Invasive Potential of “Noninvasive” Human Bladder Carcinoma

An Electron Microscopy Study

Peter J. Effert, MD, and Peter Seifert, PhD

Key Words: Bladder neoplasms; Microinvasion; Ultrastructure; Langerhans cell

Abstract

In classification systems for bladder tumors, a clear distinction between superficial noninvasive and urothelial carcinoma invasive to the lamina propria is of prognostic and therapeutic significance. However, a subset of tumors classified as noninvasive is characterized by increased recurrence and progression rates. This study was done to look for ultrastructural characteristics in histopathologically noninvasive urothelial bladder carcinomas that might predict an unfavorable prognosis. In 10 (83%) of 12 bladder tumors studied extensively, electron microscopy revealed the presence of different degrees of lamina propria penetration by individual tumor cells (microlesions and microinvasions). In 10 (77%) of 13 “normal” control tissues, no such lesions or microinvasions were detected. These findings indicate that ultrastructural analysis may contribute to more precise staging of superficial bladder carcinoma. Undetected microinvasions may explain more aggressive biologic behavior in a subset of bladder tumors classified as noninvasive by conventional histopathologic assessment.

When first diagnosed with bladder carcinoma, about 80% of patients have superficial disease. In these patients, various clinical and histopathologic variables affecting risk of tumor recurrence and progression have been studied. Prognostic significance of cytologic grading of malignancy has been demonstrated, especially in high-grade disease. However, differentiation of noninvasive (pTa) from invasive (pT1) superficial tumors is considered of particular importance. Long-term survival rates in Ta disease are significantly increased compared with T1 disease. Therefore, the International Union Against Cancer (UICC) and the World Health Organization (WHO)/International Society of Urological Pathology (ISUP) consensus classification of bladder tumors differentiate noninvasive from invasive superficial carcinoma.

Nevertheless, in a subset of superficial noninvasive carcinomas, early progression to higher stages of disease is observed. Standard histopathologic evaluation of H&E-stained tissue sections by light microscopy will not necessarily predict aggressive behavior in this subset of noninvasive tumors. Therefore, in the present study, superficial noninvasive bladder tumors were studied by electron microscopy. Initially, pTa papillary tumors had been examined by means of electron microscopy for the presence of neural tissue. During these studies, the presence of microinvasions was first observed. For the study reported herein, an extended series of pTa tumors and control tissues were analyzed with the intent to look for ultrastructural alterations at the epithelial-stromal boundary that might serve as predictors of imminent tumor progression.
Materials and Methods

Tissue Specimens

Bladder tumor specimen from 12 men were obtained by transurethral resection [Table 1]. For conventional histopathologic processing and H&E staining, tissue samples were fixed in 10% formaldehyde and embedded in paraffin. The stage (TNM) and degree of differentiation (G) of the tumors were determined by a pathologist according to the guidelines of the UICC and the WHO, taking into account the WHO/ISUP consensus classification presented in 1998.6,10 Immediately after resection, corresponding tissue samples were prepared for transmission electron microscopy.

The control group consisted of bladder biopsy specimens obtained from 13 patients for reasons of hematuria, suspect patches of reddish urothelium, or irritative voiding symptoms. Informed consent was obtained from all patients before biopsies were done.

Transmission Electron Microscopy

For conventional transmission electron microscopy, specimens were fixed with Karnovsky solution in a 0.1-mol/L concentration of phosphate buffer (pH 7.3), postfixed in a 1% solution of osmium tetroxide, dehydrated in ethanol, and embedded in Durcupan ACM. Ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate and examined under a Zeiss EM 109 transmission electron microscope (Carl Zeiss, Oberkochen, Germany). Pictures were taken with a large-format TFP camera (60/70 mm) (Carl Zeiss) on Kodak Technical Pan Film TP 120. No digitalized picture processing was used.

Results

General Morphologic Features

Conventional histopathologic assessment revealed the typical histopathologic features of urothelial carcinoma in all cases [Image II].11 Consistent with the normal layering of the urinary bladder mucosa, the neoplasms were composed of an outer epithelial layer (lamina epithelialis mucosae) and an inner connective tissue component (lamina propria mucosae). The subepithelial connective tissue extended into the papillary branches of the tumors.

Noninvasive urothelial papillary carcinoma was diagnosed in all cases (Table 1). The restriction of neoplastic growth to the epithelial layer without invasion of the lamina propria noted on H&E-stained sections by light microscopy was confirmed by a second pathologist. Light microscopy revealed superficial lamina propria invasion in 1 additional case, which was excluded from further analysis.

In H&E-stained tissue sections of control tissue samples, no malignancy or dysplasia was found. Inflammatory changes were noted in 2 of the specimens.

Ultrastructure of the Subepithelial Region

In the normal mucosa, a basal lamina consisting of lamina rara and lamina densa always marks the boundary between epithelium and subepithelial stroma. This basal lamina is visible by electron microscopy only. In tumor biopsy and

Table 1
Bladder Tumor Samples Studied

<table>
<thead>
<tr>
<th>Tumor No.</th>
<th>Patient Age (y)</th>
<th>Degree of Differentiation</th>
<th>Tumor Stage</th>
<th>WHO/ISUP Classification*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>G1</td>
<td>Ta</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>G2</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>71</td>
<td>G3</td>
<td>Ta</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>G2</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>69</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>76</td>
<td>G2</td>
<td>Ta</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>27</td>
<td>G1</td>
<td>Ta</td>
<td>2</td>
</tr>
</tbody>
</table>

ISUP, International Society of Urological Pathology; WHO, World Health Organization.

* 1, papillary neoplasm of low malignant potential; 2, papillary carcinoma, low grade; 3, papillary carcinoma, high grade.

normal control tissue specimens, lamina rara and lamina densa were clearly visible **Image 2A**. However, in neoplastic tissue, a uniform structure of the basal lamina was missing. While full, intact basal lamina was prevalent, varying changes were noted in some areas. These included a fluffy, swollen appearance and sudden, periodic changes between unilayered and multilayered basal lamina, as well as the presence of small splits or gaps (Image 2A). Even in subepithelial capillaries, foci of spongy basal lamina, splits in the basal lamina, or fusion of vascular and epithelial basal lamina were noted.

In 10 (83%) of 12 tumors studied, definite penetration of the basal lamina by individual tumor cells was noted **Table 2**. The pattern was sprout-like or other shapes of projections of basal epithelial cells through gaps of the basal lamina into the stroma **Image 2B** and **Image 2C**. In addition to such subcellular microinvasions, individual epithelial

**Image 2I** Patterns of microinvasion in urothelial carcinoma detected by electron microscopy. **A**, Basal epithelial cell in bladder carcinoma. Among normally differentiated segments of the basal lamina, gaps filled with other components of the extracellular matrix are found (asterisks). Arrow, lamina rara of the basal lamina; arrowhead, lamina densa of the basal lamina. E, epithelial cell; S, stroma with collagenous fibrils. Bar equals 0.5 µm. **B**, Basal epithelial cells in bladder carcinoma projecting (arrowheads) into the stroma through gaps in the basal lamina. Arrow, basal lamina. E, epithelial cell; S, stroma. Bar equals 0.5 µm. **C**, Protrusion of a basal epithelial cell through perforated basal lamina into the stroma. Pseudopodia of a Langerhans cell (LC) contact the subcellular microinvasion. C, capillary; E, epithelial cell; M, microinvasion; S, tumor stroma. Bar equals 1 µm. **D**, Two epithelial cells (asterisks) extending into the stroma through a broad gap (arrowheads) in the basal lamina (arrow). Other epithelial cells (x) lie apart from the epithelium on the stromal side of the basal lamina. E, epithelial cell; S, tumor stroma. Bar equals 1 µm.
cells protruded through basal lamina gaps into the stroma. Alternatively, groups of 2 to 3 epithelial cells were adjacent to the basal lamina on the stromal side. These cells showed morphologic features characteristic of apical epithelial cells. The presence of microvilli and tight junctions indicated reversed polarity. In the same areas, endothelial cells of capillaries near the epithelium showed projections into the stroma. When endothelial and epithelial basal laminae were fused, such projections reached into the intercellular space of the urothelium.

Apart from epithelial cells, Langerhans cells and their precursor cells were seen penetrating the basal lamina. Langerhans cells contained Birbeck granules, permitting unequivocal identification. In some cases, direct contacts between Langerhans cells and subcellular microinvasions were noted (Image 2C).

In control tissue samples, 10 of 13 samples were free of microinvasions (Table 2).

Discussion

As many studies have shown, recurrence and progression rates of all tumors that invade the lamina propria (pT1 in the UICC classification) are significantly increased compared with noninvasive tumors (pTa in the UICC classification). Recognition of early invasion, therefore, is considered one of the challenging areas in genitourinary pathology.

However, even in noninvasive disease, a subset of tumors has increased recurrence and progression rates comparable to those of lamina-invasive pT1 tumors. Current evidence indicates that increased risk in these tumors may be a reflection of cytologic tumor grade. Of high-grade pTa tumors, 25% to 40% were reported to progress to invasive disease. Another explanation for increased aggressiveness in a subset of tumors is provided by clinical studies evaluating the value of a second transurethral resection in superficial bladder tumors (pTa and pT1): 75% had residual tumor on second resection, and 29% were upstaged to more invasive disease.

Microinvasions detected by electron microscopy manifest as subcellular projections or individual cells penetrating the basal lamina.

By ultrastructural analysis, we were able to identify subcellular and cellular microinvasions in 10 of 12 papillary carcinomas classified as noninvasive by conventional histopathologic assessment. We are aware that a second opinion pathology review will reveal discrepancies in tumor grade and stage in a substantial number of specimens. However, because the tumors examined for the present study were evaluated by 2 independent pathologists, the risk of understaging seems to be minimized. Furthermore, when bladder tumors posing a diagnostic problem as to invasion of the lamina propria were reevaluated by several pathologists, errors were due mostly to overevaluation of microinvasion. In contrast, understaging of pTa disease has been reported to occur in up to 3% of cases only.

Subcellular microinvasions of epithelial cells are a reflection of altered tissue interaction and the result of lost regulatory capacities of the extracellular matrix. Langerhans cells are outposts of the immune system. Therefore, contacts observed between microinvasions and Langerhans cells are not likely to happen accidentally. Rather, they reflect cellular reactions to pathologic changes.

Microinvasions detected in a smaller subset of control tissues point to destructive processes of unknown origin. Corresponding patients should be subjected to surveillance.

In addition to microinvasion of cells or cell groups, gaps in the basal lamina were noted in a few areas of some of the tumors. Invasive neoplastic growth is associated with the penetration of malignant cells through the basal lamina, which is part of the basement membrane detectable by light microscopy. Therefore, the basement membrane is considered a functional boundary preventing tumor cell migration to the subepithelial stroma. It should be taken into consideration that the basement membrane does not represent a separate tissue structure but a filmy part of the stromal matrix regulating cell growth and tissue architecture.

Table 2

<table>
<thead>
<tr>
<th>Microinvasion</th>
<th>Tumor No.</th>
<th>Control tissue No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>1, 2, 3, 6, 7, 8, 9, 10, 11, 12</td>
<td>+, microinvasion detected; –, no microinvasion detected.</td>
</tr>
</tbody>
</table>
Numerous studies using immunohistochemical staining of the basement membrane components laminin and collagenase type IV have documented the loss of continuity of basement membrane in many different malignant neoplasms. In bladder tumors, patchy or absent basement membranes were associated with increasing tumor stage and progression. Nevertheless, a proportion of pT1 and muscle-invasive tumors maintained intact basement membranes. Since basement membrane production can continue even in invasive disease, basement membrane staining has turned out to be of limited additional value as a prognosticator. Instead, histopathologic determination of bladder tumor stage on H&E-stained sections remained the most important independent parameter associated with survival and progression-free survival.

Visualization of the region of the basement membrane by methenamine silver staining has been found useful for identifying early stromal invasion in doubtful cases. Similarly, it has been suggested that basement membrane staining may facilitate the assessment of microinvasion in bladder cancer, which may be overlooked or only suspected by light microscopic evaluation of H&E-stained sections. However, following basement membrane staining, only 3% of the cases were upstaged to microinvasive disease.

For selected indications, electron microscopy has an established role in surgical pathology. Electron microscopy can help reduce the risk of incorrect diagnosis and may help resolve diagnostic dilemmas with a high degree of certainty. The data presented herein would indicate that electron microscopy could contribute to more accurate tumor staging as well and, thereby, affect therapeutic strategies. However, the effort associated with ultrastructural analysis is too excessive for applicability on a routine basis. And even then, only a random test can be performed owing to the small tissue pieces. In addition, the detection rate of microinvasions...
might have been even higher if all tissue resected could have been examined by electron microscopy.

Conclusions

By ultrastructural analysis, the invasive potential of basal epithelial cells was detected in a substantial proportion of tumors considered to be noninvasive. Although not applicable on a routine basis, electron microscopy for the detection of microinvasion may help explain the increased recurrence and progression rates in a subset of bladder tumors classified as noninvasive by conventional histopathologic assessment.

From the 1Department of Urology, St Franziskus Hospital and Rheinisch Westfälische Technische Hochschule University of Aachen, Aachen, Germany; and 2Alfried-Krupp Laboratory for Electron Microscopy, Department of Ophthalmology, University of Bonn, Bonn, Germany.

Address reprint requests to Dr Effert: Department of Urology, St Franziskus Hospital and RWTH University, Triererstrasse 176/178, D-52078 Aachen, Germany.

Acknowledgments: We thank Claudine Strack and Parand Pour Nouroz for excellent technical assistance and untiring evaluation of tissue sections. Careful review of histopathologic tissue sections by R. Lindenfelsker, MD, and R. Goebbelts, MD, Institute of Pathology, Teaching Hospital Bardenberg of RWTH University of Aachen, is gratefully acknowledged.

References