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Urothelial Carcinoma With Squamous Differentiation Is Associated With High Tumor Stage and Pelvic Lymph-Node Metastasis

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Background: *Squamous differentiation occurs in up to 20% of urothelial carcinoma cases and is thought to be an unfavorable prognostic factor.*
Methods: *Data from urothelial carcinoma in patients treated with cystectomy from 2002 to 2014 at Roswell Park Cancer Institute were retrospectively reviewed. A 2-tier system was adopted for stage analysis. T1 and T2 disease were grouped in organ-confined and low-stage categories, whereas T3 and T4 disease were grouped in high-stage categories. The extent of squamous differentiation was semi-quantified as focal (≤ 20%) or extensive (> 20%).*
Results: *Squamous differentiation occurred in 19.3% (47 of 244) of cases. Urothelial carcinoma with squamous differentiation presented with a significantly higher rate of high-stage disease compared with pure urothelial carcinoma (72.3% vs 43.1%;* P < *.01). The nodal metastatic rate in urothelial carcinoma with extensive squamous differentiation was significantly higher than that seen in pure urothelial carcinoma (46.2% vs 27.0%;* P = *.04).*
Conclusions: *Urothelial carcinoma with squamous differentiation is associated with advanced tumor stage. In addition, urothelial carcinoma with extensive squamous differentiation presented with a significantly higher rate of nodal metastasis. These findings can be the contributing factors for the unfavorable clinical outcomes seen in patients with urothelial carcinoma and squamous differentiation.*

Introduction

In the United States, an estimated 76,960 new diagnoses and 16,390 deaths due to urothelial carcinoma of the urinary bladder occurred in 2016.[1] The estimated 5-year survival rate of urothelial carcinoma of the urinary bladder is 77% — a survival rate that has not changed in the last 30 years.[1] This fact motivated us to identify pathological features of urothelial carcinoma of the urinary bladder to be used for prognosis and to improve the clinical treatment of this disease.

Urothelial carcinoma is well known for its divergent differentiation resulting in distinct, morphological variants.[2] Squamous differentiation, defined by the presence of intercellular bridges, keratinization, or both, is the most common variant, occurring in up to 20% of urothelial carcinomas of the bladder, followed by glandular differentiation.[3-10] Although urothelial carcinoma with squamous differentiation may be associated with poor prognosis, conflicting data have been reported regarding the role of squamous differentiation in unfavorable clinical outcomes.[7,10-16] Because it is not uncommon for squamous differentiation to concurrently occur with other histological variants of urothelial carcinoma, such as micropapillary, glandular, and sarcomatoid differentiation, studies that report on mixed urothelial carcinoma variants may contribute to the discrepancies.[10,17,18]

In this study, our aim was to compare tumor pathological stage and status of pelvic lymph-node metastasis between invasive urothelial carcinoma and urothelial carcinoma with squamous differentiation in patients who underwent cystectomy.

Materials and Methods

Case Selection

A retrospective review was conducted of 353 patients treated with radical cystectomy from 2002 to 2014 at the Roswell Park Cancer Institute (Buffalo, NY). Exclusion criteria were: no residual urothelial carcinoma; no invasive component; presence of urothelial carcinoma variants other than urothelial carcinoma with squamous differentiation; and urethral/upper urinary tract primary cancer. Patients who had not undergone lymph-node dissection were also excluded from analysis.

Invasive urothelial carcinoma was staged and graded according to the American Joint Committee on Cancer[19] and World Health Organization Classification of Tumours[20] recommendations, respectively. Prior to cystectomy, each patient underwent biopsy or trans-

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urethral resection of a bladder tumor. No patients were given chemotherapy or radiotherapy prior to cystectomy. The study was approved by the Institutional Review Board of our institution.

The cystectomy specimens were processed using the standard procedure recommended by the College of American Pathologists.[21] If the tumor stage of cystectomy was different from the results of a prior biopsy or transurethral resection of a bladder tumor, then the higher stage was assigned to the patient for statistical analyses.

Urothelial carcinoma with squamous differentiation was defined by the presence of clear-cut intercellular bridges, intracellular keratinization, or both, or the formation of squamous pearls in a background of invasive urothelial carcinoma. For cases of urothelial carcinoma with squamous differentiation, the slides were reviewed and the extent of squamous differentiation was semi-quantified as focal if the element of squamous differentiation was equal to 20% or less of invasive carcinoma and was considered to be extensive if it was more than 20%.

To analyze tumor stage, T1- and T2-staged tumors were grouped into an organ-confined, low-stage category, whereas T3- and T4-staged tumors were grouped into high-stage disease. Nodal positivity (N+) was defined as any lymph node with metastatic urothelial carcinoma (N1–3) at any tumor stage (T1–T4).

Statistical Analysis

Differences in patient age were tested using a 2-tailed *t* test. The distributions of sex, tumor stage, and nodal involvement were analyzed using a 2-sample *Z* test for the difference between proportions. A *P* value of less than .05 was considered to be statistically significant.

Results

Of the 353 patients who underwent radical cystectomy at the Roswell Park Cancer Institute, 244 had invasive urothelial carcinoma that fulfilled our inclusion criteria, including 197 (80.7%) with "pure" urothelial carcinoma and 47 (19.3%) who had urothelial carcinoma with squamous differentiation. The clinicopathological features of the patients with pure urothelial carcinoma and urothelial carcinoma with squamous differentiation are summarized in Table 1.

No difference in age was observed between the 2 groups of patients. Male sex was predominant in both groups. Of the 197 male patients, 163 (82.7%; male-to-female ratio 4.8) had pure urothelial carcinoma, and 29 of 47 male patients (61.7%; male-to-female ratio 1.6) had urothelial carcinoma with squamous differentiation. However, the percentage of female patients (38.3%; 18 of 47) who had urothelial carcinoma with squamous differentiation was significantly higher than those with pure urothelial carcinoma (17.3%; 34 of 197; $P < .01$; Fig 1).

Table 1. — Clinicopathological Features of Patients Evaluated for Tumor Stages

Feature	Pure Urothelial Carcinoma	Urothelial Carcinoma With Squamous Differentiation	*P* Value
Patient, n (%)	197 (80.7)	47 (19.3)	
Age, y			.8000*
Mean ± SE	69.5 ± 0.8	69.7 ± 1.5	
Range	40–90	45–87	
Sex, n (%)			.0010**
Man	163 (82.7)	29 (61.7)	
Woman	34 (17.3)	18 (38.3)	
Man:woman ratio	4.8	1.6	
Stage, n (%)			.0003**
Low (T1/2)	112 (56.9)	13 (27.7)	
High (T3/4)	85 (43.1)	34 (72.3)	

**P* value was analyzed using a 2-tailed *t* test.
***P* values were analyzed using a 2-sample *Z* test for the difference between proportions.
SE = standard error of mean.

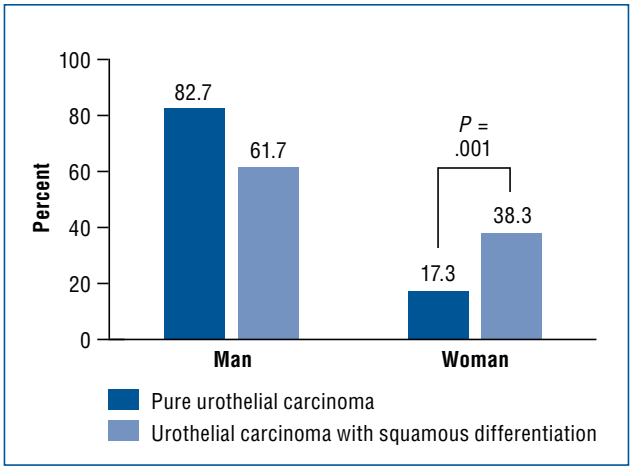

Fig 1. — Women made up a significantly higher proportion of patients with urothelial carcinoma with squamous differentiation than pure urothelial carcinoma. The *P* value was analyzed using a 2-sample *Z* test for the difference between proportions.

The percentages of the 197 patients who presented with stages T1 to T4 in the pure urothelial carcinoma group were 26.9% (53), 29.9% (59), 29.4% (58), and 13.7% (27). The corresponding percentages of the 47 patients who had urothelial carcinoma with squamous differentiation were 6.3% (3), 21.4% (10), 48.9% (23), and 23.4% (11), respectively.

Tumor stages were further grouped into organ-confined disease (T1/2) and high-stage disease (T3/4). The percentages of patients who presented with high-stage disease were 72.3% (34 of 47) among

those with urothelial carcinoma and squamous differentiation and 43.1% (85 of 197) in patients with pure urothelial carcinoma (see Table 1). These data suggest that patients who have urothelial carcinoma with squamous differentiation are more likely to present with extravesical (T3/4) disease and less likely to have organ-confined disease than those with pure urothelial carcinoma ($P = .003$; Fig 2).

Nodal status was analyzed in 196 patients with pure urothelial carcinoma and 46 patients with urothelial carcinoma and squamous differentiation. Four patients with pure urothelial carcinoma and 5 patients with urothelial carcinoma and squamous differentiation had pericystic lymph nodes present in the sections, whereas the remaining patients underwent extended pelvic lymph-node dissection.

Based on the extent of the squamous differentiation, cases of urothelial carcinoma with squamous differentiation were further stratified into focal squamous differentiation when the squamous component comprised no more than 20% of invasive carcinoma; by contrast, squamous differentiation was considered to be extensive when it made up more than 20% (Fig 3). Among the cases of urothelial carcinoma with squamous differentiation, 20 with focal differentiation and 26 with extensive differentiation were identified.

Lymph-node involvement by tumor was seen in 20.0% of patients (4 of 20) with urothelial carcinoma and focal squamous differentiation and in 46.2% of patients (12 of 26) with urothelial carcinoma and extensive squamous differentiation. Although no significant difference in nodal metastasis was observed between the urothelial carcinoma with squamous differentiation and the pure urothelial carcinoma groups (34.8% vs 27.0%), as well as between urothelial carcinoma with focal squamous differentiation and pure urothelial carcinoma (20.0% vs 27.0%), the nodal metastatic rate in urothelial carcinoma with extensive squamous differentiation was significantly higher compared with that seen in pure urothelial carcinoma (46.2% vs 27.0%; $P = .04$; Table 2, Fig 4).

Discussion

The incidence rate of squamous differentiation in urothelial carcinoma in our patient cohort is similar to the rate reported in the literature (19.3% vs 21.0%).[8] Prior studies have reported that urothelial carcinoma with squamous differentiation is more aggressive because of its resistance to radiotherapy, chemotherapy, and immunotherapy.[4,22,23] However, other studies of urothelial carcinoma treated with radical cystectomy or transurethral resection have demonstrated that the adverse outcomes associated with squamous differ-

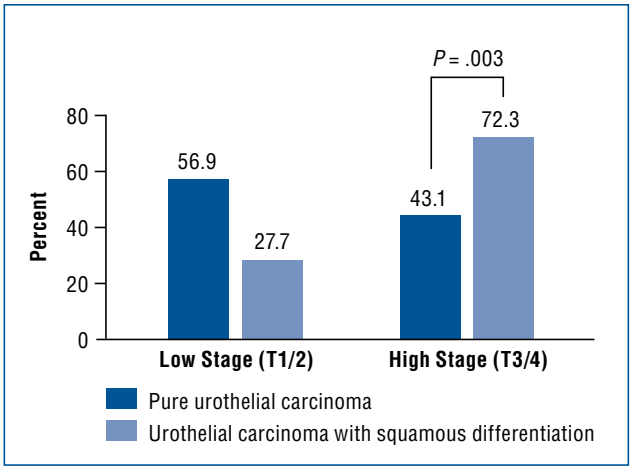

Fig 2. — Patients with squamous differentiation in urothelial carcinoma were more likely to present with high-stage (T3/4) tumors. The *P* value was analyzed using a 2-sample *Z* test for the difference between proportions.

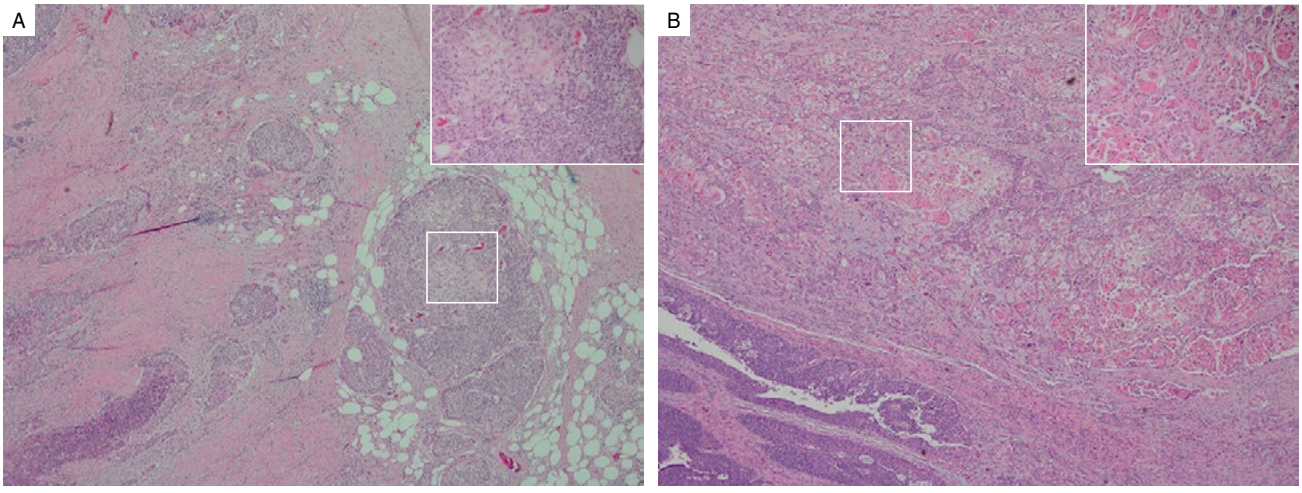

Fig 3A–B. — (A) Urothelial carcinoma with focal area of squamous differentiation showing intercellular bridges (H & E stain, ×40; inset: ×200). (B) Urothelial carcinoma with extensive squamous differentiation showing keratinization and squamous pearl formation (H & E stain, ×40; inset: ×200). H & E = hematoxylin and eosin.

Table 2. — Urothelial Carcinoma With Extensive Squamous Differentiation Associated With High Lymph-Node Positivity

Nodal Status	Pure Urothelial Carcinoma	Urothelial Carcinoma With Squamous Differentiation	Urothelial Carcinoma With Focal Squamous Differentiation	Urothelial Carcinoma With Extensive Squamous Differentiation
	No. of Patients, %			
N0	143 (73.0)	30 (65.2)	16 (80.0)	14 (53.8)
N+	53 (27.0)	16 (34.8)	4 (20.0)	12 (46.2)
Total	196 (100.0)	46 (100.0)	20 (100.0)	26 (100.0)
P value	—	.29	.50	.04

P values were analyzed using a 2-sample *Z* test for the difference between proportions.

entiation are present, even among patients who have not received radiotherapy.[7,11,17] Our study revealed that urothelial carcinoma with squamous differentiation is associated with a high tumor stage and a high rate of pelvic nodal metastasis. These data suggest that the aggressive clinical behavior of urothelial carcinoma with squamous differentiation may be attributed to lack of treatment response and to the intrinsic biological behavior of the tumor.

Although urothelial carcinoma with squamous differentiation portends a poor prognosis, it is unclear whether squamous differentiation is an independent prognostic factor.[13] By multivariate analysis, some studies have shown that the presence of squamous differentiation in urothelial carcinoma has a high propensity for local recurrence or predicts a decreased rate of cancer-specific survival,[24,25] whereas others have demonstrated that it has no predictive value on cancer-specific or recurrence-free survival and overall survival rates.[10,14] Instead, tumor stage and nodal involvement are 2 independent prognostic factors by multivariate analysis.[18,26,27] The data in the present study have demonstrated that, regardless of whether squamous differentiation is an independent predictor of clinical outcome, squamous differentiation is associated with a high tumor stage and nodal metastasis — both of which are proven prognostic factors in urothelial carcinoma.[28] These factors may account for the aggressive clinical behavior of urothelial carcinoma with squamous differentiation.

The extent of squamous differentiation in urothelial carcinoma may vary with the extreme of urothelial carcinoma in situ as the only urothelial component.[29] The association of a high-grade tumor and a shorter disease-free period with extensive squamous differentiation (defined as squamous differentiated areas ≥ 50% neoplastic surface) in urothelial carcinoma was reported as early as 1992[30] and reiterated in 2014 by Mitra et al,[18] who found that the presence of extensive squamous differentiation is associated with significantly decreased rates of overall survival. Our findings support these studies, even though the definition used in the present study is different. In this study, urothelial carcinoma with extensive squamous differentiation was associated with higher nodal positivity, whereas urothelial carcinoma with focal squamous differentiation was not, indicating that the extent of squamous differentiation plays an important role in prognosis and could explain the conflicting multivariate analysis results of previous research.[18]

Taken together, the findings of the present study emphasize the need to detect and quantitate the component of squamous differentiation in the setting of urothelial carcinoma in specimens obtained via resection and then including these features in pathology reports.

Urothelial carcinoma is a male-predominant tumor with a male-to-female ratio of 3.3 to 1.[31] Although the men in the present study predominated both the pure urothelial carcinoma and urothelial carcinoma with squamous differentiation groups, the percentage of women with urothelial carcinoma and squamous differentiation was significantly higher than the group with pure urothelial carcinoma (38.3% vs 17.3%; $P = .001$), suggesting that women are more likely to have urothelial carcinoma with squamous differentiation.

Such a finding may explain the higher rate of muscle-invasive diseases diagnosed in women than in men (85% vs 51%).[32] We speculate that the higher rate of uro-

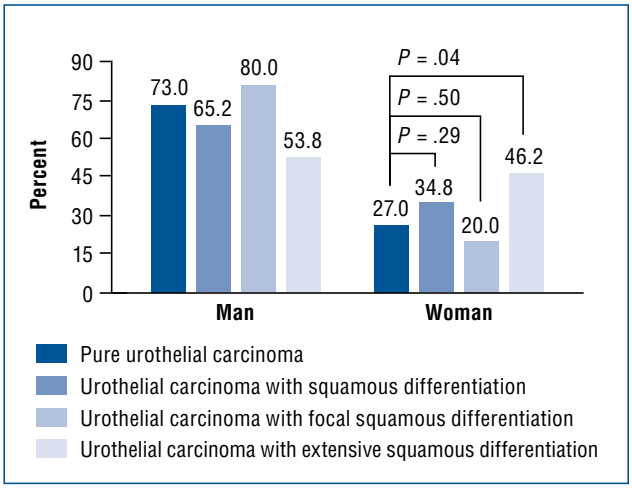

Fig 4. — Urothelial carcinoma with extensive squamous differentiation was associated with higher lymph-node positivity. *P* values were analyzed using a 2-sample *Z* test for the difference between proportions.

thelial carcinoma with squamous differentiation seen in women is due to a high incidence rate of squamous metaplasia in women in general. However, Fadl-Elmula et al[33] found that the squamous component in urothelial carcinoma contains the same cytogenetic changes as the urothelial carcinoma component, suggesting that the squamous element might be derived from urothelial carcinoma, not from squamous metaplasia. Sakamoto et al[29] demonstrated the absence of squamous metaplasia in 27 of 28 cases studied of urothelial carcinoma with squamous differentiation, supporting the suggestion that squamous metaplasia in women does not appear to be the cause of the higher rate of incidence of urothelial carcinoma with squamous differentiation seen in this population. Thus, whether squamous metaplasia or dysplasia plays a role in the squamous differentiation of urothelial carcinoma is still debatable, and more research is needed to understand the sex-specific differences in squamous differentiation.

Conclusions

The data presented in this study demonstrate that urothelial carcinoma with squamous differentiation is associated with high rates of advanced tumor stage and nodal metastasis. The present study also suggests that extensive squamous differentiation may be a contributing factor to the unfavorable clinical outcomes of urothelial carcinoma.

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