The Prognostic Role of p53, Metallothionein, P-glycoprotein, and MIB-1 in Muscle-invasive Urothelial Transitional Cell Carcinoma

Lillian L. Siu,1 Diponkar Banerjee, Reena J. Khurana, Xia Pan, Rafael Pflueger, Ian F. Tannock, and Malcolm J. Moore2

Departments of Medicine [L. L. S., I. F. T., M. J. M.] and Pathology [D. B., R. J. K.], Princess Margaret Hospital, Toronto, Ontario, M5G 2M9, and the Department of Mathematics and Statistics, York University, North York, Ontario, M3J 1P3 [X. P., R. P.], Canada

ABSTRACT

Tissue from primary tumors was analyzed for 118 patients with urothelial cancer who subsequently received cisplatin-based chemotherapy. Immunohistochemical staining was performed for nuclear p53 reactivity; for two proposed mediators of drug resistance, metallothionein (MT) and P-glycoprotein; and for the cell proliferation marker MIB-1. For each marker, immunoreactivity was expressed as a percentage of positively staining cells, and overall intensity of staining was graded on a scale from 0 to 3. The product of these two measurements was calculated to generate a percentage-intensity index. Clinical data were obtained independently via retrospective chart review. Chemotherapy regimens containing cisplatin (cisplatin, methotrexate, and vinblastine or methotrexate, vinblastine, doxorubicin, and cisplatin) were administered for metastatic disease (n = 64), for locally advanced disease (n = 45), or as an adjuvant treatment (n = 9). The overall response rate was 56% among 99 evaluable patients, and median survival was 12.7 months. By univariate analysis, Eastern Cooperative Oncology Group performance status (P = 0.0025), tumor grade (P = 0.03), percentage of MT staining (P = 0.01), and percentage-intensity index of MT staining (P = 0.04) were significant predictors of response to chemotherapy. The first three of these were significant in a multivariate model (P = 0.05, 0.04, and 0.04, respectively). By subgroup analysis, the percentage of MT staining predicted for response in metastatic disease (P = 0.03), but not in locally advanced disease (P = 0.28). Only performance status was significantly related to overall survival (P = 0.0001, log-rank test) in the whole cohort. Overexpression of MT in the 64 patients with metastatic disease was associated with a shorter survival (P = 0.04). Expression of p53, P-glycoprotein, and MIB-1 did not predict for survival. In conclusion, overexpression of MT is associated with a poorer outcome from chemotherapy, possibly due to cisplatin resistance.

INTRODUCTION

Bladder cancer accounts for projected estimates of 59,000 new cases and 13,050 deaths in North America in 1997 (1, 2). Despite the use of radical local treatments such as cystectomy, radiotherapy, or a combination of the two, about half of the patients with muscle-invasive bladder cancer relapse within 2 years of diagnosis (3, 4). The integration of adjunctive chemotherapy with surgery and/or radiation might improve patient outcome by eradicating micrometastases, although there is no definitive evidence as yet for improvement in survival from large randomized studies. Cisplatin-based chemotherapy regimens are used most widely and may be administered before, concurrently with, or after completion of local treatment. Identification of a subset of patients at the highest risk for recurrence would allow the delivery of multimodality therapy to those who would most likely benefit and avoid the unnecessary exposure of others to cytotoxic agents.

In patients who present with or develop metastatic bladder cancer, the prognosis is generally poor, with a median survival of about 1 year. Combination chemotherapeutic regimens such as MVAC3 or CMV are offered frequently to patients with good PS. In an intergroup study comparing MVAC to single-agent cisplatin, MVAC was found to be superior with respect to response rate, duration of remission, and overall survival (5). Good PS, no prior weight loss, and absence of metastases to the lung, liver, or bone were important prognostic factors for survival in a multivariate analysis. Although combination regimens offer a survival benefit in selected patients with metastatic bladder cancer, most patients who initially respond to therapy will develop and succumb to resistant disease. A better understanding of the relevant mechanisms of drug resistance would provide the basis for more rational and effective modulations of chemotherapy for bladder cancer.

Traditional pretreatment clinical and histological prognostic factors such as PS, tumor grade, and stage may not adequately predict patient outcome. Over the last decade, there has...
been increasing interest in the role of biological markers to stratify bladder cancers according to their behavior and chemosensitivity. The p53 tumor suppressor gene product regulates the cell cycle and is increased after DNA damage by genotoxic stress, resulting in arrest in the G1 phase of the cell cycle. It also mediates apoptosis induced by DNA-damaging chemotherapeutic drugs and ionizing radiation (6). Dysfunction of the p53 gene may therefore render cancer cells resistant to therapy. MT is a cysteine-rich low molecular weight protein involved in the homeostasis of essential metals and in the detoxification of toxic metals. It has been implicated in the resistance to alkylating agents and cisplatin (7). PGP is an ATP-dependent membrane efflux pump associated with multidrug resistance to a range of chemotherapeutic agents including the Vinca alkaloids and anthracyclines. MIB-1 is a cell cycle-related protein and serves as a marker of cell proliferative activity.

The present study sought to determine whether IHC assays of four biomarkers (p53, MT, PGP, and MIB-1) have independent prognostic value for tumor response and overall survival in patients with muscle-invasive urothelial TCC treated with cisplatin-based chemotherapy. Their associations with the conventional clinical and histological parameters are explored. This study also aims to provide further insight into the cellular determinants of response or resistance to drugs commonly used for this disease.

MATERIALS AND METHODS

Patients. The clinical data of 118 patients with primary urothelial TCC specimens available for IHC analysis were obtained by retrospective chart review and recorded by an observer who was unaware of the pathological information. All patients had muscle-invasive cancer and were treated with cisplatin-based chemotherapy at the Princess Margaret Hospital from January 1984 to June 1993. Demographic data and pretreatment characteristics of these patients are summarized in Table 1. More than 75% of the patients had a good baseline ECOG PS of 0 or 1. The urinary bladder was the site of the primary tumor in the majority of cases. Among the 118 tumors, most were poorly differentiated with a high histological grade. Specimens with pure adenocarcinoma, squamous cell carcinoma, or small cell carcinoma were excluded. Chemotherapy was administered for metastatic disease in 64 patients, for locally advanced disease in 45 patients, and as an adjuvant in 9 patients. Ninety-seven percent of patients received either of two commonly used cisplatin-based regimens, MVAC or CMV, at previously described conventional doses and schedules (8, 9). In cases in which more than one line of chemotherapy was delivered, only the tumor response to the first line of drug treatment was noted. Response to therapy was determined by physical examination, radiological investigation, or cystoscopic assessment and graded according to standard criteria (10).

Antibodies and Immunohistochemistry. Tumor-containing paraffin-embedded blocks or sections of the 118 cases were obtained. All specimens were of the primary urothelial tumor resected before any chemotherapeutic intervention, via either transurethral resection or cystectomy. The histological slides were reviewed to confirm the presence of tumor and graded according to the criteria of the WHO (11). The IHC analysis was performed by a single pathologist (R. J. K.) without knowledge of the tumor stage or the results of clinical follow-up. equivocal findings were resolved through consultation with a second pathologist (D. B.).

Paraffin sections were dewaxed in toluene and hydrated through graded alcohols to distilled H2O. Before incubation with the primary antibodies, the sections were treated in 3% H2O2 for 10 min to block endogenous peroxidase activities. Sections for MT staining were digested in 0.4% pepsin for 10 min at 42°C. p53, PGP, and MIB-1 slides were heated in citrate buffer (pH 6.0) inside a microwave pressure cooker for 30 mm. Slides were developed in AEC [0.0125% 3-amino-9-ethylcarbazole, w/v, in 0.2 M acetate buffer (pH 5.2)] for 20–30 min. Counterstaining was done in Mayer’s hematoxylin, and slides were coverslipped in Crystal Mount (Biomedca Corp., Foster City, CA).

The following primary antibodies and corresponding final working dilutions were used for the present study: anti-p53 monoclonal antibody D07 (Novocasta Laboratories, Ltd.,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Pretreatment characteristics of 118 patients with tumor specimens available for review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>No. of patients</td>
</tr>
<tr>
<td>Age, (yr) Median (range)</td>
<td>63 (38–79)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>89</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Primary Disease</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>103</td>
</tr>
<tr>
<td>Others*</td>
<td>15</td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>Disease type</td>
<td></td>
</tr>
<tr>
<td>Adjuvant</td>
<td>9</td>
</tr>
<tr>
<td>Locally advanced</td>
<td>45</td>
</tr>
<tr>
<td>Metastatic</td>
<td>64</td>
</tr>
<tr>
<td>Type of chemotherapy</td>
<td></td>
</tr>
<tr>
<td>MVAC</td>
<td>69</td>
</tr>
<tr>
<td>CMV</td>
<td>45</td>
</tr>
<tr>
<td>Othersb</td>
<td>4</td>
</tr>
</tbody>
</table>

* Renal pelvis (n = 10), ureter (n = 4), and urethra (n = 1).
* Methotrexate, doxorubicin, and cisplatin.
Fig. 1  a, TCC. Nuclear immunolocalization of p53 using anti-p53 monoclonal antibody D07. Magnification, $\times$100.  b, TCC. Cytoplasmic and nuclear immunolocalization of MT using anti-MT antibody E9. Magnification, $\times$250.  c, TCC. Cytoplasmic immunolocalization of PGP using anti-PGP monoclonal antibody JSB-1. Magnification, $\times$630.  d, TCC. Nuclear immunolocalization of proliferation marker MIB-1. Magnification, $\times$630.

Table 2  Distribution of the frequency of immunoreactivity for each of the four biomarkers, expressed as the number of tumor specimens in which $\leq$10 and $\geq$20% of the cells were positive for each marker

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>$\leq$10%</th>
<th>&gt;10%</th>
<th>$\leq$20%</th>
<th>$\geq$20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>28 (24%)</td>
<td>89 (76%)</td>
<td>42 (36%)</td>
<td>75 (64%)</td>
</tr>
<tr>
<td>MT</td>
<td>59 (53%)</td>
<td>53 (47%)</td>
<td>70 (63%)</td>
<td>42 (37%)</td>
</tr>
<tr>
<td>PGP</td>
<td>48 (41%)</td>
<td>69 (59%)</td>
<td>55 (47%)</td>
<td>62 (53%)</td>
</tr>
<tr>
<td>MIB-1</td>
<td>12 (10%)</td>
<td>105 (90%)</td>
<td>26 (22%)</td>
<td>91 (78%)</td>
</tr>
</tbody>
</table>

Table 3  Distribution of immunoreactivity frequency for each of the four biomarkers according to overall intensity of staining: 0, no staining; 1, weak staining; 2, moderate staining; and 3, heavy staining

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>8 (8%)</td>
<td>4 (4%)</td>
<td>67 (64%)</td>
<td>26 (25%)</td>
</tr>
<tr>
<td>MT</td>
<td>17 (16%)</td>
<td>8 (8%)</td>
<td>40 (39%)</td>
<td>39 (38%)</td>
</tr>
<tr>
<td>PGP</td>
<td>24 (23%)</td>
<td>29 (28%)</td>
<td>51 (49%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>MIB-1</td>
<td>3 (3%)</td>
<td>77 (73%)</td>
<td>25 (24%)</td>
<td></td>
</tr>
</tbody>
</table>

Three indices of immunoreactivity were generated for each of the four biomarkers. An objective index was obtained by selecting 400 tumor cells at random per slide, and immunoreactivity was expressed by the percentage of positively staining cells (percentage of the marker). A semiquantitative index was obtained by evaluating the overall intensity of immunostaining and grading it on a scale from 0 to 3: 0, no staining; 1, weak staining; 2, moderate staining; and 3, heavy staining. Finally, the product of these two measurements was calculated to produce a percentage-intensity index. Tables 2 and 3 summarize the distribution of the immunoreactivity frequency for each of the four biomarkers according to the percentage of positively staining cells and overall intensity, respectively.

Statistical Analysis. For clinicopathological variables, patients were classified into three disease types according to their management strategies: (a) adjuvant; (b) locally advanced; and (c) metastatic. In the response analysis, complete and partial response categories were grouped together as nonresponse. For ECOG PS, patients were categorized as ECOG 0, 1, 2, or 3, and an eight-cell contingency table was established using these four categories and response versus no response. For biomarker variables, the percentage of the marker was assessed as continuous data in the response analysis. In the survival analysis, a cutoff point of 10% immunostaining was used where $\leq$10% represented immunonegativity and $>10\%$ represented immunopositivity. This criterion was selected based on previously reported (12) correlation between mutations in the p53 gene and the accumulation of p53 protein in over 10% of the tumor cell nuclei. The same cutoff point was used for the other three biomarkers, because no previous data exist to suggest an appropriate discriminating value. Other cutoff points (20 and 50%) were examined in an exploratory analysis for potential use in future studies. Survival was calculated from the date of initiation of chemotherapy to the date of death or censoring.

Contingency tables, Pearson’s $\chi^2$ test and logistic regression (13) were used to explore in univariable and multivariable analysis the relationship between biomarker and clinicopathological variables and response to chemotherapy. Statistical significance was reported using two-sided Ps, their corresponding ORs, and 95% CI for the ORs. The correlation among biomarker...
variables was explored using correlation coefficient analysis. The Kaplan-Meier method (14) was used to derive the survival function. The log-rank test was used to determine whether overexpression of each of the biomarkers, age, gender, PS, and tumor grade correlated with survival.

RESULTS

Response to Chemotherapy. Ninety-nine of 118 patients had disease that could be assessed for response. Of the remaining 19 patients, 9 received chemotherapy on an adjuvant basis, whereas the others had nonevaluable lesions such as bony metastasis. Among the 99 evaluable patients, 13 patients (13%) achieved a complete response, and 43 patients (43%) achieved a partial response, resulting in an overall response rate of 56%. Clinicopathological parameters including age, gender, PS, disease type, and tumor grade as well as biomarker variables including percentage staining, overall intensity, and percentage-intensity index for each of the four biomarkers were examined for their role in predicting tumor response. By univariate analysis using logistic regression, good PS ($P = 0.0025$; OR = $2.62$; 95% CI, 1.45–5.12), low percentage of MT staining ($P = 0.01$; OR = $1.02$; 95% CI, 1.00–1.03), high tumor grade ($P = 0.03$; OR = $0.26$; 95% CI, 0.07–0.79), and low percentage-intensity index of MT staining ($P = 0.04$; OR = $1.005$; 95% CI, 1.00–1.01) were significant positive predictors of response to chemotherapy. Only the first three of these factors were significant independent predictors of response in a multivariate model. PS ($P = 0.05$; OR = $2.18$; 95% CI, 1.05–4.96); percentage of MT staining ($P = 0.04$; OR = $1.07$; 95% CI, 1.01–1.16); and tumor grade ($P = 0.04$; OR = $0.26$; 95% CI, 0.07–0.86)) were significant positive predictors of response to chemotherapy. The semi-quantitative percentage-intensity index of MT staining did not demonstrate independent prognostic value. By subgroup analysis, all of the three significant prognostic factors in multivariate analysis (percentage of MT staining, tumor grade, and PS) seemed to be more predictive of response in patients with metastatic as compared to locally advanced disease [percentage of MT staining (locally advanced disease, $P = 0.28$; metastatic disease, $P = 0.03$); tumor grade (locally advanced disease, $P = 0.57$; metastatic disease, $P = 0.04$); and PS (locally advanced disease, $P = 0.96$; metastatic disease, $P = 0.07$)].

An exploratory analysis looking at the ability of the biomarkers to predict complete response was also undertaken, although the small number of complete responses (13) made statistical analysis of doubtful validity. The results were similar to that seen when complete responses and partial responses were combined as described above.

Correlation Analysis. Linear regression analysis was used to test for any correlation between individual pairs of the biomarkers under study. Correlation was observed between the percentage of MT staining and the percentage of MIB-1 staining ($r = 0.32; P = 0.005$) as well as between the percentage of PGP staining and the percentage of MIB-1 staining ($r = 0.22; P = 0.02$). No correlation was found between PS or tumor grade with any of the four biomarkers.

Survival Analysis. At the time of reporting, 19 patients (16%) were alive, and 99 patients (84%) were dead. The median survival duration for the entire cohort of 118 patients was 12.7 months. When separated by stage of disease, the median duration of survival for patients with adjuvant disease, locally advanced disease, and metastatic disease was 27.8, 17.4, and 10.4 months, respectively.

For the entire cohort of 118 patients, only PS was related significantly to overall survival ($P = 0.0001$; Fig. 2), whereas expression of p53, MT, PGP, and MIB-1 were not predictive of overall survival, regardless of stratification by either percentage of staining or overall intensity. By subgroup analysis, overexpression of MT (>10% immunostaining) was associated with poorer overall survival in patients with metastatic disease ($P = 0.04$; Fig. 3). There was no difference in outcome when cutoff points of 20 and 50% were used for the four biomarkers in the survival analysis.

DISCUSSION

This study confirms that PS is a paramount factor in determining response to chemotherapy and survival for patients with muscle-invasive urothelial TCC treated with cisplatin-based chemotherapy. As seen in many other tumor sites, patients’ pretreatment functional capacity is a more reliable indicator of their disease outcome than physical variables such as age or gender. With respect to pathological classification, high-

![Fig. 2](image1.png)  
*Fig. 2* Probability of survival in patients ($n = 118$) with urothelial TCC according to ECOG PS.

![Fig. 3](image2.png)  
*Fig. 3* Probability of survival in patients ($n = 59$) with metastatic urothelial TCC according to expression of MT ($\leq 10\%$ versus $>10\%$).
grade tumors had better responses to chemotherapy than did those of lower grade in the present study. Over 80% of the specimens were classified as grade 3, and only 2% were grade 1; this skewed distribution makes it difficult to conclusively associate tumor differentiation with treatment response. However, it is possible that poorly differentiated tumors with a rapid rate of cell proliferation respond better to chemotherapy, due to the effective killing of actively cycling tumor cells (15).

Among the four biomarkers evaluated in this study of primary bladder cancers, the prognostic significance of p53 overexpression has been reported most extensively in the literature. The p53 tumor suppressor gene, located on chromosome 17p13.1, encodes for a 53-kDa nuclear phosphoprotein. Mutations at this locus are among the most frequent genetic defects found in human malignant tumors (16), including bladder cancers (17). Inactivation of the p53 gene can occur by mutation or deletion, and structural alterations are often accompanied by the loss of heterozygosity of alleles on chromosome 17p (6, 17). Also, cellular or viral proteins have been shown to bind to the p53 protein and abrogate its normal function (18).

Mutant p53 protein complexes with a heat shock protein to form a metabolically stable product and therefore possesses a prolonged half-life in comparison to its wild-type counterpart (19). This longer half-life results in the accumulation of mutant p53 protein in the nucleus, allowing its detection by IHC assay. In bladder cancer, nuclear overexpression of p53 demonstrated strong association with mutations in the p53 gene (18). This longer half-life results in the accumulation of mutant p53 protein complexes with a heat shock protein to form a metabolically stable product and therefore possesses a prolonged half-life in comparison to its wild-type counterpart (19). This longer half-life results in the accumulation of mutant p53 protein in the nucleus, allowing its detection by IHC assay. In bladder cancer, nuclear overexpression of p53 demonstrated strong association with mutations in the p53 gene (18).

Table 4 Summary of recent studies of the prognostic significance of p53 overexpression measured by IHC

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Specimen/stage(s)</th>
<th>Assay and antibody/immunoreactivity cutoff</th>
<th>Results</th>
<th>Significance in multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas et al. (Ref. 22)</td>
<td>21</td>
<td>Bx/pT1</td>
<td>IHC with CM-1/cutoff = 10%</td>
<td>N/S</td>
<td>DFS+</td>
</tr>
<tr>
<td>Gardiner et al. (Ref. 23)</td>
<td>28</td>
<td>Bx/pT1</td>
<td>IHC with CM-1, PAB1801, D07/6 groups of immunoreactivity</td>
<td>N/S</td>
<td>DFS+</td>
</tr>
<tr>
<td>Sarkis et al. (Ref. 24)</td>
<td>43</td>
<td>Bx/pT1</td>
<td>IHC with PAB1801/cutoff = 20%</td>
<td>+</td>
<td>DFS, OS</td>
</tr>
<tr>
<td>Nakopoulou et al. (Ref. 25)</td>
<td>87</td>
<td>Bx/T2-T4 (n = 45); T2-T4 (n = 42)</td>
<td>IHC with PAB1801/cutoff = 10%</td>
<td>-</td>
<td>DFS, OS</td>
</tr>
<tr>
<td>Sarkis et al. (Ref. 26)</td>
<td>90</td>
<td>Bx/T2-T4</td>
<td>IHC with PAB1801/cutoff = 20%</td>
<td>+</td>
<td>DFS, OS</td>
</tr>
<tr>
<td>Soini et al. (Ref. 27)</td>
<td>42</td>
<td>Bx/cystx/all stages</td>
<td>IHC with CM-1/5 groups of immunoreactivity</td>
<td>+</td>
<td>DFS, OS</td>
</tr>
<tr>
<td>Lipponen et al. (Ref. 28)</td>
<td>212</td>
<td>Bx/all stages</td>
<td>IHC with CM-1/cutoff = 20%</td>
<td>+</td>
<td>DFS, OS</td>
</tr>
<tr>
<td>Esrig et al. (Ref. 21)</td>
<td>243</td>
<td>Cystx/all stages</td>
<td>IHC with PAB1801/cutoff = 10%</td>
<td>+</td>
<td>DFS, OS</td>
</tr>
</tbody>
</table>

* DFS, disease-free survival.
* OS, overall survival.
* Bx, biopsy.
* +, nonsignificant; pT1, pathological stage T1.
* P not given.
* Cystx, cystectomy.

Mutant p53 protein complexes with a heat shock protein to form a metabolically stable product and therefore possesses a prolonged half-life in comparison to its wild-type counterpart (19). This longer half-life results in the accumulation of mutant p53 protein in the nucleus, allowing its detection by IHC assay. In bladder cancer, nuclear overexpression of p53 demonstrated strong association with mutations in the p53 gene identified by sequencing analysis (12).

Using DNA sequencing analysis, mutations of the p53 gene have been detected in a high percentage of invasive TCC of the bladder (17), but less frequently in noninvasive papillary and superficial tumors (12, 20). This suggests that p53 mutations may be one of the important biological events in the multistep process of tumor progression. Studies have attempted to determine the relationship between nuclear accumulation of p53 protein and clinical outcome in patients with bladder cancer (Table 4). In the largest of these series, Esrig et al. (21) concluded that overexpression of p53 in the tumor cell nuclei as detected by IHC methods predicts for increased risk of recurrence and death independently of tumor grade, stage, and lymph node status. Nuclear p53 reactivity was in fact the only independent predictor of recurrence and overall survival in patients with TCC confined to the bladder, suggesting that this subgroup may benefit from adjuvant therapy. In some studies, however, overexpression of p53 oncoprotein had no independent prognostic value. From Table 4, it seems that positive immunostaining with the mouse monoclonal anti-p53 antibody PAB1801 was more predictive of clinical outcome. Other antibodies to p53 gene products, which include the polyclonal antibody CM-1 and the antibody D07 used in this study, are perhaps less discriminatory in their predictive functions. Furthermore, the absence of nuclear reactivity does not rule out mutation of the p53 gene. Rarely, genetic aberrations such as nonsense mutations may yield truncated p53 proteins that lack the nuclear localization signal (26). Inactivation of p53 wild-type gene products may also occur through complex formation with cellular or viral proteins, and such functional impairment would not be detected by IHC methods. Although p53 immunostaining might miss p53 mutations that result in a lack of expression of the protein, studies in bladder cancer have demonstrated that this is infrequent, with only 2–7% of patients who have mutations by DNA sequencing being negative by immunohistochemistry. (12, 29). Overall, between 6 and 15% of tumors with negative IHC analysis may have p53 mutations.
Thus, given that only 8% of our specimens had negative staining for p53, only about 1% of the cases may represent a false negative with a missed mutation.

MT was the only biomarker demonstrated to be an independent predictor of tumor response in the present study. MTs are a family of cytosolic proteins rich in sulphydryl-containing cysteine residues, whose major physiological function seems to involve the absorption, transport, and metabolism of essential trace metals such as copper and zinc. In addition, they play a role in the detoxification of heavy metals such as cadmium (30). They are normally found at low concentrations in various tissues but are readily inducible by a variety of stimuli, including steroids, heavy metals, and lymphokines (31). Experimental evidence has linked the overexpression of cellular MT with resistance to alkylating agents and cisplatin (7), but the precise mechanism of action is unknown. Various potential mechanisms have been postulated, which include direct binding of MT to cisplatin, free radical scavenging, and alteration of the body metal levels that may be important in the formation of lethal cisplatin-DNA cross-links (32).

There have been few reports addressing the prognostic role of MT in urothelial cancers. Bahson et al. (32) evaluated the prechemotherapy transurethral resection biopsy specimens of 21 patients with locally advanced bladder cancer who received subsequently neoadjuvant treatment with the CMV regimen. Eight of these patients had no detectable immunostaining for MT and were more likely to sustain a complete pathological response after chemotherapy than those with any detectable degree of staining. There was no significant relationship between MT expression and overall survival or cancer-specific survival in this study, but the small number of patients made it impossible to draw any firm conclusions. Our present study in 118 patients complements these findings, suggesting that MT may be of prognostic importance in predicting tumor response to cisplatin-based chemotherapy. Although there are other known mechanisms of cisplatin resistance, further study of the contribution of MT to tumor cell resistance might improve treatment strategies.

By subgroup analysis, overexpression of MT in patients with metastatic disease was associated with poorer overall survival. Given the smaller number of patients included in this subset, these observations should be regarded as hypothesis generating. It is possible that in the setting of locally advanced disease, MT-mediated tumor resistance did not influence survival outcome, because definitive treatment with surgery and/or radiotherapy was generally available for salvage. On the other hand, chemotherapy was the mainstay of therapy in the metastatic setting, such that patients with drug-resistant tumors were destined to fare poorly.

PGP is not constitutively expressed in normal urothelium and is detected at diagnosis in about one-third of untreated human bladder cancers (33, 34). Up-regulation in the proportion of tumor cells expressing PGP occurs after exposure to combination chemotherapy regimens containing drugs known to select for PGP expression in vitro (35). The lack of correlation between PGP expression and clinical outcome seen in the present study is consistent with the results of others (36, 37). Because multidrug resistance is a complicated phenomenon, the presence of other factors such as the non-PGP-mediated multidrug resistance-associated protein may partly explain this phenomenon.

Overexpression of MT and PGP are both correlated with an increased MIB-1 proliferative index, suggesting that rapidly dividing tumors may have increased levels of these markers of intrinsic drug resistance. However, as noted previously, high-grade tumors in the present study seemed to have better responses to therapy. Therefore, tumor response and chemosensitivity are likely the result of an intricate interplay of multiple factors. Similar to Ki-67, MIB-1 immunostaining is a measure of cell proliferative activity, but it can be performed readily on paraffin-embedded sections without requiring fresh or frozen tissues. The Ki-67 index reflects the biological aggressiveness of tumors and has been shown to correlate with known prognostic factors such as tumor grade and stage (38–40) in bladder carcinoma. The independent prognostic value of these proliferative markers has yet to be determined.

In conclusion, the strongest predictive factor for response and survival in patients with urothelial cancer treated with cisplatin-based combination chemotherapy is baseline PS. Nuclear p53 reactivity, PGP expression, and cell proliferation as measured by the marker MIB-1 were not associated with response to chemotherapy or survival. The overexpression of MT in primary tumor specimens is associated with a poorer outcome from chemotherapy, possibly due to cisplatin resistance. A greater understanding of the factors associated with drug resistance in patients with urothelial cancer will assist in the development of better systemic therapies for this disease.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of E. Lee in the development of the database of patients used in this study, and we thank Dr. P. Ng for advice on the statistical analyses in this study.

REFERENCES

expression of p53 in primary pT1 transitional cell bladder cancer in
73:
23. Gardiner, R. A., Walsh, M. D., Allen, V., Rahman, S., Samaratunga,
overexpression of p53 protein in transitional cell bladder carcinoma: a
men for metastatic transitional cell carcinoma of the urinary tract: a
Harker, W., Meyers, F. J., Freiha, F. S., Palmer, J. M., Shortliffe,
36. Sandlow, J., Cohen, M. B., Robinson, R. A., Dreicer, R., and
35. Petrylak, D. P., Scher, H. I., Reuter, V., O’Brien, J. P., and
33. Bahnson, R. R., Becich, M., Ernstoff, M. S., Sandlow, J., Cohen,
32. Bahnson, R. R., Becich, M., Ernstoff, M. S., Sandlow, J., Cohen,
31. Kagi, J. H. R., and Kojima, Y. (eds.). Metallothionein. II. Proceed-
29. Cardon-Cardo, C., Dalbagni, G., Saez, G. T., Oliva, M. R., Zhang,
28. Lipponen, P. K. Over-expression of p53 nuclear oncoprotein in
27. Soini, Y., Tsurupeniemi-Hujanen, T., Kallman, D., Autoio-Harmanen,
26. Sarkis, A. S., Bajorin, D. F., Reuter, V. E., Herr, H. W., Metto, G.,
25. Nakopoulos, L., Constantinides, C., Papandropoulos, J., Theodorop-
24. Sarkis, A. S., Dalbagni, G., Cardon-Cardo, C., Zhang, Z-F., Sheinfeld,
23. Esrig, D., Spruck, C. H., III, Nichols, P. W., Chiwun, B., Steven,
22. Bahnson, R. R., Becich, M., Ernstoff, M. S., Sandlow, J., Cohen,
17. Flesher, J. H. R., and Kojima, Y. (eds.). Metallothionein. II. Proceed-
16. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53
14. Kaplan, E. L., and Meier, P. Nonparametric estimation from incom-
13. Fienberg, S. E. The Analysis of Cross-Classified Categorical Data,
11. Mostofi, F. K. Histological typing of urinary bladder tumors. In:
10. Oken, M. M., Creech, R. H., Tormey, D. C., Horton, J., Davis, T. E.,
8. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53
7. Sarkis, A. S., Dalbagni, G., Cordon-Cardo, C., Zhang, Z-F., Schultz,
6. Sandlow, J., Cohen, M. B., and Williams, R. D. Absence of immunohistochemical metallo-
thionein staining in bladder tumor specimens predicts response to neo-
5. Vahakangas, K. p53 immunohistochemistry in transitional cell carcinoma and dysplasia of
3. Bahnson, R. R., Becich, M., Ernstoff, M. S., Sandlow, J., Cohen,
2. Esrig, D., Spruck, C. H., III, Nichols, P. W., Chiwun, B., Steven,
1. Harker, W., Meyers, F. J., Freiha, F. S., Palmer, J. M., Shortliffe,
The prognostic role of p53, metallothionein, P-glycoprotein, and MIB-1 in muscle-invasive urothelial transitional cell carcinoma.


Updated version  Access the most recent version of this article at:  
http://clincancerres.aacrjournals.org/content/4/3/559

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.