Prognostic Value of Combined p53 and Survivin in pT1G3 Urothelial Carcinoma of the Bladder

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Key Words: Bladder cancer; Superficial urothelial tumor; pT1G3; pT1a/pT1b substaging; Micrometric measurement; Survivin; p53

Abstract

pT1G3 bladder tumors have a high tendency to recur and progress. We evaluated the prognostic values of the depth of submucosal invasion and immunostaining with survivin and p53 in 30 pT1G3 urothelial carcinomas at the first endoscopic resection. The depth of invasion was evaluated toward the muscularis mucosa and measured using a micrometer. Survivin and p53 immunostaining were performed using an automated immunostainer. Of the patients, 19 (63%) had tumor recurrence, 11 (37%) had tumor progression, 10 (33%) had metastatic spread, and 10 (33%) died of the disease. Infiltration of deep lamina propria (pT1b) and a micrometric measure of 1.5 mm or more were associated with an increased risk of tumor local and/or metastatic progression (P = .03 and P = .02, respectively). A combined high expression of survivin (≥20%) and p53 (≥50%) was associated with an increased risk of tumor local progression (P = .0007). We showed that combined p53 and survivin immunostaining could be helpful in distinguishing patients with a high risk of tumor progression.

Materials and Methods

This retrospective study concerned 30 patients who underwent first transurethral resection between January 1994 and December 2003 at our institution, University Hospital.
Lille, France. All cases were pT1G3 urothelial carcinomas according to the 1973 WHO classification and high-grade according to the 2004 WHO/ISUP classification. Patient information and consent were obtained by the referring physician. A follow-up, including physical examination, cystoscopy, and cytologic examination during more than 2 years, was required for all cases. The presence of one or multiple recurrences (at least 1 tumor in the same location as the first tumor according to the cystoscopic investigation), local tumor progression (appearance of muscle invasion at transurethral resection, ie, at least pT2 stage), metastatic evolution, and cause of death (related to the disease or not) were noted for each patient.

Tissue samples were obtained from endoscopic transurethral resection. Specimens were formalin fixed and paraffin embedded. All histologic slides were available and reviewed by 2 pathologists (S.G. and X.L.). The following histologic parameters were reviewed: tumor necrosis, vascular invasion, abnormal mitotic figures, and mitotic rate. Consistent with the literature, tumors manifesting with invasion of the lamina propria above the level of muscularis mucosa (or the large vessels present at this level) were subclassified as pT1a and those with invasion to or beyond the muscularis mucosa were subclassified as pT1b.5 Depth of invasion of the lamina propria was measured with an ocular micrometer from the basement membrane to the deepest invasive cancer cells, following the method proposed by Cheng et al.6 For pT1b tumors, a second early resection was performed to avoid muscular invasion.

Immunohistochemical analysis was performed on 4-µm-thick sections from specimens on an automated immunostainer (Benchmark XT, Ventana, Strasbourg, France). The primary antibodies used were anti-p53 antibody (clone DO7, dilution 1:20; DAKO, Trappes, France) and antisurvivin antibody (clone D8 sc-17779, 1:20 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Appropriate positive and negative control samples were added for each run.

Immunostaining was evaluated by counting more than 500 tumor cells for each case at high power (×400). Staining for p53 and survivin was considered positive when more than 5% of tumor nuclei were immunostained. A high p53 index was defined when 50% or more nuclei were stained and a high survivin index when 20% or more nuclei were stained, as previously described.7,8 Statistical analyses were performed by using the χ² test and the Fischer exact test. The level of significance was set at .05.

Results

The study comprised data for 1 woman and 29 men with a median age of 70 years (range, 33-87 years). The median follow-up period was 57 months. The clinical evolution of the disease in these patients is summarized in Table 1. Four patients had metastatic evolution without local progression.

None of the morphologic parameters assessed (abnormal mitotic figures, tumor necrosis, and vascular invasion) alone was statistically correlated with tumor recurrence, local tumor progression, metastases, or death related to the disease. However, when at least 2 of the following parameters—abnormal mitotic figures, areas of tumor necrosis or vascular invasion—were observed in tumors, the progression rate was significantly higher (7/9 tumors [78%]) than in tumors with only 1 of these parameters (4/21 tumors [19%]; P = .0042).

Histologic and immunohistochemical results are summarized in Table 2.

Of 30 cases, 16 had superficial invasion of the lamina propria (pT1a) and 14 were staged as pT1b Image 1 and Image 2. Patients with pT1b tumors had a significantly increased risk of local progression and/or metastases in comparison with patients with pT1a tumors (71% vs 31%; P = .03). Indeed, at the end of the first 2 years of follow-up, 6 of 14 pT1b tumors showed progression compared with only 1 of 16 pT1a tumors (43% vs 6%; P = .03). No significant results between pT1 substaging and recurrence or death related to the disease were noted.

The mean depth of invasion measured in specimens was 1.49 mm (range, 0.076-5 mm). Tumors with local progression had a mean ± SD depth of invasion of 2.25 ± 1.52 mm compared with 1.05 ± 1.33 mm for cases without progression (P = .014). For cases with metastatic spread, the mean ± SD depth was 2.18 ± 1.54 vs 1.08 ± 1.38 mm for nonmetastatic cases (P = .02). For local tumor progression and/or metastases, the mean ± SD depth was higher (2.42 ± 1.58 vs 0.57 ± 0.54 mm; P = .0004). The cutoff value of 1.5 mm proposed by Cheng et al6 was reached in 10 (33%) of 30 patients. Among these patients, 9 had local progression and/or metastatic evolution. In contrast, 6 of 20 patients with a depth of invasion of less than 1.5 mm had tumor progression (P = .002).

Nuclear immunostaining for survivin was observed in 24 (80%) of 30 cases Image 3. Very weak cytoplasmic staining was noted in these cases but was not readily assessable. The

<table>
<thead>
<tr>
<th>Event</th>
<th>No. (%) of Cases</th>
<th>Median (Range) Time to Event (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td>19 (63)</td>
<td>13 (3-98)</td>
</tr>
<tr>
<td>Local progression</td>
<td>11 (37)</td>
<td>16 (4-83)</td>
</tr>
<tr>
<td>Metastatic evolution</td>
<td>10 (33)</td>
<td>39 (11-96)</td>
</tr>
<tr>
<td>Progession and/or metastases</td>
<td>15 (50)</td>
<td>24 (4-96)</td>
</tr>
<tr>
<td>Death</td>
<td>15 (50)</td>
<td>44 (24-99)</td>
</tr>
<tr>
<td>Death due to the disease</td>
<td>10 (33)</td>
<td>60 (24-99)</td>
</tr>
</tbody>
</table>
The percentage of tumor nuclei stained ranged from 0% to 61% (mean, 24.8%). The percentage of stained nuclei was higher for patients with tumor progression and/or metastatic evolution (30% ± 19% vs 19% ± 23%; \( P = .03 \)). But, considering tumor progression alone, this mean ± SD percentage of stained nuclei tended only to be statistically higher (32% ± 10% vs 21% ± 22%; \( P = .05 \)). Among the 24 cases with positive immunostaining, 18 cases demonstrated high survivin expression (≥20% of stained nuclei). The risk of progression was significantly higher in tumors with high survivin expression (10/18 progressed [56%]; \( P = .02 \)). No relationship was found between survivin immunostaining and recurrence or death related to the disease.

Nuclear staining with p53 was observed in 23 (77%) of 30 cases \( \text{Image 4} \). The proportion of stained nuclei ranged from 0% to 100% (mean, 62%). The mean ± SD percentage of stained nuclei was higher for patients with tumor progression (68% ± 30%) than for patients without tumor progression (47% ± 41%; \( P = .0065 \)). Of 23 p53+ tumors, 20 had high expression (≥50% of stained nuclei). The risk of progression was significantly higher for patients with high p53 expression.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Histologic and Immunohistochemical Results in 30 Cases of pT1G3 Bladder Carcinoma*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Local Progression</strong></td>
<td><strong>Local Progression and/or Metastases</strong></td>
</tr>
<tr>
<td><strong>Yes</strong></td>
<td><strong>No</strong></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
</tr>
<tr>
<td>Muscularis mucosa</td>
<td></td>
</tr>
<tr>
<td>pT1a (n = 16)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>pT1b (n = 14)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>by Micrometer</td>
<td></td>
</tr>
<tr>
<td>&lt;1.5 mm (n = 20)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>≥1.5 mm (n = 10)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Percentage of stained nuclei</td>
<td></td>
</tr>
<tr>
<td>Survivin</td>
<td></td>
</tr>
<tr>
<td>&lt;20 (n = 12)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>≥20 (n = 18)</td>
<td>10 (56)</td>
</tr>
<tr>
<td>p53</td>
<td></td>
</tr>
<tr>
<td>&lt;50 (n = 10)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>≥50 (n = 20)</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Survivin ≥20% and p53 ≥50%</td>
<td></td>
</tr>
<tr>
<td>No (n = 15)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Yes (n = 15)</td>
<td>10 (67)</td>
</tr>
</tbody>
</table>

* Data are given as number (percent).
(10/20 patients [50%]) than for patients with low or no p53 expression (1/10 patients [10%]; \( P = .048 \)).

The coexpression of survivin and p53 was observed in 22 (73%) of 30 tumors. High survivin expression associated with high p53 expression was observed in 15 of 30 cases. This group of patients had a statistically higher risk of disease progression (10/15 [67%] vs 1/15 [7%]; \( P = .0007 \)). No relationship was found between p53 immunostaining and recurrence or death related to the disease.

**Discussion**

Deep subepithelial invasion beyond the level of muscularis mucosa (pT1b stage) seemed to be a very useful criterion for staging bladder carcinoma. Substaging as pT1a or pT1b is being used more frequently by pathologists and urologists.\(^5\) Previously, Hölmang et al\(^9\) showed that patients with pT1bG3 tumors had a greater risk of progression and disease-related death than patients with pT1a tumors (56% vs 36%). The present study strengthens the prognostic value of pT1a/pT1b substaging. Indeed, we showed that patients with pT1b tumors had an increased risk of tumor progression and/or metastases compared with pT1a stage (71% vs 31%; \( P = .028 \)). Substaging as pT1a or pT1b is not yet recommended in the international pathologic classifications\(^1,10\) or in clinical guidelines.\(^11\) The main criticism is the absence of reproducibility. Indeed, the muscularis mucosa is inconsistently represented on resection specimens. Its presence varies from 33% to 66% according to different authors.\(^12,13\) This difficulty is overcome by using the large arteries present in the lamina propria as landmarks.\(^14\) Therefore, we think that substaging as pT1a and pT1b could be performed in almost all cases, as in our study.

Cheng et al\(^6\) proposed another approach to evaluate the level of invasion. This approach relies on the measurement of the depth of invasion by using a micrometer: “The depth of stromal invasion in the specimens obtained by transurethral resection of the bladder was measured from the basement membrane of the bladder mucosa to the deepest invasive tumor cells. When tissue fragments contained tumor without intervening stroma (usually high grade) or the specimens were not oriented, the depth of invasion was measured from the shorter distance to avoid overestimation of the depth of invasion.”\(^6\) This study comprised 55 patients with a pT1 bladder tumor detected by transurethral resection who had undergone cystectomy a second time. The depth of invasion at resection was correlated with the final pathologic stage on the cystectomy specimen. Indeed, 95% of patients with a depth of invasion of 1.5 mm or more at transurethral resection had finally an invasive carcinoma (\( \geq pT2 \)). In another study by the same authors,\(^15\) including 83 patients in whom stage T1 bladder carcinoma was diagnosed at transurethral resection, the depth of invasion was predictive of cancer progression: the 5-year progression-free rate for patients with a depth of invasion of 1.5 mm or more was 67% compared with 93% for patients with a depth of invasion of less than 1.5 mm (\( P = .009 \)).

In our study, patients with tumor progression had deeper invasion at initial diagnosis than patients without progression (\( P = .014 \)). By using the cutoff of 1.5 mm proposed by Cheng et al,\(^6\) 90% of the patients experienced tumor progression (local and/or metastatic) when the tumor depth was 1.5 mm or more and only 30% when the depth was less than 1.5 mm (\( P = .002 \)). However, in our experience, this measure was often
difficult and required meticulous technique because of the unpredictable orientation of the specimens. When tissue fragments contained tumor without intervening stroma, Cheng et al\textsuperscript{6} proposed that the depth of invasion be measured from the shortest distance to avoid overestimation. But transurethral resection often involves papillary fragments, making it impossible to clearly define the true level of the basement membrane from these fragments. Moreover, the urothelium is often present on several sides of the fragment, leading to an erroneous measure. Thus, this technique seems difficult in everyday practice, which is probably why use of the micrometric measure of depth of invasion is infrequently described in the literature.

van der Aa et al\textsuperscript{16} proposed separating “microinvasive” from “extensive” pT1 carcinoma by using a micrometric parameter. They defined microinvasion as a single focus of invasion of the lamina propria over a maximum distance of 0.5 mm (within 1 high-power field, \( \times 400 \)). Extensive infiltration could be multifocal, microinvasive areas or invasion that could not fit within 1 high-power field. In their study, rapid progression was observed in extensive pT1 carcinomas. In our study, the number of pT1G3 microinvasive carcinomas was insufficient to statistically assess the proposed method.

To date, many potential prognostic markers have been described in the literature. Survivin belongs to the family of inhibitor of apoptosis proteins and is a short-lived protein that has an important role during mitosis and apoptosis.\textsuperscript{17} Survivin expression has been reported to be associated with an unfavorable prognosis in several cancers.\textsuperscript{18} Immunohistochemical studies investigating the prognostic value of survivin in bladder cancer have yielded variable results. Swana et al\textsuperscript{19} were the first to report preferential expression of survivin in high-grade urothelial carcinomas compared with low-grade tumors. The mean time to recurrence in patients with grade G1 tumors was shorter with survivin+ tumors than with survivin– tumors. Ku et al\textsuperscript{20} showed that cytoplasmic survivin expression was an independent prognostic factor for disease-free survival.

In another study, Lehner et al\textsuperscript{20} observed cytoplasmic staining for survivin in normal and neoplastic urothelium. However, in this study, patients with nuclear survivin staining had longer disease-free survival. Nakahashi et al\textsuperscript{21} studied survivin expression in transitional cell carcinomas of the upper urinary tract. The cytoplasmic expression of survivin did not represent a prognostic factor. In the present study, 80% of the tumors were immunopositive for survivin. The location of the staining was nuclear and very weakly cytoplasmic. By using a cutoff point of 20% as previously proposed,\textsuperscript{7} we were able to individualize a subgroup of high-risk tumors with a high progression rate.

The most widely studied molecular marker in bladder cancer is p53. Esrig et al\textsuperscript{22} showed that p53 changes were predictive of the outcome in patients with bladder cancer who underwent radical cystectomy. In the case of superficial bladder tumors (pTa, pT1), some authors showed that p53 immunoexpression was an independent factor for disease progression.\textsuperscript{23,24} Combined alterations of p53 and p21, p16, or Rb have been described.\textsuperscript{25,26} A poor response to bacillus Calmette-Guérin therapy also was predicted by p53.\textsuperscript{27} However, these results have not been confirmed by other studies.\textsuperscript{28-30} In our study, high expression of p53 was associated with an increased risk of progression.

In a recent meta-analysis, no definite evidence of p53 overexpression as an independent prognostic factor was established.\textsuperscript{31} The reasons for the lack of evidence include many factors such as variability of immunohistochemical assays for tissue fixation, different pretreatments and primary antibodies used, and different cutoff points for scoring. Molecular sequencing is the referent method for establishing p53 status, but no exact correlation between p53 immunodetection and \( Tp53 \) mutations by molecular analysis has been demonstrated.\textsuperscript{32} In their study, Hernandez et al\textsuperscript{32} found that even if alterations of the p53 pathway are essential components of bladder cancer progression, the molecular analysis of \( Tp53 \) could not be an independent factor of local progression.

By using combined survivin and p53 immunostaining, we demonstrated that patients with high survivin and p53 expression had a significant risk of progression (\( P = .0007 \)). In a recent study, Karam et al\textsuperscript{33} showed that overexpression of several antiapoptosis markers (p53, survivin, bcl-2, and caspase-3) was associated with a worse prognosis in patients treated by cystectomy. A positive correlation between p53 and survivin expression has been noted in gastric,\textsuperscript{34} pancreatic,\textsuperscript{35} breast,\textsuperscript{36} lung,\textsuperscript{37} hepatocellular,\textsuperscript{38} and laryngeal\textsuperscript{39} carcinoma. Negative regulation of survivin may be induced by p53, but the exact mechanism of this regulation remains uncertain.\textsuperscript{39}

The present study confirms the prognostic value of the pT1a/pT1b subclassification for tumor risk progression in high-grade papillary urothelial carcinomas. The measurement of the depth of invasion by using a micrometer seems to be valuable but remains difficult in everyday practice. We also demonstrated the value of the association of immunostaining against survivin and p53. Of course, further studies are required to validate these data with larger series of patients.

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Acknowledgment: We thank N. Ramdane for the statistical analysis, and the Immunohistochemistry Unit of the Department of Pathology, Lille University Hospital, for its technical ability.

References


