Tissue Inhibitor of Metalloproteinase-2 as a Multifunctional Molecule of Which the Expression Is Associated with Adverse Prognosis of Patients with Urothelial Bladder Carcinomas

Hariklia Gakiopoulou, Lydia Nakopoulou, Argyris Siatelis, Ioannis Mavrommatis, Effie G. Panayotopoulou, Ioanna Tsirmpa, Constantinos Stravodimos, and Aris Giannopoulos


ABSTRACT

Purpose: Tissue inhibitors of metalloproteinases (TIMPs) regulate matrix metalloproteinase (MMP) activity controlling the breakdown of extracellular matrix components and, thus, play an important role in the process of invasion and metastasis. Moreover, there are several new functions, growth control, apoptosis, and angiogenesis, in which TIMPs seem to be involved. The aim of this study was to elucidate the role of TIMP-2 in human urothelial cancer assessing TIMP-2 protein expression in 106 urothelial bladder carcinomas and evaluating its importance relative to clinicopathologic parameters (age, gender, histological grade, and stage) and patient survival, as well as to markers associated with cell growth and apoptosis (Ki-67, p53, and bcl-2).

Experimental Design: Immunohistochemistry (avidin-biotin complex method-horseradish peroxidase) was performed to detect TIMP-2, Ki-67, p53, and bcl-2 proteins using monoclonal and polyclonal antibodies. Statistical analysis was univariate and multivariate.

Results: TIMP-2 immunohistochemical expression was observed in stromal fibroblasts and in cancerous cells in 26.4% and 69.8% of cases, respectively. TIMP-2 stromal but not cancerous cell expression associated significantly with the high histological grade of carcinomas (P < 0.0001) and the advanced stage of the disease (P = 0.001). TIMP-2 either stromal or cancerous cell expression correlated significantly with the expression of Ki-67 proliferation index (P = 0.02 and P = 0.044, respectively) and the mutant p53 protein (P = 0.043 and P = 0.045, respectively). In univariate survival analysis patients with positive TIMP-2 stromal cell immunohistochemical expression had a significantly worse overall survival in comparison with TIMP-2 stromal cell-negative patients (log rank test: P = 0.0002). However, in multivariate survival analysis the only independent survival factors were the stage of the disease and patient age.

Conclusions: TIMP-2 protein expression in either the stromal or cancerous cells is associated with the proliferation index Ki-67 and the apoptosis-related protein p53. These findings are in keeping with in vitro studies reporting a growth-promoting ability of TIMP-2 and its involvement in apoptosis regulation. On the other hand, TIMP-2 stromal cell expression only was associated with adverse prognosis of urothelial bladder cancer patients.

INTRODUCTION

To invade and metastasize, cancer cells must penetrate the ECM barriers in a process involving, among other factors, the proteolytic degradation of ECM components (1). MMPs consist of a family of at least 16 structurally related enzymes capable of degrading ECM components (2). Once activated (3), the MMPs are subject to control by endogenous inhibitors such as α2 macroglobulin and more specifically by TIMPs (4). Four different TIMPs have been described: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (4). TIMPs are low molecular weight secreted proteins that bind to the active form of the MMPs at a 1:1 stoichiometric ratio, inhibiting enzymatic activity (4, 5).

TIMP-2 is a Mr 21,000 protein that forms a noncovalent stoichiometric complex with both the latent and active forms of MMP-2 (6). Although in general TIMPs inhibit MMPs, TIMP-2 binds selectively to pro-MMP-2 (6, 7). It has been reported that although the NH2-terminal domain of TIMP-2 binds to the NH2-terminal domain of active MMP-2, the COOH-terminal domain of TIMP-2 binds specifically to the COOH-terminal domain of pro-MMP-2 (8). This pro-MMP-2/TIMP-2 complex can still be activated and shows proteolytic activity (9). There is also evidence that MT-MMP acts as a receptor for TIMP-2 and can complex with pro-MMP-2 leading to subsequent activation of pro-MMP-2 (10). According to previous studies, MMP-2 protein seems to play an important role in urothelial cancer progression, because its levels have been associated with high-

Received 11/9/02; revised 4/10/03; accepted 4/21/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Dr. Lydia Nakopoulou, Department of Pathology, University of Athens–Medical School, 75, M. Asias str., 115 27 Athens, Greece. Phone: 30-210-7489649; Fax: 30-210-7462157; E-mail: lnakopou@cc.uoa.gr.

2 The abbreviations used are: ECM, extracellular matrix; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; MT, membrane type; ABC, avidin biotin-peroxidase complex; df, degrees of freedom.
grade and high-stage carcinomas, as well as with recurrence of patients with advanced urothelial carcinomas after complete resection (11, 12).

From the above mentioned data, it is evident that much progress has been made in explaining the interactions between TIMP-2 and MMPs. However, these interactions still remain a controversial matter, and this controversy is reflected in the results of studies investigating the role of TIMP-2 expression in the progression of various carcinomas. At first, TIMP-2 was considered as a suppressor of invasion and metastasis because of the general concept that TIMPs inhibit MMPs (13–16). However, the complexity of TIMP-2/MMP interactions in combination with the multiple new functions that have been attributed to TIMPs, for instance, cell growth control, relation to apoptosis, and angiogenesis (17–20), have led to a reconsideration of the role of TIMP-2 in cancer (21). In fact, there are several recent studies demonstrating a relation between TIMP-2 expression and progression of carcinomas (21–25).

In view of the above, we investigated TIMP-2 immunohistochemical expression in a series of 106 bladder carcinomas in relation with clinicopathological parameters and patient survival. Moreover, prompted by studies pointing out the multiple functions of TIMPs (17–20), we investigated, for the first time in bladder cancer, the possible correlations between TIMP-2 expression and the Ki-67 proliferation indice, the inhibitory of apoptosis bcl-2 protein, and the mutant p53 protein.

**PATIENTS AND METHODS**

**Patients and Tumor Specimens.** Specimens were obtained from 106 patients with urothelial bladder carcinomas. The age of patients ranged from 42 to 89 years (mean age: 71 years) and the male:female ratio was 92:14. Transurethral biopsy was performed in all of the patients. None of the patients had received prior chemotherapy, intravesical instillation therapy, or radiation. Ta and T1 tumors were treated by transurethral resection and intravesical instillations of either bacillus Calmette-Guerin or epirubicin, whereas for muscle-invasive carcinomas, cystectomy, radiation, or systemic chemotherapy was used according to our clinical protocols.

Urothelial carcinomas were graded histologically according to the criteria of the Ancona 2001 Refinement of the WHO 1973 classification (Ref. 26; Table 1). Pathological staging of Ta-T1 tumors was based on the results of transurethral resection. All of the T1 tumors of this study have had muscle in the specimen. Tumor staging was performed based on the results of excreting pyelography, transurethral biopsy and resection, bimanual palpation under anesthesia, computed tomography, and ultrasonography, and was assessed according to the criteria of the International Union Against Cancer Staging System (27). For patients with muscle-invasive tumors who underwent cystectomy, tumor stage was confirmed by cystectomy. Finally, 22 patients underwent cystectomy, whereas the remainder 41 tumors were muscle-invasive urothelial carcinomas (20 T2, 15 T3, and 6 T4; Table 1).

Survival analysis was based on data available from 103 patients. The follow-up of patients ranged from 2 months to 144 months with a median follow up of 43 months (interquartile range: 17 months-71 months). Thirty-nine of 103 patients died from the disease (cause-specific deaths) during the observation period.

**Immunohistochemistry.** All of the immunohistochemical analyses were performed on routinely processed formalin-fixed, paraffin-embedded 4-µm thick tissue sections. The im-

---

**Table 1** TIMP-2 immunohistochemical expression in cancerous cells and stromal cells in relation with clinicopathological parameters (gender, histological grade, stage), Ki-67, p53, and bcl-2 immunohistochemical expressions.

<table>
<thead>
<tr>
<th></th>
<th>TIMP-2 cancerous cells</th>
<th>TIMP-2 in stromal cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>92</td>
<td>40</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ta–T1</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td>T2–T3–T4</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>56</td>
<td>31</td>
</tr>
<tr>
<td>Positive</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>p53&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>49</td>
<td>28</td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>bcl-2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>a</sup> NS, not significant.

<sup>b</sup> Total number of cases stained for p53 and bcl-2 was 105 and 104, respectively.
munohistochemical demonstration of TIMP-2 was achieved using the avidin-biotin immunoperoxidase method as described previously (28). A monoclonal antibody against TIMP-2 (Medicorp, Montreal, Quebec, Canada) was used at a dilution of 1:200. The clone used was the 67–4H11, corresponding to the oligopeptide YRGAAPRKQELDIED (residue 178–193) of the human TIMP-2.

The other immunomarkers assessed in this study in combination with TIMP-2 (p53, Ki-67, and bcl-2) have also been detected immunohistochemically with the following antibodies: (a) anti-p53, clone BP53.12.1 (Oncogene, Cambridge, MA) at a dilution of 1:50; (b) rabbit antihuman Ki-67 (DAKO, Glostrup, Denmark) at a dilution of 1:200; and (c) anti-bcl-2 clone 124 (DAKO) at a dilution of 1:100. A standard ABC method (Vectastain Elite, Vector Laboratories, Burlingame, CA) was used for visualization with diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin and mounted.

To enhance antigen retrieval for p53, Ki-67, and bcl-2, sections were microwave-treated in 0.01 M citrate buffer (pH 6.0) at 700 Watt (two cycles of 5 min for each antigen). Positive controls for TIMP-2 included tissue from breast carcinomas with known immunoreactivity for TIMP-2 (29), whereas positive controls for p53, Ki-67, and bcl-2 included bladder cancer tissue with known immunoreactivity for the above markers (30). Negative controls had the antibodies replaced by PBS.

**Evaluation of Immunohistochemistry.** A semiquantitative evaluation of TIMP-2 immunostaining in either the stromal or cancerous cells was carried out by two independent observers according to the percentage of positive cells. Staining intensity was analogous to the percentage of positive cells. Thus, we established the cutoff of 10% as a point to separate negative from positive tumors, because the former group included tumors either totally negative or tumors with immunoreactivity of weak intensity in a few cells. In contrast, positive tumors demonstrated a clear-cut, moderate immunoreactivity in ≧10% of cells, which was even stronger in tumors with positive reaction in ≧40% of cells. Using these criteria both observers agreed about the positivity or negativity of each case and the degree of positivity in the positive cases. Precisely, tumors were scored using a scale of 0–2 as follows: score 0: <10%, positive cells per 10 high power fields (×400), score 1: 10–40% positive cells, and score 2: >40% positive cells per 10 high power fields (×400). For purposes of statistical analysis TIMP-2-positive cases formed one group.

The extent of Ki-67 expression was evaluated using a score of 0–3 as follows: score 0: ≤10% positive cells per 10 high power fields (×400), score 1: 11–30% positive cells, score 2: 31–50% positive cells, and score 3: >50% positive cells, as reported previously (30). Bcl-2 expression was scored as negative (score 0) if <10% of tumor cells were positive, slightly positive (score 1) if 10–50% of tumor cells were positive, and as strongly positive (score 2) if >50% of neoplastic cells showed cytoplasmic staining, as described previously (30). The fraction of p53-positive stained nuclei was scored in a scale of 0–3 as follows: 0 (negative) = <10% of positive tumor nuclei, 1 = 10–25% positive tumor nuclei, 2 = 26–50% of positive tumor nuclei, and 3 = >50% of positive tumor nuclei as described previously (31).

**Statistical Analysis.** Pearson’s χ² test with continuity correction and Mann-Whitney test was used to assess correlations between TIMP-2 stromal or cancerous cell expression and clinicopathological parameters of bladder carcinomas, as well as p53, Ki-67, and bcl-2 expressions. The association between stromal cell and cancerous cell TIMP-2 expression was investigated through the McNeamar’s test. Multivariate logistic regression analysis was performed to evaluate which of the above parameters correlated independently with TIMP-2 expression. The effect of TIMP-2 expression on postoperative survival rates was assessed by univariate analysis (log rank test) and multivariate analysis (Cox proportional hazard regression model). A P ≤ 0.05 was considered as statistically significant.

**RESULTS**

In the present study, positive TIMP-2 immunohistochemical expression was observed in stromal fibroblasts in 26.4% of cases and in cancerous cells in 69.8% of cases (Figs. 1 and 2). In cancerous cells, TIMP-2 protein was localized in the cytoplasm demonstrating a granular pattern of expression (Fig. 1).
whereas in some cases a nuclear localization was also observed.
A statistically significant difference was observed between
TIMP-2 stromal and cancerous cell expression (McNeamar’s
test: \( P < 0.0001 \)).
TIMP-2 immunohistochemical expression in either the
cancerous or stromal cells did not associate with patient age
(Mann-Whitney test: \( P = 0.64 \) and \( P = 0.96 \), respectively) and
gender (\( P = 0.50 \), \( \chi^2 = 0.44 \) and \( P = 1.00 \), \( \chi^2 = 0.0001 \),
respectively; Table 1). On the other hand, TIMP-2 stromal cell
immunohistochemical expression demonstrated a statistically

---

**Fig. 3**  Schematic representation of TIMP-2 stromal cell expression in relation to A. histological grade; B. stage of the disease and TIMP-2 either stromal or cancerous cell expression in relation to Ki-67 proliferation indice (C and D) and p53 mutant protein (E and F).
Table 2  Multivariate logistic regression analysis of TIMP-2 stromal cell expression: among the various parameters included in the analysis, the model demonstrated a statistical significant association between TIMP-2 stromal cell expression and the histological grade of urothelial carcinomas

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(B)</th>
<th>95% confidence interval for Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>2.095</td>
<td>0.546</td>
<td>14.732</td>
<td>1</td>
<td>0.0001</td>
<td>8.124</td>
<td>2.787, 23.679</td>
</tr>
<tr>
<td></td>
<td>-6.342</td>
<td>1.533</td>
<td>17.116</td>
<td>1</td>
<td>0.0001</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

B. Variables not in the equation

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step</td>
<td>Variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Gender</td>
<td>0.001</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.857</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ki-67</td>
<td>0.936</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>bcl-2</td>
<td>0.298</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stage</td>
<td>1.487</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>p53</td>
<td>1.263</td>
<td>1</td>
</tr>
<tr>
<td>Overall statistics</td>
<td>4.311</td>
<td>6</td>
<td>0.635</td>
</tr>
</tbody>
</table>

The above mentioned significant associations between TIMP-2 either stromal or cancerous cell expression prompted us to investigate coexpression patterns of these proteins in relation with histological grade and stage. Simultaneous positive expression of TIMP-2 in stromal cells, Ki-67 and p53 [TIMP-2(+)/Ki-67(+)/p53(+)] was observed in 13 cases of which 12 (92.3%) were grade III carcinomas and 8 (61.5%) were muscle-invasive carcinomas. On the other hand, simultaneous negative expression [TIMP-2(-)/Ki-67(-)/p53(-)] of all of the three proteins was found in 28 cases of which 5 (17.8%) were grade III carcinomas and only 3 (10.7%) were muscle-invasive carcinomas. The rest of the cases demonstrated positivity for two of the above proteins or for only one protein. Coexpression of TIMP-2 in stromal cells with Ki-67 and p53 in cancerous cells associated significantly with the higher histological grade and stage of urothelial carcinomas ($P < 0.0001$ and $P < 0.0001$, respectively).

Survival analysis demonstrated that patients with positive TIMP-2 stromal cell immunohistochemical expression had a significantly worse overall survival in comparison with TIMP-2 stromal cell negative patients (log rank test: $P = 0.0002$; $\chi^2 = 13.51$). Twenty-two of 75 (29.3%) patients with negative TIMP-2 stromal cell expression were dead at the end of the follow-up period, whereas 17 of 28 (60.7%) patients with positive TIMP-2 stromal cell expression were dead at the end of the same period. Mean survival time of TIMP-2-negative patients was 103 months, whereas mean survival time of TIMP-2-positive patients was 40 months. Kaplan-Meier survival curves of patients according to positive or negative TIMP-2 stromal cell expression are shown in Fig. 4. TIMP-2 cancerous cell immunohistochemical expression did not associate significantly with patient survival. Multivariate Cox proportional hazard regression analysis, adjusted for all of the parameters investigated in this study, demonstrated as the only independent survival factors, the stage of the disease ($P < 0.0001$, $B = 1.046$, $SE = 0.158$, Wald = 43.665, $df = 1$, Exp(B) = 2.846, and 95% confidence interval for Exp(B) = 2.087–3.881) and the age of...
patients \( P < 0.0001, B = 0.081, SE = 0.018, \text{Wald} = 19.386, \ df = 1, \ Exp(B) = 1.084, \text{and 95% confidence interval for} \ Exp(B) = 1.046–1.124 \).

Moreover, in an attempt to determine the possible prognostic value of the different coexpression patterns among TIMP-2, p53, and Ki-67 we performed multivariate Cox proportional hazard regression analysis adjusted for the following parameters: age, gender, grade, stage, bcl-2 expression, stromal TIMP-2 expression, cancerous TIMP-2 expression, simultaneous positive expression of stromal TIMP-2, p53, and Ki-67 [TIMP-2 St(+)/p53(+)/Ki-67(+)], simultaneous negative expression of all these markers [TIMP-2 St(-)/p53(-)/Ki-67(-)], negative stromal TIMP-2 expression with positivity of both p53 and Ki-67 [TIMP-2 St(-)/p53(+)/Ki-67(+)], and negative stromal TIMP-2 with positivity of either p53 or Ki-67 [TIMP-2 St(-)/p53(+) or Ki-67(+)]. However, the only independent prognostic factors were again the stage of the disease \( P < 0.0001, B = 1.379, SE = 0.207, \ df = 1, \ Exp(B) = 3.971, \text{and 95% confidence interval for} \ Exp(B) = 2.649–5.952 \) and patient age \( P < 0.0001, B = 0.083, SE = 0.018, \ df = 1, \ Exp(B) = 1.086, \text{and 95% confidence interval for} \ Exp(B) = 1.048–1.125 \).

**DISCUSSION**

In the present study, TIMP-2-positive immunohistochemical expression in stromal fibroblasts was observed in 26.4% of urothelial carcinomas and in cancerous cells in 69.8% of the cases. Whereas in the past, TIMP-2 expression was mainly localized to the tumor stroma (32–37), cancerous cell TIMP-2 immunostaining has also been reported in several carcinomas (13, 25, 33, 38). In bladder cancer, Grignon et al. (39) observed TIMP-2 protein localization in cancerous cells in 62% of cases, which is in keeping with our results. On the other hand, the same authors observed TIMP-2 protein localization in stromal cells in 74% of cases, a percentage significantly higher than the observed in our study (39). This discrepancy may be because in the study by Grignon et al. (39), all of the carcinomas were invasive, which, as it is known, contain a higher amount of neoplastic stroma. In contrast, our study included noninvasive carcinomas (pTa) and a high percentage of superficially invasive carcinomas (pT1).

Explanations for TIMP-2 cancerous cell localization comes from *in vitro* studies demonstrating that MT-MMP may act as a cell surface receptor for TIMP-2 (32). According to Hewitt and Danø (32), TIMP-2 immunostaining in cancerous cells may represent TIMP-2 produced in the stroma and then bound to MT-MMP on the surface of neoplastic cells. Moreover, specific TIMP-2 receptors responsible for cell growth have been identified on the surface of cancerous cells (40). An alternative explanation for TIMP-2 localization in cancerous cells is the production of TIMP-2 by the cancerous cells themselves, a
suggestion supported by the identification of TIMP-2 mRNA in cervical squamous carcinoma cells (25).

In the present study, a significant association was observed between TIMP-2 stromal cell immunostaining and high grade as well as high stage urothelial carcinomas (P < 0.0001 and P < 0.0001, respectively). Moreover, TIMP-2 immunolocalization in either the stromal or cancerous cells correlated significantly with Ki-67 proliferation index immunohistochemical expression (P = 0.02 and P = 0.044, respectively). In a previous study, we have demonstrated a strong correlation between Ki-67 immunohistochemical expression and high-grade as well as high-stage urothelial carcinomas suggesting that uncontrolled proliferation contributes to the dedifferentiation and progression of urothelial cancer (30). The observed positive correlation between TIMP-2 either stromal or cancerous cell expression and Ki-67 proliferation index in this study raises the possibility that TIMP-2 in both of these localizations is involved in growth control. Furthermore, it could be speculated that stromal TIMP-2 is involved in growth control, particularly in high-grade and high-stage urothelial carcinomas. Our suggestion of TIMP-2 participation in growth control is supported by several in vitro studies in recent literature (17, 18, 40, 41). Apart from the well-established erythroid-potentiating activity of TIMP-2 and TIMP-1, i.e. support of the growth of erythroid precursors (18), a feedback loop that regulates TIMP-2 expression via cyclic AMP-dependent mechanism and influences cell growth, has been described (19, 41, 42). Moreover, in a previous study in breast cancer, we have reported a statistically significant association between TIMP-2 protein expression and the proliferation index topoisomerase IIα (29). It has been suggested that the domains that are responsible for the growth factor activity of TIMPs are distinct from those responsible for their MMP inhibitory activity (40). Hayakawa et al. (40) demonstrated the presence of specific TIMP-2 receptors on cell surfaces responsible for mediating the growth-promoting activity of TIMP-2. Similarly, the cell growth-promoting activity of TIMP-1 is suggested to be a direct cellular effect mediated by a cell surface receptor not occurring through the inhibition of MMPs (43, 44). The study of TIMP-2-mediated signal transduction and TIMP-2 cross-linking to the cell surface have led to speculation that the TIMP-2 receptor may be a seven-pass transmembrane-type receptor similar to that for other peptide growth factors (19).

According to recent studies, the MMPs and their inhibitors (TIMPs) have been suggested to regulate apoptosis (20). In the present study, TIMP-2 immunohistochemical expression in either stromal or cancerous cells associated significantly with mutant p53 protein expression (P = 0.043 and P = 0.045, respectively), whereas no association was observed between TIMP-2 and bcl-2 expressions. These findings seem contradictory, taking into account that both mutant p53 and bcl-2 can inhibit apoptosis. However, as it is known, mutant p53 protein expression is associated with high-grade and high-stage urothelial carcinomas, whereas, as we have shown in a previous study, bcl-2 protein expression is associated with low-grade and low-stage urothelial carcinomas (30, 31, 45). As in the case of growth control, it seems that TIMP-2 involvement in apoptosis control consists of a late event in urothelial cancer progression. In keeping with our results, Li et al. (20) reported that bcl-2 overexpression has no effect on TIMP-2 expression in breast epithelial cell lines, whereas, to the contrary, it induced TIMP-1 expression in the same cell lines. Our suggestion of TIMP-2 involvement in growth control and apoptosis regulation as a late event, at least as stromal TIMP-2 is concerned, was supported by the finding that simultaneous positive expression of stromal TIMP-2, p53, and Ki-67 associated significantly with both muscle-invasive and high-grade carcinomas (P < 0.0001 and P < 0.0001, respectively).

In the present study, a statistically significant association was observed between TIMP-2 stromal cell expression and advanced stages of the disease (P = 0.001). High TIMP-2 expression levels, determined by Northern blot, slot blot, and reverse transcription-PCR analysis, have been associated previously with advanced stage of bladder carcinomas (24, 46, 47). There is only one immunohistochemical study investigating TIMP-2 protein expression in bladder cancer in which no association is reported between TIMP-2 expression and stage, probably because of the small sample and the fact that all of the carcinomas were invasive (39).

On the other hand, in some previous studies in other tumor types like colorectal, gastric, breast cancer, and neuroblastomas, high TIMP-2 levels have been associated with less advanced stages of the disease (13–16). These associations have been attributed to the generally held view that the primary action of TIMP-2 is inhibition of MMP activity. However, the role of TIMP-2 in the interactions with MMPs and especially with gelatinase A (MMP-2) is a controversial matter. Although in general, active MMPs are inhibited by TIMPs, progelatinase A (pro-MMP-2) binds selectively to TIMP-2 through their COOH-terminal domains (6–8). This progelatinase A/TIMP-2 complex can still be activated and shows gelatinase activity, thus favoring proteolytic degradation of ECM, which is necessary for tumor cell invasion and metastasis (9).

In the present study, we observed a significant association between TIMP-2 stromal cell expression and poor patient survival (P = 0.0002). In contrast with other tumor types in which conflicting results are reported as far as TIMP-2 prognostic significance is concerned, in bladder cancer, there is agreement, in general, about the adverse TIMP-2 prognostic significance (24, 39). In the only one previous immunohistochemical study TIMP-2 expression was also associated with poor outcome (39). However, a discrepancy was observed between our results and the results of Grignon et al. (39) in that the association observed in our study between TIMP-2 expression and poor survival concerned only TIMP-2 stromal cell expression. In contrast, Grignon et al. (39) reported an association between either stromal or cancerous TIMP-2 expression and poor survival. However, as already mentioned, the study by Grignon et al. (39) was based on a significantly smaller and different sample, containing only 42 patients with invasive cancer, and was performed on frozen sections not permitting direct comparison to our results. Some previous studies in other tumor types have also reported an adverse prognostic significance of TIMP-2 expression. For instance, in human breast, colorectal, and cervical carcinomas high levels of TIMP-2 were associated with shortened disease-free or overall patient survival (21, 23, 25, 36, 48).

The adverse prognostic significance of TIMP-2 stromal cell expression that we observed is supported by the other findings of this study, i.e. the associations between TIMP-2
TIMP-2 Expression in Urothelial Bladder Cancer

stromal cell expression, and the advanced grade and stage of urothelial carcinomas, suggesting that stromal TIMP-2 expression plays a role in tumor progression and may prove to be a useful prognostic factor in urothelial bladder cancer. On the other hand, the observed associations between TIMP-2 expression in either the stromal or cancerous cells and Ki-67 proliferation index or mutant p53 protein suggest that TIMP-2 is involved in tumor growth control and apoptosis supporting previous in vitro studies reporting the growth-promoting activity of TIMP-2 and arguing for the multifunctional nature of this molecule. Additional studies with larger series of patients, higher representation of all stages of carcinomas, and even longer follow-up periods are needed to establish the possible utility of TIMP-2 as a prognostic marker and to reveal whether this marker might carry an independent prognostic value over stage or inside subgroups of staging.

REFERENCES
Clinical Cancer Research

Tissue Inhibitor of Metalloproteinase-2 as a Multifunctional Molecule of Which the Expression Is Associated with Adverse Prognosis of Patients with Urothelial Bladder Carcinomas

Hariklia Gakiopoulou, Lydia Nakopoulou, Argyris Siatelis, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/15/5573

Cited articles
This article cites 43 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/15/5573.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/15/5573.full#related-urls

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, use this link
http://clincancerres.aacrjournals.org/content/9/15/5573.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.