Int J Clin Exp Pathol 2013;6(8):1458-1466
www.ijcep.com /ISSN:1936-2625/IJCEP1305035

Original Article

Urothelial eddies in papillary urothelial neoplasms: a distinct morphologic pattern with low risk for progression

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Received May 25, 2013; Accepted July 3, 2013; Epub July 15, 2013; Published August 1, 2013

Abstract: We encountered an undescribed histologic feature of papillary urothelial neoplasms: "urothelial eddy", which was histologically reminiscent of squamous eddy of irritated follicular keratosis of the skin. A review of 756 patients with transurethral resection of bladder tumor revealed 10 patients (1.3%) of papillary urothelial neoplasms with urothelial eddies. All cases were male with a median age of 65 years. Urothelial eddies were characterized by small ovoid nests of ovoid to spindle cells arranged in an onion-skin pattern with fine cytoplasmic processes within wide intercellular space. The cytoplasmic processes mimicked intercellular bridges but ultrastructurally were cytoplasmic microvillous projections. They were of papillary urothelial neoplasm of low malignant potential in seven patients and low-grade urothelial carcinoma in three patients. Nine patients presented as non-invasive tumor and one patient showed microinvasion within papillary stalks. Six patients showed an inverted growth pattern. Their immunoprofile was more similar to that of conventional urothelial carcinoma rather than squamous cell carcinoma: high expressions of GATA3, S100P, uroplakin III, and cytokeratin 7; and low expressions of high molecular weight cytokeratin and p53. The Ki-67 labeling index was low (mean and median values, 2% each). During the follow-up period (mean, 88.7 months), four patients, including the microinvasive patient, showed recurrence with the same grade and stage but neither progressed into muscle-invasive tumor nor caused death. Our results suggest that urothelial eddy is a rare aberrant histology of papillary urothelial neoplasms with indolent behavior and should be discriminated from squamous differentiation of urothelial carcinoma, which has a poor prognosis.

Keywords: Urothelial carcinoma, variant, urothelial eddy, indolent

Introduction

The prognosis of urothelial carcinoma is dependent on tumor stage and grade, as well as specific morphologic features including histologic variants showing divergent differentiation [1]. Urothelial carcinoma has a propensity for aberrant differentiation and the most common histologic variant is squamous differentiation, which is characterized by intercellular bridges and/or keratinization [1-3]. Because it is a poor prognostic factor in urothelial carcinoma and portends resistance to conventional anticancer treatment for urothelial carcinoma, recognizing the squamous differentiation has important clinical significance for proper management of these patients [2, 4, 5].

During a histologic review of bladder cancer patients, we encountered a distinct morphologic pattern of papillary urothelial neoplasm. We designated it as urothelial eddy, because it is morphologically similar to squamous eddy seen in irritated follicular keratosis of the skin. Although it is reminiscent of squamous differentiation, urothelial eddy is urothelial in nature and the tumor appears to follow an indolent clinical course. The aim of this study was to characterize in detail both the pathologic features and clinical outcomes of papillary urothelial neoplasms with urothelial eddies.

Table 1. Primary antibodies studied in this study and their subcellular expression

Antibody	Clone	Dilution	Company	Subcellular location
GATA3	L50-823	1:600	Biocare Medical, Concord, CA	Nucleus
S100P	Polyclonal	Prediluted	Cell Marque, Rocklin, CA	Nucleus
Uroplakin III	AU1	Prediluted	Cell Marque, Rocklin, CA	Cell membrane
Thrombomodulin	1009.3	1:50	Dako Corp., Carpinteria, CA	Cell membrane
Cytokeratin 7	OV-TL 12/30	1:200	Dako Corp., Carpinteria, CA	Cytoplasm
Cytokeratin 20	Ks 20.8	1:200	Dako Corp., Carpinteria, CA	Cytoplasm
High molecular weight cytokeratin	34bE12	1:100	Dako Corp., Carpinteria, CA	Cytoplasm
p63	7JUL	1:200	Novocastra, Newcastle, UK	Nucleus
p53	DO-7	1:100	Dako Corp., Carpinteria, CA	Nucleus
Ki-67	7B11	1:200	Zymed, CA	Nucleus

Materials and methods

Patient selection and clinical data

This study was approved by the Institutional Review Board of Asan Medical Center (2013-0027). A slide review of 756 patients who underwent transurethral resection of bladder tumor (TURBT) at Asan Medical Center from 1996 to 2006 revealed 10 patients with papillary urothelial neoplasms with urothelial eddies. To characterize the immunohistochemical profile of these tumors, 22 patients with conventional urothelial carcinoma, which were randomly matched to patients of papillary urothelial neoplasms with urothelial eddies for age, sex, tumor-node-metastasis (TNM) stage, tumor grade, and TURBT year, were included as a control group. In addition, 11 patients with pure squamous cell carcinoma of the urinary bladder were included as control group of malignant counterpart of squamous eddy.

Patient clinical information, including follow-up data and cystoscopic and radiological findings, was obtained from electronic medical records. Survival data were retrieved from the Department of Medical Records at the Asan Medical Center. The tumor grade and stage were assigned according to the WHO 1973 and WHO/ISUP 2004 classifications and the American Joint Committee on Cancer (AJCC) Staging System, 7th edition, respectively [1, 6].

Tissue microarray construction

Tissue microarray (TMA) blocks were generated from the formalin-fixed paraffin-embedded tissue blocks of TURBT specimens, which had been harvested at initial diagnosis. They were constructed using a manual tissue microarrayer (Beecher Instruments, Silver Spring, MD) and composed of 1 mm diameter cores of papillary urothelial neoplasms with urothelial eddies and 2 mm diameter cores of control tumors. A representative three cores of the tumor tissues for each patient were included in the TMA blocks.

Immunohistochemistry

An immunohistochemical study was performed using urothelial lineage markers (GATA3, S100P, and uroplakin III) and markers expressed in various neoplasms, including urothelial neoplasm (thrombomodulin, cytokeratins 7 (CK7) and 20 (CK20), high molecular weight cytokeratin (HMWCK), p53, p63, and Ki-67). The primary antibodies used in this study are summarized in **Table 1**. Immunohistochemical staining was performed using a polymer-based system and an autostainer (Ventana Medical Systems, Inc., Tucson, AZ). Diaminobenzidine was used as a chromogen and the tissues were counterstained with Mayer's hematoxylin. Appropriate positive control tissues for each antibody were included.

All TMA slides were evaluated by two independent pathologists (MSK. and YMC.), both of whom were blinded to the associated clinical and pathological information. The expression of the markers was recorded as average percentages of positive cells in all cores using semiquantitative eyeball measurement. Patients with greater than or equal to 5% positive

cells were regarded as positive except for uroplakin III, CD20, and Ki-67, for which the presence of any positive cells was regarded as positive patient.

Electron microscopy

Three representative patients with papillary urothelial neoplasms with urothelial eddies were studied by electron microscopy using standard techniques. One patient with normal urothelial mucosa of the urinary bladder and one patient with normal squamous mucosa of the esophagus were also included for comparison. The submitted tissues were retrieved from paraffin blocks, deparaffinized, post fixed in 1% buffered osmium tetroxide, dehydrated, and embedded in Epon. Ultrathin sections of 1 μm thickness were stained with uranyl acetate-lead citrate and examined with a JEOL 1200 EX-II transmission electron microscope (Jeol, Tokyo, Japan).

Statistical analysis

The data were analyzed by PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) software. Crosstabs and one-way analysis of variance were used as required. Differences were regarded as statistically significant when P-values < 0.05.

Results

Clinical features of papillary urothelial neoplasms with urothelial eddies

The clinicopathologic features of the 10 patients (1.3%) of papillary urothelial neoplasms with urothelial eddies are summarized in **Table 2**. All patients were male with a median age of 65 years (range, 45-79 years) and admitted to this hospital because of gross hematuria. On cystoscopic examination, all patients demonstrated papillary to pedunculated mass in the urinary bladder (**Figure 1A**). Six patients presented with a single mass, three patients with two masses, and one patient had multiple masses. The tumors varied in size from 3 mm to 45 mm with a mean size of 28 mm. All patients underwent TURBT to completely remove the grossly visible tumor. Four patients underwent subsequent intravesical instillation of Bacillus Calmette-Guérin (BCG), two of whom received mitomycin C. One additional patient was treated with intravesical epirubicin alone. The remaining five patients did not receive any intravesical treatment after TURBT. No patient underwent radical cystectomy.

Histologic features of papillary urothelial neoplasms with urothelial eddies

The 10 patients with papillary urothelial neoplasms with urothelial eddies demonstrated areas of both urothelial eddies and conventional urothelial tumor (**Figure 1B-F**). The urothelial eddy area ranged between 10% and 95% (mean, 66.5%). Six patients showed an inverted growth pattern of the tumor (mean, 40%; range, 0-95%; **Figure 1C**).

The urothelial eddies were characterized by small ovoid nests of cells arranged in an onion-skin pattern (**Figure 1D**). Because of the peculiar cellular arrangement, tumor cells varied in shape, from ovoid to spindle to polygonal with indented nuclei (**Figure 1E**). The cells showed wide intercellular space and fine cytoplasmic processes, which were projecting into the intercellular space on high magnification and superficially mimicking intercellular bridges (**Figure 1E**). However, none of the patients showed keratinization.

Eight patients were originally diagnosed as low-grade papillary urothelial carcinoma (1973 WHO grade 2) and two patients as papillary urothelial neoplasm of low malignant potential (PUNLMP, 1973 WHO grade 1) by non-genitourinary (GU) pathologists. When reviewed by a GU pathologist (YMC) for this study, five out of the eight low-grade patients were downgraded to PUNLMP. As a result, seven patients were graded as PUNLMP and three patients as low-grade urothelial carcinoma (**Table 2**). No patient was classified as high grade on original or revised grading. One patient showed multiple foci of microinvasion of tumor cells within the stalk of the papillae (**Figure 1F**). However, an in situ urothelial carcinoma component, lymphatic tumor emboli, or proper muscle invasion was not observed in any patient.

Immunohistochemial features of papillary urothelial neoplasms with urothelial eddies

The immunoprofile of papillary urothelial neoplasms with urothelial eddies is summarized in **Table 3**. Among the urothelial lineage markers,

Table 2. Clinicopathologic features of 10 patients with papillary urothelial neoplasms with urothelial eddies

Patient no.	Age (yr)	Cystoscopic features		Pathologic features				Post-TURBT intravesical instillation	Follow-up			
		Tumor no.	Tumor Size (mm)	pT stage	Grade† (original /revised)	Inverted pattern (%)	Urothelial eddy (%)		Period (mo)	Recurrence no.	Progression	Survival
1	52	Single	20	Ta	1/1	95	95	None	28	0	No	AWOD
2	64	Single	15-20	Ta	2/1	20	20	None	121	0	No	DOOC (TA)
3	79	Multiple	Up to 30	Ta	2/2	0	10	BCG	77	1	No	AWOD
4	79	Single	30	Ta	2/2	0	90	None	95	1	No	AWOD
5	56	Two	10 and 30	T1	2/2	10	70	BCG & mitomycin C	115	2	No	AWOD
6	65	Two	3 and 15	Ta	1/1	95	70	BCG & mitomycin C	233	3	No	AWOD
7	71	Two	10 and 30	Ta	2/1	90	80	None	2	0	No	AWOD
8	65	Single	10	Ta	2/1	90	80	None	20	0	No	DOOC (AGC)
9	45	Single	20	Ta	2/1	0	70	Epirubicin	99	0	No	AWOD
10	66	Single	45	Ta	2/1	0	80	BCG	97	0	No	AWOD

†1973 WHO classification is used for tumor grade because old patients were graded according to the classification. yr, years; no., number; mo, months; AWOD, alive without disease; DOOC, Died of other cause; TA, traffic accident; BCG, Bacillus Calmette-Guérin; AGC, advanced gastric cancer.

Table 3. Comparison of immunohistochemical profiles of papillary urothelial neoplasms with urothelial eddies, conventional urothelial carcinoma, and squamous cell carcinoma of the urinary bladder

	Papillary urothelial neoplasms with urothelial eddies n = 10		Urothelial carcinoma n = 22		Squamous cell carcinoma n = 11		*P*-value	
	Positive patient no. (%)	Mean expression % ± SD	Positive patient no. (%)	Mean expression % ± SD	Positive patient no. (%)	Mean expression % ± SD	Positive patient no. (%)†	Mean expression % ± SD‡
GATA3	8 (80)	34 ± 31	21 (96)	52 ± 32	0 (0)	0 ± 0	< 0.001	< 0.001
S100P	10 (100)	68 ± 11	22 (100)	67 ± 10	2 (18)	8 ± 9	< 0.001	< 0.001
Uroplakin III	5 (50)	2 ± 3	10 (46)	3 ± 4	0 (0)	0 ± 0	0.022	0.035
Thrombomodulin	4 (40)	12 ± 16	13 (59)	18 ± 10	7 (63)	13 ± 7	1.000	0.941
CK7	10 (100)	73 ± 12	21 (96)	90 ± 23	5 (46)	20 ± 31	0.001	< 0.001
CK20	4 (40)	1 ± 3	6 (28)	4 ± 13	0 (0)	0 ± 0	0.162	0.503
p63	10 (100)	55 ± 27	20 (91)	49 ± 36	7 (63)	47 ± 32	0.074	0.210
HMWCK	5 (50)	14 ± 24	10 (46)	19 ± 32	11 (100)	78 ± 33	0.005	< 0.001
p53	3 (30)	3 ± 4	5 (23)	6 ± 17	6 (55)	27 ± 35	0.086	0.017
Ki-67	5 (50)	2 ± 1	12 (55)	7 ± 11	6 (55)	9 ± 12	0.717	0.001

no., number; CK, cytokeratin; HMWCK, high molecular weight cytokeratin. †The significance was evaluated by linear by linear association. ‡The significance was evaluated by one-way analysis of variance.

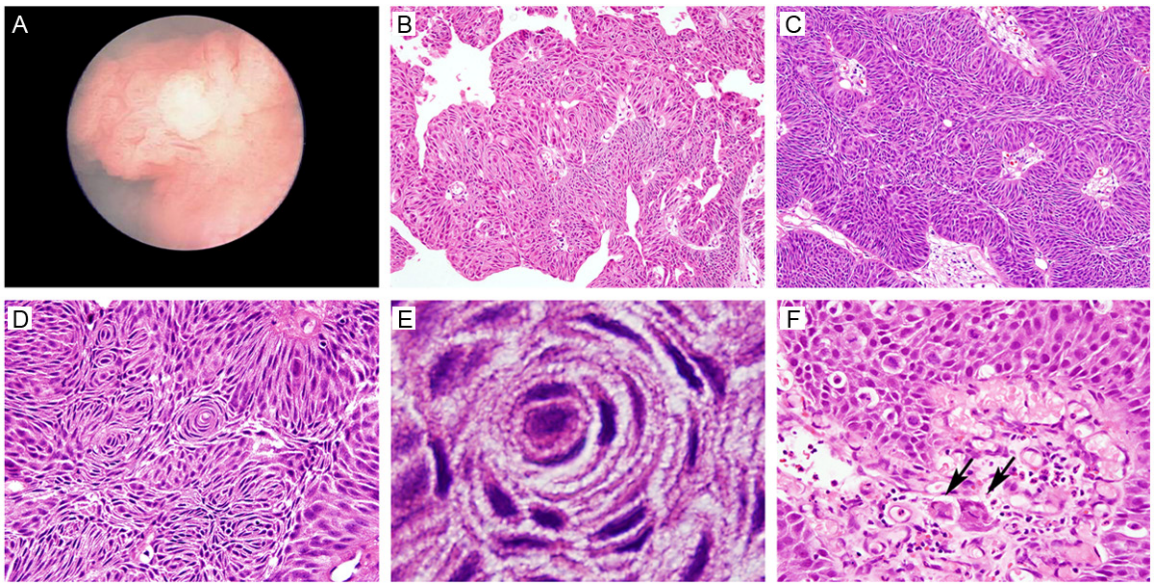

Figure 1. Cystoscopy and histology of papillary urothelial neoplasms with urothelial eddies (patients 9 and 7). A: Cystoscopic photograph shows papillary mass (patient 9). B-F: The histologic examination revealed papillary proliferation of tumor cells (B) with frequent inverted growth pattern (C), urothelial eddies, which are small ovoid nests of cells arranged in an onion-skin pattern (D), wide intercellular space and cytoplasmic processes within the intercellular spaces (E), and microinvasion of tumor cells within the stalk of the papillae (F). (B and F: patient 5; C-E: patient 7; hematoxylin and eosin stain; original magnification: B and C: ×200; D and F: ×400; E: ×1000).

papillary urothelial neoplasms with urothelial eddies revealed a high expression of S100P in all patients (mean expression, 68%; range, 50-100%) and GATA3 in eight patients (mean expression, 34%; range, 2-77%). Five patients showed uroplakin III expression, which was of a surface or focal membranous pattern (mean expression, 2%; range, 0-8%) as previously described [7]. Among the markers expressed in various tumors, papillary urothelial neoplasms with urothelial eddies showed strong and diffuse expressions of CK7 (mean expression, 73%; range, 57-100%) and p63 (mean expression, 55%; range, 27-95%) in all patients and focal expressions of thrombomodulin in four patients (mean expression, 12%; range, 0-57%) and HMWCK in five patients (mean expression, 14%; range, 0-80%). CK20 and p53 were barely expressed in four patients (mean expression, 1%; range, 0-8%) and three patients (mean expression, 3%; range, 0-10%), respectively. Five patients showed Ki-67 expression with a mean labeling index of 2% (median expression, 2%; range, 0-4%). In the same tumors, immunohistochemical findings were identical in eddy areas and non-eddy areas.

The immunoprofile of papillary urothelial neoplasms with urothelial eddies was similar to conventional urothelial carcinoma cases but different from squamous cell carcinoma cases in expressions of GATA3, S100P, uroplakin III, CK7, HMWCK, and p53 (**Figure 2**). There were no differences in expressions of thrombomodulin, CK20, and p63 between the papillary urothelial neoplasm group with urothelial eddies and two control groups of conventional urothelial carcinoma and squamous carcinoma. Interestingly, papillary urothelial neoplasms with urothelial eddies did not share a similar immunoprofile to that of squamous cell carcinoma of the urinary bladder. The Ki-67 labeling index of papillary urothelial neoplasms with urothelial eddies was the lowest among the three tested groups.

Electron microscopic features of papillary urothelial neoplasms with urothelial eddies

The papillary urothelial neoplasms with urothelial eddies showed wide intercellular space (**Figure 3A**) with many cytoplasmic microvillous projections (**Figure 3B**). In the normal urothelium, however, the intercellular space was inconspicuous and the microvillous projections were not as prominent as papillary urothelial neoplasms with urothelial eddies (**Figure 3C** and **3D**). Furthermore, well-developed desmosomes

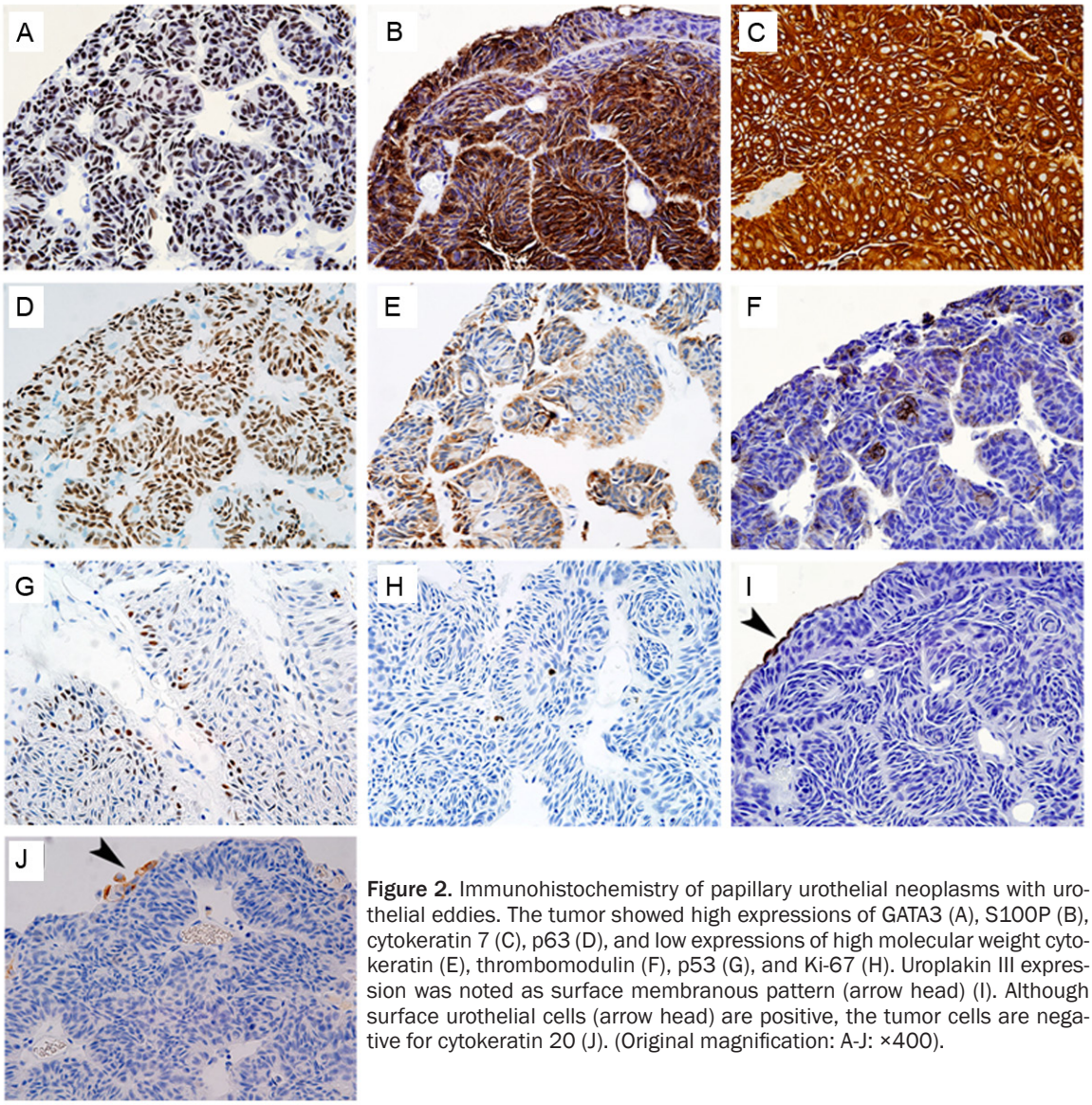

Figure 2. Immunohistochemistry of papillary urothelial neoplasms with urothelial eddies. The tumor showed high expressions of GATA3 (A), S100P (B), cytokeratin 7 (C), p63 (D), and low expressions of high molecular weight cytokeratin (E), thrombomodulin (F), p53 (G), and Ki-67 (H). Uroplakin III expression was noted as surface membranous pattern (arrow head) (I). Although surface urothelial cells (arrow head) are positive, the tumor cells are negative for cytokeratin 20 (J). (Original magnification: A-J: ×400).

and keratinization, diagnostic and characteristic ultrastructural features of squamous cells as shown in **Figure 3E** and **3F**, were not evident.

Clinical behavior of papillary urothelial neoplasms with urothelial eddies

During the mean follow-up period of 88.7 months (range, 2-233 months), four patients of papillary urothelial neoplasms with urothelial eddies developed tumor recurrence: two patients experienced one recurrence, one patient experienced two recurrences, and another one patient experienced three recurrences (**Table 2**). All recurrences were non-invasive papillary tumors except for patient five, whose original and recurrent tumors were of low grade with microinvasion within the papillary stalks. Two patients showed similar histologic features to their original tumor with wide intercellular space but the onion-skin pattern was inconspicuous. The recurrent tumors of the other two patients were conventional papillary urothelial carcinoma without urothelial eddies. None of the patients developed tumor progression in terms of grade and stage. Neither muscle-invasive recurrence nor urothelial carcinoma specific death was noted; however, two patients died of other causes: one of gastric adenocarcinoma and the other one in a traffic accident.

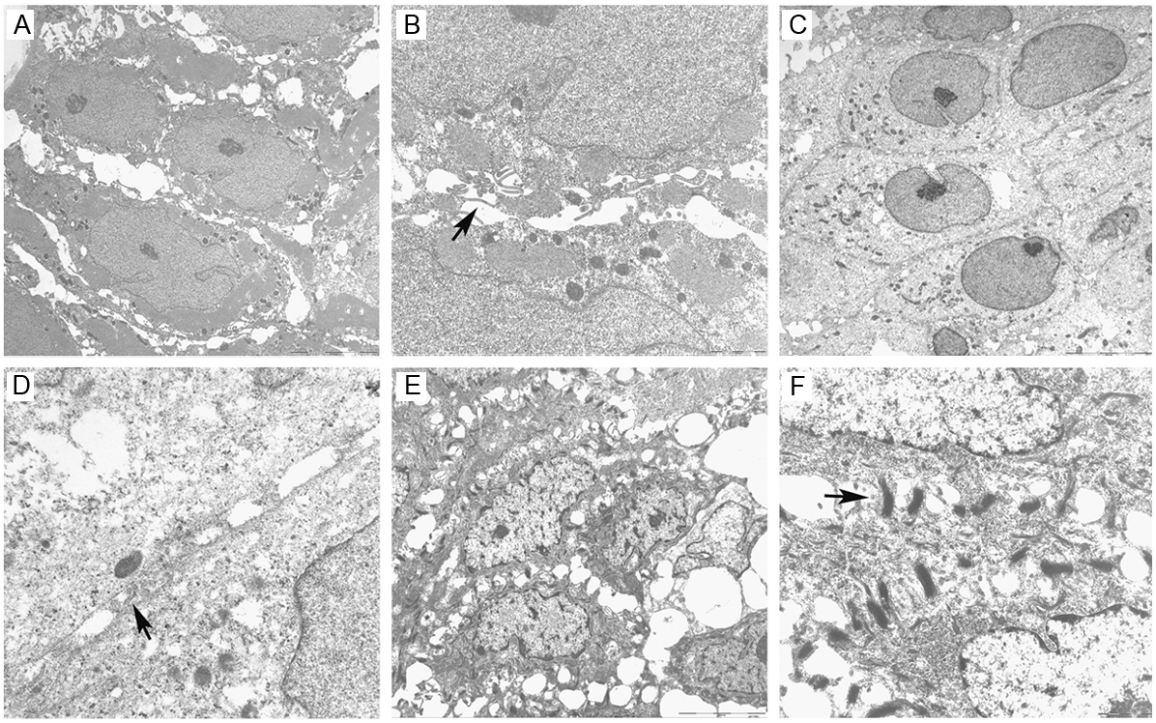

Figure 3. Electron microscopy of papillary urothelial neoplasms with urothelial eddies (A and B), normal urothelium of the urinary bladder (C and D) and normal squamous epithelium of the esophagus (E and F). Arrows indicate cytoplasmic microvillous projections of papillary urothelial neoplasms with urothelial eddies (B) and the normal urothelial cells (D) and desmosomes of normal squamous cells (F). (Original magnification: left column, ×8000; left column, ×15000).

Discussion

In this report, urothelial eddy, a distinct histologic pattern of papillary urothelial neoplasm, is presented for the first time. Although urothelial eddy is morphologically reminiscent of squamous differentiation, it is urothelial in nature and the tumor appears to follow an indolent clinical course with a low risk of tumor progression and cancer-related death. Awareness of papillary urothelial neoplasms with urothelial eddies has two pathologic implications: discriminating urothelial eddies from squamous differentiation of urothelial carcinoma and the correct grading of the tumors.

Squamous differentiation, defined by the presence of intercellular bridges and/or keratinization, is a relatively common phenomenon in urothelial carcinoma and is identified in up to 60% of patients with this disease [1, 3]. It is associated with high tumor grade and advanced tumor stage [8]. Although it is not clear whether the poor clinical outcome is a reflection of the histology or the advanced stage of the disease, squamous differentiation portends high rates of tumor recurrence and progression and poor cancer specific survival [8, 9]. Furthermore, squamous differentiation appears to predict a poor response to chemotherapy, radiotherapy, and surgery in urothelial carcinoma patients [2, 4]. Therefore, the presence or absence of squamous differentiation should be included in the surgical pathology report [1].

By contrast, papillary urothelial neoplasms with urothelial eddies appears to have indolent clinical course given that none of the 10 patients progressed to muscle-invasive tumor or died of the disease during the mean 88.7 months follow-up period. The wide intercellular spaces with cytoplasmic processes mimicking intercellular bridges in urothelial eddies may cause confusion with squamous differentiation of urothelial carcinoma on histologic examination. On electron microscopy, normal urothelial cells showed inconspicuous intercellular spaces with a few small cytoplasmic microvillous projections. By contrast, papillary urothelial neoplasms with urothelial eddies demonstrated

wide intercellular spaces and many prominent cytoplasmic microvillous projections, which created superficial resemblance of intercellular bridges on histologic examination. However, keratinization and well-formed desmosomes, as seen in squamous cells, were not evident in papillary urothelial neoplasms with urothelial eddies. Furthermore, at the immunohistochemical level, papillary urothelial neoplasms with urothelial eddies has a different immunoprofile from squamous cell carcinoma, but is similar to conventional urothelial carcinoma with a high expression of GATA3, S100P, CK7, and uroplakin III, and low expressions of HMWCK and p53. Based on our immunohistochemical and ultrastructural findings, papillary urothelial neoplasms with urothelial eddies appears to be of urothelial rather than squamous origin.

There was a tendency for papillary urothelial neoplasms with urothelial eddies to be diagnosed as a higher grade. After the review by a GU pathologist, five patients were downgraded from low grade (1973 WHO grade 2) to PUNLMP (1973 WHO grade 1). This was probably due to the architectural and nuclear distortions as a result of onion-skin pattern and indented nuclei. Nevertheless, these patients did not show further architectural and nuclear atypia of higher grade tumor, including conspicuous loss of polarity at low magnification, chromatin clumping, prominent nucleoli, or brisk mitotic activity [10]. Therefore, this study suggests that squamous differentiation should be obvious to be stated in the pathology report and we recommend to avoid the term of "squamoid" differentiation based on polygonal cell appearance only without convincing evidence of intercellular bridges, keratinization or both.

The squamous eddy is a characteristic feature of irritated follicular keratosis, which is a benign inverted proliferative lesion of squamous cells in the skin and considered as a variant of seborrheic keratosis [11]. Previous reports suggest that squamous eddies formed by focal maturation of resting basaloid cells into squamous cells by irritation-induced activation [12, 13]. An increased apoptotic activity was reported in the squamous eddy [14]. Based on these findings, squamous eddies are suggested to be an involution phase of the skin lesion [14]. Interestingly, papillary urothelial neoplasms with urothelial eddies patients also frequently showed an inverted growth pattern (6 out of 10 patients, 60%) similar to irritated follicular keratosis. Although apoptotic activity was not assessed in this study, papillary urothelial neoplasms with urothelial eddies showed the lowest Ki-67 labeling index among the three groups tested. These findings may point to the non-aggressive nature of the disease.

Limitations of this study are its retrospective design and small number of patients. We did not provide a mechanistic explanation of urothelial eddy formation, although apoptosis with low proliferative activity as demonstrated by the low Ki67 activity in this study might have a role in the pathogenesis of the unusual histology. Therefore, further studies on a large number of patients are warranted to confirm its indolent clinical course.

Acknowledgement

This study was supported by a grant from Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0013063).

Disclosure of conflict of interest

None.

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