by Mostofi et al., 1988). Muscle-invasive tumours (T2, T3 and T4) are routinely treated using chemotherapy regimens based on the cytotoxic agents cisplatin, methotrexate, vinblastine and doxorubicin, with total response rates of 60% (30% complete) commonly observed in previously untreated patients (Harker et al., 1985; Sternberg et al., 1988). However, a large proportion (approximately 40%) of bladder tumours do not respond to chemotherapy, and the reasons underlying this treatment failure remain to be elucidated. Molecular mechanisms that mediate cellular drug resistance may play a role in the differential responsiveness of individual bladder tumours to chemotherapy.

The multidrug resistance-associated protein (MRP) is a 190 kDa membrane phosphoglycoprotein belonging to the ATP-binding cassette family of transporter proteins, to which the transmembrane drug efflux pump, P-glycoprotein (encoded by the MDR1 gene), also belongs (Cole et al., 1992; Endicott and Ling, 1989). However, while MRP shares many of the structural and functional features of P-glycoprotein, the two proteins share only 15% amino acid identity (Cole et al., 1994; Almquist et al., 1995). Since its cloning and identification from the doxorubicin-selected small-cell lung cancer (SCLC) cell line, H69/AR (Cole et al., 1992), the degree of overexpression of the 6.5 kb MRP mRNA has been shown to correlate well with the multidrug resistance (MDR) phenotype of a number of doxorubicin-selected cell lines of different origin that do not overexpress P-glycoprotein (P-gp), including H69/AR, GLC4/ADR (SCLC; Zaman et al., 1993), H1.L0/ADR (glioma) and H1666/ADR (fibrosarcoma; Marshall et al., 1991), HT1080/DR4 (fibrosarcoma; Slovak et al., 1993), COR-L23/R (large-cell lung carcinoma; Barrand et al., 1994) and T24/ADM-2 (TCC of the bladder, Hasegawa et al., 1995), with MRP overexpression widely associated with reduced intracellular drug accumulation.

Gene transfection studies (Grant et al., 1994; Cole et al., 1994) have demonstrated that introduction of the full-length MRP cDNA into HeLa cells confers a typical MDR phenotype, displaying cross-resistance to the anthracyclines (doxorubicin, daunorubicin, epirubicin), vinca alkaloids (vincristine, vinblastine), VP16 and taxol, but not mitoxantrone, 9-alkyl anthracyclines or cisplatin. ATP-dependent reduced drug accumulation was also observed in these transfectants which, coupled with recent monoclonal antibodies studies demonstrating localisation of the MRP protein to the plasma membrane in MRP transfectants and overexpressing cells by Flens et al. (1994), Hnipfer et al. (1994) and Almquist et al. (1995), is highly suggestive of a role for MRP as an energy-dependent transmembrane drug efflux pump. Recent studies have suggested that in some cells, MRP may alternatively or in addition be involved in the intracellular sequestration and redistribution of drugs away from their site of action (Cole et al., 1992; Almquist et al., 1995), and in the ATP-dependent transport of glutathione S-conjugates across the cell membrane, thus providing a potential role for MRP in the removal of glutathione-conjugated xenobiotics from the cell (Muller et al., 1994).

Expression of the MRP gene is implicated in tumour cell resistance to many of the drugs currently used in the chemotherapy of bladder cancer (epirubicin, doxorubicin and vinblastine). However, the clinical relevance of MRP gene expression in human tumours remains unclear, with few studies having investigated the variation and pattern of MRP
expression within a specific tumour type, or related this to the subsequent response to chemotherapy. This study reports the determination of MRP gene transcript levels in a series of previously untreated bladder TCC, and relates these levels to clinical and histopathological features including tumour stage, grade, progression and recurrence, as well as expression levels in the normal, non-neoplastic bladder. In addition, the relationship between inter-tumour variation in pretreatment tumour MRP mRNA levels and the response to chemotherapy is examined, and MRP mRNA levels are evaluated in bladder tumour biopsies taken following treatment by chemotherapy. Associations between the relative transcript levels of the MDR1 and MRP genes in a cohort of untreated bladder tumours are also investigated.

Materials and methods

Tumours, tissues and cell lines

TCC tumour samples were obtained at presentation by local resection or cystectomy from the primary tumour site. The mean tumour sample mass was 1.35 (± 0.21 s.e.) g with a minimum value of 0.18 g and a maximum of 5.46 g. A portion of the sample was sent for histological evaluation of tumour differentiation, and the extent of tumour invasion was assessed according to UICC (1978) criteria by histopathological examination, computerised tomography (CT) scan and bimanual palpation. Patient metastases were assessed by chest radiograph and CT scan of the regional lymph nodes. Care was taken to limit the proportion of tumour sample contaminated by normal tissue. The papillary growth pattern of superficial tumours allowed their routine resection without contamination by underlying normal tissue. Despite their involvement with the underlying lamina propria and muscle, contamination of muscle-invasive tumours with normal tissue was minimised by only selecting material from the protruding tumour mass. Although it was more difficult to ensure complete elimination of normal tissue from these samples, histological examination indicated an upper limit of 10–15% contamination by normal tissue. None of the patients described had received prior treatment by chemotherapy.

Normal bladder samples from patients undergoing radical cystectomy for non-neoplastic conditions were obtained as either whole bladder wall or as pure urothelium, which was carefully stripped away from its underlying tissue. Testicular tissue from normal non-urothelial healthy individuals was obtained by orchidectomy and normal testicular mononuclear cells were used as positive controls during the validation of the MRP transcript assay, since they had been described in the literature as tissues showing high MRP expression. All tumour and tissue samples were immediately snap frozen in liquid nitrogen and transferred to a −70°C freezer for storage. Peripheral mononuclear cells were separated from whole blood taken from blood donors by centrifugation (1100 g, 15 min) over a sodium metrizoate/Ficoll solution (Lymphoprep; Nycomed UK), followed by washing in phosphate-buffered saline (PBS) and pelleting by centrifugation (550 g, 5 min), before storage at −70°C. All procedures were performed at 4°C.

GLC4 SCLC cells and their doxorubicin-selected multidrug-resistant variant, GLC4/ADR (Zijlstra et al., 1987), which overexpresses MRP mRNA in the absence of P-glycoprotein overexpression (Zaman et al., 1993), were used as additional validation controls. Both lines tested mycoplasma-negative, and were routinely grown as adherent cells in RPMI-1640 medium (Gibco) supplemented (final concentrations) with 10% fetal calf serum, L-glutamine (2 mM), sodium bicarbonate (0.2%) (all Gibco) and sodium hydroxide (120 mM). Doxorubicin was added to the media at a final concentration of 0.64 μg mL−1 (GLC4/ADR cells only). Both lines were grown at 37°C in a humidified incubator at a 5% carbon dioxide concentration, and subcultured twice weekly. Cells were harvested at 70% confluence by scraping in PBS at 4°C, and pelleted by low-speed centrifugation (550 g, 5 min) before freezing at −70°C.

Chemotherapy protocols and post chemotherapy tumour samples

Patients with muscle invasive bladder tumours received first-line chemotherapy using either the CMV (3 consecutive 21 day cycles comprising: 100 mg m−2 cisplatin (day 1), 30 mg m−2 methotrexate (days 1 and 8), 4 mg m−2 vinblastine (days 1 and 8) or EPICM (2 consecutive 21 day cycles comprising: 50 mg m−2 epirubicin (day 1), 70 mg m−2 cisplatin (day 1), 40 mg m−2 methotrexate (days 8 and 15)) regimens. Tumour response was assessed 1–3 weeks following the end of the final treatment cycle by histopathological examination, CT scan and bimanual palpation, at which time post chemotherapy tumour biopsies were obtained from partially responding patients. Tumour response was categorised as complete response (CR; complete disappearance of all clinical evidence of tumour), partial response (PR; >50% reduction in the tumour mass, with no simultaneous increase in size of any lesion), no change (NC; <50% reduction or <25% increase in tumour mass) or progressive disease (PD; >25% increase in tumour mass or development of metastatic disease). All assessments were based on the product of the two largest diameters of the bladder tumour mass.

Determination of mRNA levels by quantitative PCR-based gene transcript assay

MRP and MDR1 mRNA levels were determined in all samples relative to those of 18S rRNA as an internal reference standard, using normal tissue, normal tissue from cystectomy TCC, and antisense strand AACTAGCTCATGCTGTTACC and antisense strand AAGTATCCTATGCTGTGCG (residues 2545–2565 and 2674–2692 respectively; Cole et al., 1992), and 18S rRNA, sense strand ATGCTCTAGCTAGAGTGTCC and antisense strand AACTACGACGGTATCTGATC (residues 864–883 and 1154–1175 respectively; Gonzalez and Schmikel, 1986). The MDR1 primer sequences used were those previously described by Noonan et al. (1990). The primers yielded PCR products of 148 bp (MRP), 167 bp (MDR1) and 311 bp (18S rRNA), and optimal magnesium chloride concentrations of 1 mM, 2.5 mM and 1 mM were determined for each of the primer pairs respectively. MRP and MDR1 primers were each shown to produce differentially sized PCR products when cDNA and genomic DNA were used as reaction templates, and thus distinguish and discriminate any contaminating genomic DNA present in cDNA stocks by virtue of their spanning an intronic region.

Optimisation of the polymerase chain reaction

Oligonucleotide primers that recognise the MRP and 18S rRNA cDNA sequences were selected using the computer program designed by Lowe et al. (1990). These were (in 5' to 3' orientation): MRP, sense strand GCTTGAACATGGCATTTACC and antisense strand AAGTATCCTATGCTGTGCG (residues 2545–2565 and 2674–2692 respectively; Cole et al., 1992), and 18S rRNA, sense strand ATGCTCTAGCTAGAGTGTCC and antisense strand AACTACGACGGTATCTGATC (residues 864–883 and 1154–1175 respectively; Gonzalez and Schmikel, 1986). The MDR1 primer sequences used were those previously described by Noonan et al. (1990). The primers yielded PCR products of 148 bp (MRP), 167 bp (MDR1) and 311 bp (18S rRNA), and optimal magnesium chloride concentrations of 1 mM, 2.5 mM and 1 mM were determined for each of the primer pairs respectively. MRP and MDR1 primers were each shown to produce differentially sized PCR products when cDNA and genomic DNA were used as reaction templates, and thus distinguish and discriminate any contaminating genomic DNA present in cDNA stocks by virtue of their spanning an intronic region.

Determination of gene transcript levels

Serial cDNA dilutions for each species of interest were simultaneously and independently amplified over 25 PCR cycles (94°C for 1 min, 56°C for 1 min, 72°C for 1 min) using otherwise fixed reaction conditions, followed by electrophoretic separation and quantitative analysis of the radio-labelled PCR products using a Phosphorimagor (Molecular Dynamics), as previously described (Clifford et al., 1994). A typical Phosphorimagor image of the separated products is shown in Figure 1a. For each species, the amount of PCR product (incorporated radioactivity) was plotted against input cDNA dilution (Figure 1b). Regression analysis was performed on the plots falling on the linear range of amplification for each species. The gene transcript level relative to that of a reference gene is essentially measured as the ratio of cDNA dilutions required to yield a given amount of PCR product on the linear portion of the product vs input cDNA curve (see Clifford et al., 1994). MRP and MDR1 mRNA levels
measured relative to 18S rRNA were thus expressed as MRP/18S rRNA and MDR1/18S rRNA ratios respectively. For each cDNA sample, at least three replicates of the assay were performed, and the results analysed to give a mean (± s.e.) ratio, which represents the relative mRNA levels of the target and reference genes for the RNA sample. Extensive validations of this technique with respect to sensitivity, reproducibility and PCR amplification efficiency have previously been reported (Clifford et al., 1994).

Results

MRP gene transcript levels in normal and TCC bladder samples

MRP mRNA levels were determined in 24 previously untreated TCC (by stage; T1, n = 8; T2, n = 1; T3, n = 9; T4, n = 1; and by grade; G1, n = 4; G2, n = 9; G3, n = 11) and 5 normal bladder samples. In addition, one testis sample and two white blood cell samples (tissues previously reported to express high levels of MRP mRNA (Cole et al., 1992)) and cell lines with differential MRP mRNA levels (GLC4 and GLC4/ADR; Zaman et al., 1993) were also assayed as positive controls. Results are shown in Figure 2. The mean standard error for repeat determinations (n = 3) on any given sample was 27%, based on all samples analysed (n = 40).

Quantifiable levels of MRP mRNA were detected in all samples analysed. The pooled mean MRP mRNA level (MRP/18S rRNA ratio) for all untreated bladder tumour samples was 2.0 (± 2.2) x 10⁻³ (mean ± s.d., n = 24), with considerable variation observed between individual tumours (over a 189-fold range; highest, 1.1 x 10⁻¹; lowest, 5.9 x 10⁻⁵; coefficient of variation, 111%). The pooled mean MRP mRNA level determined for normal bladder samples was 1.06 (± 2.06) x 10⁻⁵ (mean ± s.d.), with the levels observed in individual samples varying over a comparable range (31-fold; high, 4.9 x 10⁻⁵; low, 1.6 x 10⁻⁶; coefficient of variation, 70%) to that observed for bladder tumours. Based on the limited numbers of normal bladder samples analysed, no differences were apparent between the MRP mRNA levels observed in groups of whole bladder wall and stripped urothelium normal bladder samples. This was reflected by no significant difference (t-test, P = 0.64) being observed between paired urothelium and bladder wall samples taken from a single individual patient [4.94 (± 1.23) x 10⁻⁵ vs 4.28 (± 3.99) x 10⁻⁵; mean ± s.e., n = 3]. MRP mRNA levels in tissues previously reported to show high levels of expression were higher than the mean level of expression found in bladder tumours (white blood cells, approximately 2.5-fold higher; testis, approximately 4.1-fold higher). MRP mRNA levels in drug-resistant MRP-overexpressing GLC4/ADR cells were 24-fold higher than those detected in their drug-sensitive parental GLC4 cells. This result is in close agreement with the 25-fold overexpression of MRP mRNA previously reported in these cells (by RNAase protection assay; Zaman et al., 1993), and the 20-fold overexpression that we have independently detected by Northern blot analysis (SC Clifford, 1994).

Relationships to tumour stage and grade

Figure 3a shows MRP mRNA levels determined for all TCC and normal bladder samples analysed, displayed with respect to histological grade and degree of invasiveness. With the exception of two tumours which presented as T3 G2 and T1 G3, all invasive (T2/3/4) tumours also presented as high grade (G3). These results suggest a cluster of tumours with low-MRP mRNA levels (MRP/18S ratio < 1 x 10⁻³) in the high-grade and invasive groups of tumours, and demonstrate that low-MRP mRNA levels (MRP/18S ratio < 1 x 10⁻³) were found in 55% (6/11) of G3 tumours, compared with only 8% (1/13) of G1/2 tumours (P = 0.018, Fisher’s exact test). By pooled mean analysis, similar MRP mRNA levels were found in groups of high-grade (G3, n = 13) and low-grade (G1/2, n = 11) tumours (t-test, P = 0.007), and groups of superficial (Ta/1, n = 13) and invasive (T2/3/4, n = 11) tumours (t-test, P = 0.996). However, these results are biased by the inclusion in the analysis of an individual high-grade/ invasive tumour with very high MRP mRNA levels (4-fold higher than any other high-grade/invasive tumour). The high MRP mRNA levels observed in the outlying sample were confirmed upon repeat investigation (data not shown), and removal of this single point from the stage and grade analyses revealed that both pooled mean and median MRP mRNA levels are significantly lower in high-grade tumours than low- and moderate-grade tumours (t-test, P = 0.014; Mann–Whitney test, P = 0.014). Similar results are obtained in comparisons of superficial and invasive groups of tumours, although an equivalent level of significance is not reached (t-test, P = 0.062; Mann–Whitney test, P = 0.058). Thus, these results indicate a trend towards lower MRP mRNA expression in a subset of high-grade bladder tumours.

Relationships to tumour recurrence and progression

The potential usefulness of MRP mRNA levels as a marker of superficial tumour behaviour and prognosis was investigated. No evidence was found to suggest a relationship between MRP mRNA levels and the rate of tumour recurrence (linear regression analysis, r² = 0.007, n = 12) in the group of superficial bladder tumours analysed. Similarly,
no significant differences (t-test, \(P = 0.38\)) were found between pooled mean \(MRP\) mRNA levels in groups of progressing [1.5 (± 0.9) \(\times 10^{-5}\), \(n = 4\)] and non-progressing [2.1 (± 1.3) \(\times 10^{-5}\), \(n = 9\)] superficial bladder tumours (both mean ± s.e.).

**MRP mRNA levels and the response to chemotherapy**

\(MRP\) mRNA levels varied over a 45-fold range (5.9 ± 10\(^{-7}\) to 2.69 ± 10\(^{-7}\)) in patients proceeding to receive the CMV chemotherapy regimen \((n = 6)\), and a 56-fold range (1.95 ± 10\(^{-6}\) to 1.10 ± 10\(^{-6}\)) in patients receiving EPICM therapy \((n = 4)\). Figure 4a demonstrates that in the limited number of patients investigated to date, no evidence was found to suggest an association between high pretreatment \(MRP\) mRNA levels and resistance to either chemotherapy regimen. Furthermore, it is notable that the patient with the highest tumour \(MRP\) mRNA levels had a partial response to the CMV regimen.

**MRP mRNA levels following treatment by chemotherapy**

In four cases, \(MRP\) mRNA levels were determined in paired post chemotherapy samples taken 2–3 weeks following the end of chemotherapy from partially responding patients. Results are summarised in Figure 4b. Three patients had marginally increased \(MRP\) mRNA levels following treatment (patient 1, 2.4-fold; patient 2, 1.6-fold; patient 4, 1.7-fold), however for only one of these (patient 1) was the difference of borderline significance \((P = 0.075)\). None of the elevated post treatment levels observed exceeded the mean \(MRP\) mRNA level found in untreated tumours. The most striking difference was observed in patient 3, who had significantly reduced (26.6-fold, \(P = 0.014\)) tumour \(MRP\) mRNA levels following treatment. Results therefore did not suggest any consistent overall difference between pre- and post-chemotherapy \(MRP\) mRNA levels for either regimen.

**Correlations between mRNA levels of the \(MRP\) and \(MDR1\) genes in untreated TCC samples**

\(MDR1\) mRNA levels were also determined in each of the untreated TCC samples analysed \((n = 24)\), and were found to vary over a 32-fold range (low, 3 ± 10\(^{-7}\); high, 9.52 ± 10\(^{-4}\)). No significant correlations were observed between \(MRP\) and \(MDR1\) mRNA levels in untreated TCC (Figure 5; linear regression analysis, \(r^2 = 0.002\); log regression analysis, \(r^2 = 0.162\)). These results provide no evidence to suggest co-expression or common regulation of the \(MDR1\) and \(MRP\) genes in TCC of the bladder.

**Discussion**

We have demonstrated that \(MRP\) mRNA is widely expressed in both transitional cell carcinoma and normal bladder tissue, with quantifiable gene transcripts detectable in all samples analysed, and a considerable level of variation (190-fold) observed between individual untreated bladder tumours. No other studies could be found that have investigated variations in \(MRP\) mRNA levels within a given solid tumour type, although recent studies in acute myeloid leukaemia, acute lymphocytic leukaemia (Schneider et al., 1995) and chronic lymphocytic leukaemia (Burger et al., 1994) have reported variation of \(MRP\) mRNA levels between individual untreated patients (53% \((n = 29)\), 128% \((n = 15)\) and 63% \((n = 17)\)

**Figure 2 \(MRP\) mRNA levels measured relative to 18S rRNA in tumour, normal tissue and cell line samples (shown as mean \(MRP/18S\) ratio (± s.e.), n=3). Bladder tumours are shown grouped according to their degree of invasion (Ta to T4), and shaded with respect to their degree of differentiation (G1, ☐; G2, ☐; G3, ☐).**
coefficients of variance respectively) similar to those presently reported in untreated bladder tumours [111% (n = 24) coefficient of variance].

By Northern blot analysis, Cole et al. (1992) found that MRP mRNA was detectable in testis, peripheral blood mononucleocytes, but was not detectable in placenta, brain, kidney, salivary gland, uterus, liver and spleen. Similarly, using an RNAase protection assay, Zaman et al. (1993) reported MRP mRNA to be readily detectable in a wide range of normal tissues, with highest levels detected in lung, spleen, thyroid, testis, bladder, adrenal gland and gall bladder, and lowest levels detected in kidney, colon, nerve, ovary, duodenum, placenta, liver and brain. Our results confirm that MRP gene transcripts are readily detectable in the normal bladder, and indicate that the pooled mean MRP mRNA level detected in TCC samples is respectively 2.3- and 4.1-fold lower than those detected in normal peripheral mononucleocyte and testis samples. In the small number of normal bladder samples analysed (n = 5), MRP gene transcript levels varied over a comparable range and level to those observed in bladder tumours (70% coefficient of variance reported in untreated bladder).

![Figure 3](image3.png)

**Figure 3** (a) Mean MRP mRNA levels for each TCC and normal bladder sample analysed, with each sample represented by a single point. Tumour samples are shown grouped according to stage (A) and grade (Δ), and normal bladder samples are shown as stripped urothelium (○) or whole bladder wall (●). (b) Histograms showing the distribution of individual tumour MRP mRNA levels in grade-G3 and grade-G1/2 bladder tumours. Tumours are grouped according to their level of MRP mRNA expression. Population mean and median MRP mRNA levels are shown for each group of tumours.

![Figure 4](image4.png)

**Figure 4** (a) The relationship between pretreatment MRP mRNA levels and the response to chemotherapy. Tumour MRP mRNA levels (mean MRP/18S ratio ± s.e.) are shown grouped according to regimen received and response to chemotherapy (CR, complete response, □; PR, partial response, □□; NR, no response (no change or progressive disease), □□□□). (b) MRP mRNA levels (mean MRP/18S ratio ± s.e.) in paired samples taken before (□) and following (■) chemotherapy from four individual patients, shown grouped according to chemotherapy regimen received. Biopsies were taken from patient 4 at 3 and 6 weeks following the end of treatment. Probability values (by t-test or Welch's t-test for unequal variances) for increases/decreases in expression observed following treatment are shown.

![Figure 5](image5.png)

**Figure 5** Correlation between the mean tumour MRP/18S (y-axis) and MDR/18S (x-axis) ratios in all samples.
Reduced MRP mRNA levels observed in high-grade tumours are uncertain. Reduced MRP mRNA levels may represent a marker of advanced disease in TCC of the bladder, and raises questions about their prognostic significance. The preliminary survival data accumulated to date (28.1 month mean follow-up in surviving patients) suggest that low MRP mRNA levels do not identify a subgroup of high-grade tumours with worse prognosis, with equivalent mortality rates observed in subgrouping of high-grade tumours with high (MRP/18S \( \geq 1 \times 10^{-3} \)) and low (MRP/18S \( < 1 \times 10^{-3} \), n = 6) MRP mRNA levels (four out of six patients died from their disease in each case). MRP mRNA expression did not predict tumour recurrence and progression within the group of superficial bladder tumours studied, however the numbers involved to date (n = 13) are small, and only two tumours in this group had low MRP/18S ratios (\(< 1 \times 10^{-2}\)). Further clinical studies are therefore required to assess whether low MRP mRNA levels predict an adverse prognosis in superficial disease, as well as data on longer term survival follow-up in high-grade tumours.

The pattern of MRP mRNA expression observed with respect to tumour grade is markedly different from that which we have previously reported for the MDRI gene in untreated bladder tumours, where pooled mean MDRI mRNA levels were significantly higher in high-grade tumours than low-grade tumours, and associated with a poorer patient prognosis (Clifford et al., 1994). Thus, despite an apparent structural and functional similarity, the expression patterns of the MDRI and MRP genes appear to be markedly different in bladder cancer, and reflect the differences in their pattern of expression observed between different tissues (Cole et al., 1992). The present study demonstrates no correlation between the mRNA levels of the MDRI and MRP genes and thus provides no evidence to suggest co-regulation or expression of the two genes.

No evidence was found to support a role for high pretreatment MRP mRNA levels as a negative determinant of the response of untreated bladder tumours to the CMV or EPICM regimes, based on the limited number of samples investigated. Similarly, no consistent evidence was found to suggest that MRP-mediated drug resistance is selected for during treatment with either the EPICM or CMV regimes. Several reasons may potentially explain the lack of associations observed between MRP mRNA levels and response. Firstly, that MRP-mediated drug resistance is not functional at the MRP mRNA levels reported and is therefore not relevant to bladder cancer, with other mechanisms of resistance involved in the determination of response. This question could be further and specifically tested in vitro using MRP-expressing cell lines derived from MRP-positive untreated tumour cell lines. Secondly, that with the regimes used, the MRP-substrate drugs (epirubicin and vinblastine) are not being administered at sufficiently efficacious concentrations, and other drugs contained in the regimen (cisplatin and methotrexate) are responsible for any tumour responses observed. A critical examination of this question would require extension of these studies to a larger population of patients, with chemotherapy regimens involving therapeutic dose monitoring to achieve controlled significant doses of those agents to which MRP can confer resistance. Finally, the timing of post chemotherapy tumour sampling (at 2–3 weeks following therapy) may not have allowed sufficient time for tumour repopulation by any chemotherapy-selected resistant cell subpopulations.

The wide variation in MRP mRNA levels (190-fold) observed in the high-grade, invasive group of bladder tumours that are routinely treated by chemotherapy remains potentially very important in terms of their differential chemosensitivity. Studies with MRP transfecteds and in vitro selected cell lines (e.g. Grant et al., 1994; Cole et al., 1992; Zaman et al., 1993; Slovak et al., 1993) have shown that for smaller increases (approximately 10- to 100-fold) in MRP mRNA levels are associated with marked increases in resistance in MDR cells. However, the ranges over which these variations occur may be different to those observed in bladder tumours. Some useful initial evidence in this regard comes from a recent study by Hasegawa et al.
(1995), who reported the presence of detectable levels of the MRP protein (by Western blot) in the unselected TCC cell line, KK47. Using our PCR-based gene transcript assay, we have independently (Clifford, 1994) demonstrated this cell line to have an MRP/18S ratio of 8.2 (± 2.7) x 10^-3 (mean ± s.e.), which lies within the range of MRP mRNA levels presently reported in untreated bladder tumours, thus suggesting that the MRP mRNA levels observed in bladder tumours may indeed encode significant levels of the MRP protein. Further studies are required to determine whether the variations in MRP mRNA and protein levels observed in bladder tumours occur over a functionally significant range. In summary, this study reports a pilot investigation demonstrating extensive variation in the levels of MRP mRNA and protein determined in TCC of the bladder, with a trend towards low levels to be found more frequently in high-grade invasive TCC of the urinary bladder. However, despite encouraging in vitro and initial clinical findings, both the role of the MRP gene in clinical drug resistance and its role in the normal, non-neoplastic urothelium (and the consequence of variations in expression) still remain to be defined. The recent development of discriminatory anti-MRP monoclonal antibodies (Flens et al., 1994; Hipfner et al., 1994) should aid the progress of investigations regarding the relationship between MRP mRNA, protein levels/localisation and drug resistance in both tumour and cell line samples, and help to elucidate their significance in terms of tumour response to chemotherapy.

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