Original Article

Upper urinary tract urothelial carcinoma with intratubular spread

Judy Sarungbam1, Boaz Kurtis1, John Phillips1, Dongming Cai3, David Zhang3, Islam Humayun1, Ximing Yang2, Minghao Zhong1

1Department of Pathology, Westchester Medical Center, New York Medical College, Valhalla, NY, USA; 2Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL; 3Mount Sinai School of Medicine, New York, NY, USA

Received April 7, 2014; Accepted June 23, 2014; Epub July 12, 2014; Published July 15, 2014

Abstract: Upper urinary tract urothelial cell carcinomas (UUT-UCs) are uncommon and are defined as urothelial carcinoma involving the urinary tract from the renal calyces, renal pelvis to the distal ureter. One well-known and peculiar histopathological finding in UUT-UC is urothelial carcinoma with intratubular spread (retrograde spread within renal tubules). However, this special feature has not been systematically studied. We therefore collected a total of 53 consecutive cases of upper urinary tract urothelial carcinomas (UUT-UCs), and studied the clinical and pathological features of intratubular spread (IS). A cocktail stain comprised of antibodies PAX8 and p63 together with PAS was validated and employed to facilitate the study of intratubular spread. Seventeen cases (31.5%) showed intratubular spread demonstrated by either H&E stain and/or the cocktail stain. All of the 17 cases with intratubular spread had tumor involvement of the renal calyx; the majority of these (14/17, 82.4%) were high grade urothelial carcinoma and the remainder (3/17, 17.6%) were low grade. 4 of 17 cases (23.5%) were non-invasive. We classified intratubular spread into 4 different types, based on histopathological patterns: pagetoid, typical, florid, and secondary invasion from intratubular spread. In conclusion, our study shows intratubular spread of urothelial carcinoma is fairly common phenomenon in UUT-UC and is associated with a variety of clinical-pathological features. High grade UUT-UC tends to have more extensive intratubular spread and secondary invasion into renal parenchyma. Distinct morphological characteristics as well as the staining pattern from a unique cocktail stain help to identify and evaluate intratubular spread of urothelial carcinoma. Recognizing these different types of intratubular spreading (IS) is crucial for accurate staging of some upper urinary tract urothelial carcinomas (UUT-UCs).

Keywords: Upper urinary tract urothelial carcinoma, intratubular spread, tissue microarray

Introduction

Upper urinary tract urothelial carcinomas (UUT-UCs) are defined as malignant neoplasms of the urothelium involving from the renal calyces, renal pelvis to the distal ureter. UUT-UCs are uncommon and account for only 5-10% of urothelial carcinomas and they account for about 7% of all kidney tumors [1-3]. The natural history of UUT-UCs differs from that of bladder cancer: 60% of UUT-UCs are invasive at diagnosis compared with only 15-25% of bladder tumors. In terms of epidemiology and risk factors, there is some similarity between UUT-UCs and those associated with bladder urothelial carcinoma [4-6]. The urothelial carcinomas are well known for multicentricity and high incidence of recurrence. The presence of upper urinary tract tumor progressively increases the risk of bladder cancer. In addition, due to the technical difficulty of conservative measures of upper tract disease, the majority of UUT-UCs is treated by radical nephroureterectomy with excision of the bladder cuff regardless of tumor grade or stage [1, 7, 8].

The histopathological features of this UUT-UCs are generally similar, if not identical, to those in the urinary bladder. The World Health Organization 2004/International Society of Urological Pathology system used to evaluate bladder tumors has been widely adopted for the grading of urothelial tumors of upper urinary tract as well [9, 10]. The pathologic stage is the single
most important prognostic factor for UUT-UCs [2, 8, 11]. However, lamina propria, muscularis propria, and adventitia - the important anatomical landmarks for staging - vary considerably along the renal pelvis and ureter [1, 12]. Thus, the current staging system, while prognostically significant, has pitfalls and challenges in its application for surgical pathologists [1, 12].

One important consideration when staging these tumors, involves the identification of the peculiar histopathological finding of intratubular spread (retrograde spread within renal tubules). Intratubular spread (IS) remains an in situ process (pTa/pTis) and can be mistaken for renal parenchyma invasion, which would significantly upgrade the stage (pT3 disease) [1]. This entity has been described in many genitourinary pathology books and reviews [1, 13]. Only one paper has studied 3 cases of intratubular spread from a series of 108 high grade urothelial carcinomas of the renal pelvis [13]. In this report, we systematically studied and describe the clinical-pathological features of intratubular spread in a series of 53 UUT-UCs cases.

Materials and methods

Material

After Institutional Review Board approval (IRB# WMC, L10.884), electronic surgical pathology records were retrospectively searched for all cases of upper urinary tract urothelial carcinoma with nephrectomy, from January 2006 to August 2012 at the Department of Pathology, Westchester Medical Center, Valhalla, NY. Fifty three cases were identified and all pathological reports and slides were reviewed. Clinico-pathological information including sex, age, tumor laterality, focality, size, histological grade, pathological stage, surgical margins, and lymphovascular invasion were evaluated. In all cases, tissues were fixed in neutral-buffered formalin and embedded in a paraffin block as part of a routine surgical pathology procedure. The tumors were graded using the World Health Organization/International Society of Urologic Pathologists (WHO/ISUP) grading system as either papillary urothelial neoplasm of low malignant potential (PUNLMP), low-grade urothelial carcinoma, or high grade urothelial carcinoma. Staging was reported based on the 2010 AJCC/TNM staging system.

The cocktail stain of p63, PAX8 and PAS

This cocktail stain begins with sequential immunohistochemical stains of p63 and PAX8 and followed by Periodic Acid Schiff (PAS) special stain. The procedures are outlined as follows: Immunohistochemical stains were performed on 5 micron (μm)-thick sections using the BenchMark ULTRA IHC/ISH stainer (Ventana Medical Systems, Oro Valley, AZ). The sections are deparaffinized and subjected to heat-induced antigen retrieval using EDTA buffer at pH 7.9. A prediluted anti-p63 (VENTANA, 4A4) mouse monoclonal primary antibody was applied for 32 minutes at 37 degree Celsius. Ventana Ultra view universal DAB detection kit with brown chromogen was used to visualize the reaction. The section was then incubated with prediluted rabbit polyclonal anti PAX8 (Cell Marque, MRQ-50) for 40 minutes at 42 degree Celsius. Ventana Ultra view universal DAB detection kit with red chromogen was used to visualize the reaction. For negative controls, the primary antibodies are replaced with phosphate-buffered saline. PAS special stain: After sequential immunohistochemistry stains of p63 and Pax8, the same slides are subjected for staining with Periodic Acid Schiff/Hematoxylin using Ventana stainer. PAS periodic acid is applied to the sections for 4 minutes; the sections are then rinsed and followed by incubation with PAS SCHIFF for 4 minutes and then with PAS neutralizer for 8 minutes. The sections are finally counter stained with PAS hematoxylin.

Construction of tissue microarrays (TMA)

The tissue microarray was constructed as previously described [14]. The formalin-fixed, paraffin-embedded tissue blocks and the corresponding histological H&E-stained slides were overlaid for tissue TMA sampling. Pathologists reviewed the slides and marked the representative areas of tumor tissues. We used a tissue arraying instrument (Beecher Instruments, Silver Spring, MD, USA) to punch triplicate 1.0 mm diameter cylinders of tissue from selected cancer areas of individual donor tissue block and re-embed into a recipient paraffin block at a predefined position. Subsequently, multiple sections (5 μm) were cut from the TMA tissue array for H&E stain and immunohistochemistry stains.
Intratubular spread of urothelial carcinoma

**Table 1. Pathological features of different types of intratubular spread**

<table>
<thead>
<tr>
<th>Type</th>
<th>Location</th>
<th># of tubules involvement</th>
<th>Recognizable on H&amp;E architecture</th>
<th>Association of grade</th>
<th>Association of invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical</td>
<td>Close to calyx</td>
<td>Few</td>
<td>Yes</td>
<td>Tubular, pseudoglandular</td>
<td>High/Low</td>
</tr>
<tr>
<td>Pagetoid</td>
<td>Close to calyx</td>
<td>Few</td>
<td>Maybe</td>
<td>Pagetoid, few cells</td>
<td>High/Low</td>
</tr>
<tr>
<td>Florid</td>
<td>Extended to medulla/cortex junction</td>
<td>More than 10</td>
<td>Yes</td>
<td>Pseudoglandular, solid nest with smooth contour</td>
<td>High</td>
</tr>
<tr>
<td>Invasion from tubular spread</td>
<td>May reach cortex</td>
<td>Multi-foci, more than 10 on each focus</td>
<td>Yes</td>
<td>Solid nest with irregular contour</td>
<td>High</td>
</tr>
</tbody>
</table>

**Table 2. Clinical-pathological features of the cases**

<table>
<thead>
<tr>
<th></th>
<th>All Cases</th>
<th>Cases with IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Age</td>
<td>70.7 yrs (39-88)</td>
<td>67.6 yrs (50-84)</td>
</tr>
<tr>
<td>Right/Left</td>
<td>22/31</td>
<td>11/17</td>
</tr>
<tr>
<td>Mean tumor size</td>
<td>3.76 cm (0.4-10 cm)</td>
<td>3.76 cm (0.4-10 cm)</td>
</tr>
<tr>
<td>Multifocality</td>
<td>18 (33.9%)</td>
<td>6 (35.3%)</td>
</tr>
<tr>
<td>Low grade</td>
<td>12 (22.7%)</td>
<td>3 (17.6%)</td>
</tr>
<tr>
<td>High grade</td>
<td>41 (77.3%)</td>
<td>14 (82.4%)</td>
</tr>
<tr>
<td>Stage:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pTa</td>
<td>17 (32%)</td>
<td>4 (23.5%)</td>
</tr>
<tr>
<td>pT1</td>
<td>3 (5.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>pT2</td>
<td>7 (13.2%)</td>
<td>1 (5.9%)</td>
</tr>
<tr>
<td>pT3</td>
<td>23 (43.4%)</td>
<td>11 (64.7%)</td>
</tr>
<tr>
<td>pT4</td>
<td>3 (5.7%)</td>
<td>1 (5.9%)</td>
</tr>
</tbody>
</table>

**Result**

**Clinical and pathological information**

A total of 53 cases of upper urinary tract urothelial carcinoma were identified. The important clinical findings are summarized in Table 1. The mean age of the patients was 70.7 years (range, 39-88 years). Majority of the patients were males (41/53, 77.4%) and the remaining 12 (22.6%) were females and the male:female ratio is 3.4:1. The mean tumor size was 3.76 cm (range, 0.4-10 cm). Forty-one (77.4%) cases were high grade urothelial carcinoma and the remaining 12 (22.6%) were low-grade. We did not identify any papillary urothelial neoplasm of low malignant potential (PUNLMP). The majority of the cases were staged pTa, 17 cases (32%) and pT3, 23 cases (43%).

Seventeen cases (31.5%) showed intratubular spread by H&E stain and/or the cocktail stain. The clinical features of these 17 cases are also summarized in Table 1. The majority of the cases were seen in males with a male:female ratio of 16:1. The mean age at presentation (67.6 years) is similar to the overall presentation and the left kidney (11 cases, 64.7%) is affected more than the right kidney (6 cases/35.3%). All 17 cases with tumor involvement of the renal calyx; 14 of 17 (82.4%) were high grade urothelial carcinoma and the remaining 3 (17.6%) cases were low grade; 4 of 17 cases (23.5%) were non-invasive.

**Histopathological features of intratubular spread**

Of these 53 UUT-UCs cases, 17 presented with intratubular spread. We further classified intratubular spread into 4 different sub-types: pagetoid; typical; florid; and secondary invasion from intratubular spread (Table 2). In order to study intratubular spread better, we developed a cocktail stain which included PAX8 and p63 IHC stains and PAS special stain. This cocktail stain was designed so PAX8 would highlight renal tubule cells, P63 will label urothelial carcinoma cells, and PAS will stain basement membrane; the cocktail thus can be used to evaluate tumor invasion from intratubular spread.

Pagetoid intratubular spread (Figure 1A & 1B) involves only a few renal tubules which are usually close to the calyx. The involved tubules contain predominantly renal tubule cells with only a few or even single urothelial carcinoma cell(s). The renal tubules maintain the usual morphology without dramatic distortion or extension. The urothelial carcinoma cell(s) are usually located between renal tubular cells and basement membrane. This type of IS can be identified by H&E section. However, recut and the cocktail stain can increase the yield of detection. In our study series, there are 4 cases of pagetoid spread; 2 of them are low grade; 2 of...
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Figure 1. Histopathological features and cocktail stains of 4 different types intratubular spreading (IS). A: Pagetoid IS. The involved tubules contain predominantly renal tubule cells with only a few urothelial carcinoma cells. The renal tubules maintain the usual morphology without dramatic distortion or expansion. C: Classical IS. The involved tubules are extended and contain predominantly urothelial carcinoma cells with the architecture ranging from tubular, pseudoglandular to solid nest and some showing contraction effect around the smooth renal tubular space. E: Florid IS. The involved tubules are significantly enlarged and exclusively occupied by urothelial carcinoma with solid nest pattern and smooth contour. G: Secondary invasion from IS compose of both non-invasive IS and invasive component. The invasive component is usually single cell or small cluster cells with irregular contour and desmoplastic reaction. B, D, F and H: Are cocktail stain (brown: P63; Red: Pax8 and PAS stain) of corresponding IS.

Intratubular spread involves more renal tubules than does pagetoid IS, though usually less than 10-15 tubules. The involved tubules contain both renal tubule cells and predominantly urothelial carcinoma cells. The renal tubules show slightly distortion and expansion. The architecture of urothelial carcinoma cells ranges exhibits tubular, pseudoglandular, or solid nests. This type of IS can be easily spotted on H&E section. The cocktail stain only provides limited information when section is not in optimal condition due to poor tissue processing. In our series, we identified 5 cases of classical IS as follows: 1 low grade; 3 high grade and 1 carcinoma in situ. Among these five cases, only one showed invasion in other area.

Florid intratubular spread is similar to typical IS. This type of IS extensively involves the renal tubules. The urothelial carcinoma cells exclusively occupy preexisting tubular space with a solid nesting pattern and smooth contours, often with contraction effect around the smooth renal tubular space. The involved tubules usually are significantly enlarged compare to non-involved tubules. The cocktail stain was particularly useful in evaluating possible invasion from IS. In our series, we identified three cases of florid IS, all of which were high grade: one was non-invasive and two showed invasion in other area.

In contrast to the preceding 3 types of IS which are inherently non-invasive. and located in the medullary area and usually close to the calyx, the fourth subtype of IS, secondary invasion from intratubular spread, is markedly different. It represents true invasion of renal parenchyma stroma from the IS component, which can be located in either the medulla or cortex. The invasive component is usually single cells or small clusters of cells with irregular contour and desmoplastic reac-

Figure 2. Non-invasive urothelial carcinoma with glandular differentiation involving renal pelvis and showing unique morphology of intratubular spread. The urothelial carcinoma cells are columnar with glandular (A), flat (B) and papillary (not shown) pattern. Usual urothelial carcinoma in situ has been seen in other area (not shown). IS show true gland formation (C). The urothelial cells are columnar and perpendicular to basement membrane with subnuclear vacuoles mimicking secretory endometrium.
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Unusual morphological variants of urothelial carcinoma

We also carefully evaluated the morphologic features in our case series. The broad spectrums of cyto-pathological variants of UUC-UC previously described by others were also observed in our cases, including: squamous differentiation, glandular differentiation, micropapillary type, and plasmacytoid type. However, here we report a unique case of urothelial carcinoma of upper ureter. In this case, there was no grossly visible tumor in the renal pelvis. On microscopic examination, only urothelial carcinoma in situ (CIS) was seen at the renal pelvis, and urothelial carcinoma cells were columnar, forming glandular (Figure 2A), flat (Figure 2B), and papillary architectures (not shown). This morphology is diagnostic of high grade, non-invasive urothelial carcinoma with glandular differentiation [15] also known as urothelial adenocarcinoma in situ (AIS) [16], previously described only in bladder [15-17]. In current case, we have seen 3 out of 4 types of architectures (flat, glandular and papillary), except cribriform type. Interestingly, this case also showed IS. The urothelial cells completely replace tubular cells in several renal tubules and forming tubules/glands. The cytology of these cells is quite unique: the nucleus is located at center of columnar cells with supra- and sub-nucleus clearing; mimicking secretory endometrium (Figure 2C).

Since PAX8-positive urothelial carcinoma has been reported previously [18], we evaluated PAX8 staining in all of our cases using the tissue microarray. We found two cases of urothelial carcinoma were positive for PAX8, and we repeated the stains on the respective full section slides. In both cases, urothelial carcinoma cells were partially positive (10-30% of tumor cells) for PAX8 with stronger staining at peripheral tissue (Figure 3). Of note, normal urothelium was also positive for Pax8 in one of these two cases.

Discussion

Tumor stage remains the most important prognostic factor in upper urinary tract urothelial cell carcinomas (UUT-UCs) [2, 3, 7, 8, 11, 19, 20]. Intratubular Spread (IS) is a unique morphological phenomenon and also has an important impact on tumor staging [1, 20]. However, this well-recognized pathological entity has not been systematically studied. In contrast with previous impression that IS is a relatively rare and homogenous pathological phenomena, our study showed that IS is a relative common event that presents with a broad spectrum of morphological variants and potential prognostic values in UUT-UC.

In the 53 consecutive UUT-UCs cases in our series, we identified 17 cases with IS. Compared to all patients, the patients with IS showed significant male predominance (M:F=16:1 vs.
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3.4:1 in all patients); relatively younger average age (67.6 years vs. 70.7 in all patient); slightly higher association with high grade (82.4% vs. 77.3% in all patients) and increased association with deeper invasion (76.5% with above pT2 vs. 68% in all patients). Other features such as right/left distribution, average tumor size, and multifocality did not significantly differ between the group with IS and the remainder of patients in the series.

Morphologically, other entities can mimic IS. 1) Atypical renal tubules resulting from obstruction, inflammation, and/or native kidney diseases, are commonly associated with UUT-UCs and can appear similar to pagetoid type IS. The cocktail stain is very useful to distinguish between these two entities as the p63-positive and PAX8-negative UC cells are very easily identified in the cocktail stain. 2) Invasive urothelial carcinoma with nested pattern or glandular differentiation can mimic florid or typical type IS. However, invasive carcinoma can be located at either medulla or cortex or both whereas florid or typical IS usually involve only the medulla near the tip of the pyramid. Furthermore, invasive carcinoma will cause desmoplastic reaction whereas IS typically does not. The cocktail stain can also be helpful in some cases: UC cells are variably positive for p63 and usually negative for PAX8 [18]; PAS stain can help to evaluate invasion. One potential pitfall in the interpretation of the PAS stain is that some tumors can produce a PAS-positive basement membrane-like material. Carefully morphological evaluation is still the gold standard for determining invasion. 3) Renal cell carcinoma (RCC) with tubular differentiation (e.g. collecting duct cell carcinoma [18], mucinous tubular and spindle cell carcinoma) can also be confused with IS. Several hints can help to distinguish these two entities. First, a lower grade or non-invasive portion of urothelial carcinoma will favor a diagnosis of urothelial carcinoma with IS. Second, IS is usually a small portion of whole tumor and IS has different architecture from rest of tumor, whereas RCC with tubular differentiation are usually extensive with similar morphology in different areas. In the case of a high grade tumor with extensive invasion, the PAX8 and p63 IHC stains will be helpful and critical to distinguish between RCC and UC. This has been demonstrated in the previous study from Netto group [18].

The broad spectrum of morphological appearance of IS appears to be quite specific for urothelial carcinoma. We have not found any other kidney neoplasm showed intratubular spread in our practice or literature search. Recognizing this unique morphology will also help pathologists in the diagnosis of urothelial carcinoma, especially on biopsy specimens. Suspected upper tract urothelial carcinomas are usually evaluated with retrograde ureteropyelography, upper urinary tract cytology, and cystourethroscopy with biopsy. These biopsies are usually small and superficial and IS portion is unlikely to be captured. Very rarely, percutaneous biopsy will be performed on large urothelial carcinoma which is hard to distinguish from renal cell carcinoma radiologically. This type of biopsy will have a higher chance of capturing IS. Although there is no mention in the literature of IS present on a biopsy specimen, we found it discussed in the blog of one patient (http://www.debbisjourney.com/?cat=5). According to the pathology report description, this biopsy showed urothelial carcinoma with intratubular spread and probably florid type. Our study indicates that florid IS is frequently associated with high grade urothelial carcinoma with conventional invasion in other areas. Even there is no evidence of invasion in the biopsy, we can predict that the possibility of invasion in this patient is high.

The incidence of IS in our study is much higher than expected or reported in literature. This is partially explained by our inclusion of the two extreme ends of this entity: pagetoid IS and secondary invasion from IS. Pagetoid IS is often very subtle and sometimes is hard to be definitely diagnosed on H&E sections. Indeed, at least in 2 of our cases, diagnosis depended on a cocktail stain. This type of IS usually does not present with invasion in other areas. In real practice, pagetoid IS has been ignored and its incidence is underestimated. Conversely, invasion from IS represents true invasion and was always associated with conventional invasion in other areas. This type of IS has also has been overlooked, likely because of the presence of more obvious invasion elsewhere in the specimen.

Secondary invasion from IS is an interesting and distinct progressive type of IS and is newly proposed by us in this study. It is truly invasive with unclear staging ramifications. While the
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invasive urothelial carcinoma cells just microscopically break through the renal tubular basement membrane and could thus be interpreted as pT1, their extension into renal parenchyma could also be considered as pT3 [1, 12]. This dilemma mirrors previous staging challenges of UUT-UC, which arose from the fact that the presence and thickness of epithelial layers in the renal pelvis and ureter are widely variable. In this study, we have identified 5 cases with secondary invasion from IS which are all high grade with deeper invasion (pT3 or pT4) in other areas. Therefore, we recommend that it is appropriate to categorize secondary invasion from IS as pT3. However, it will be interesting to study and to see any prognostic differences between cases with pure secondary invasion from IS vs. conventional pT3 invasion in large cohorts.

The diagnosis of IS can largely be relied on by careful evaluation of H&E section. The cocktail stain with PAX8, p63 and PAS has certain diagnostic value on some cases. However, the pathologist has to be aware some pitfalls of this stain: First, it is well known that urothelial carcinoma; especially high grade or variant differentiations can be partially or completely negative for p63. Secondly, some rare urothelial carcinomas can be positive for PAX8. In our study, we found 2 cases to be positive for PAX8. Third, invasive urothelial carcinoma can produce basement membrane-like material which is positive for PAS and mimic non-invasion.

In summary, we systemically studied intratubular spread (IS) in upper urinary tract-urothelial carcinoma (UUT-UC) and found IS presence in about 30% of all cases and demonstrated a broad spectrum of morphological and prognostic features. Together with careful evaluation of H&E sections, the cocktail stain (p63, PAX8 and PAS) can be a valuable test for evaluation of intratubular spread.

Acknowledgements

The author would like to thank Ms. Olena Ardacheva for technical support. This study is supported by resident research fund from Westchester Medical Center, Pathology Department. We would like to dedicate this work to Dr. Myron Melamed.

Disclosure of conflict of interest

All authors declare no conflict of interest.

Address correspondence to: Dr. Minghao Zhong, Department of Pathology, Westchester Medical Center, New York Medical College, 100 Woods Road, Valhalla, NY 10595, USA. E-mail: ZhongM@WCMC.com

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