Prospective Comparison of Molecular Signatures in Urothelial Cancer of the Bladder and the Upper Urinary Tract—Is There Evidence for Discordant Biology?

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Abbreviations and Acronyms

BC = urothelial carcinoma of the bladder
CSS = cancer specific survival
LND = lymph node dissection
LVI = lymphovascular invasion
MS = biomarker score
RC = radical cystectomy
RFS = recurrence-free survival
RNU = radical nephroureterectomy
UC = urothelial carcinoma
UTUC = upper tract urothelial carcinoma

Purpose: Upper tract urothelial carcinoma is rare and less well studied than bladder cancer. It remains questionable if findings in bladder cancer can safely be extrapolated to upper tract urothelial carcinoma. We prospectively evaluate molecular profiles of upper tract urothelial carcinoma and bladder cancer using a cell cycle biomarker panel.

Materials and Methods: Immunohistochemical staining for p21, p27, p53, cyclin E and Ki-67 was prospectively performed for 96 patients with upper tract urothelial carcinoma and 159 patients with bladder cancer with nonmetastatic high grade urothelial carcinoma treated with extirpative surgery. Data were compared between the groups according to pathological stage. Primary outcome was assessment of differences in marker expression. Secondary outcome was difference in survival according to marker status.

Results: During a median followup of 22.0 months 31.2% of patients with upper tract urothelial carcinoma and 28.3% of patients with bladder cancer had disease recurrence, and 20.8% and 27.7% died of upper tract urothelial carcinoma and bladder cancer, respectively. The number of altered markers was not significantly different between the study groups. Overall 34 patients (35.4%) with upper tract urothelial carcinoma and 62 (39.0%) with bladder cancer had an unfavorable marker score (more than 2 markers altered). There were no significant differences between upper tract urothelial carcinoma and bladder cancer in the alteration status of markers, the number of altered markers and biomarker score when substratified by pathological stage. There were no

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significant differences in survival outcomes between patients with upper tract urothelial carcinoma and those with bladder cancer according to the number of altered markers and biomarker score.

Conclusions: Our results demonstrate the molecular similarity of upper tract urothelial carcinoma and bladder cancer in terms of cell cycle and proliferative tissue markers. These findings have important implications and support the further extrapolation of treatment paradigms established in bladder cancer to upper tract urothelial carcinoma.

Key Words: urinary bladder neoplasms; biological markers; carcinoma, transitional cell

UROTHELIAL carcinoma is a disease of the entire urothelium that may occur anywhere along the urinary tract from the renal pelvis to the urethra. The majority of UC arises in the bladder, whereas only about 5% to 10% of UC occurs in the upper urinary tract.[1] The rarity of upper tract urothelial carcinoma leads to difficulties in producing high quality evidence for clinical decision making. As such, findings from bladder cancer, which is more common and better studied than UTUC, are often extrapolated to UTUC. However, this is done without sufficient knowledge of the similarities or differences between UTUC and BC. Furthermore, anatomical differences in UTUC and BC produce unique diagnostic and therapeutic challenges with respect to accurate staging, intraluminal therapy, lymphadenectomy and integration of systemic chemotherapy in patients with UTUC.

Controversy remains whether upper tract and lower tract UC have inherently different biological behavior, and whether anatomical location really impacts clinical outcomes.[2–4] To date, to our knowledge, no prospectively collected molecular data are available to address this question. Therefore, we conducted a molecular comparison of BC and UTUC using a validated marker panel of cell cycle regulators and markers of proliferation.[5]

MATERIALS AND METHODS

This prospective study was performed after receiving institutional review board approval at UT Southwestern Medical Center Dallas, Texas. Immunohistochemical staining for p21, p27, p53, cyclin E and Ki-67 was performed of pathological specimens of 159 patients with nonmetastatic high grade UC of the bladder after undergoing radical cystectomy and bilateral LND. The same marker panel was evaluated using 96 consecutive pathological specimens from patients with nonmetastatic high grade UTUC who underwent radical nephroureterectomy or partial ureterectomy.

Each patient was generally seen at least every 3 to 4 months in the first year, semiannually in the second year and annually after that. Data points were entered into institutional review board approved standardized forms and transferred into 1 database. Reviews and quality assurance checks were performed multiple times throughout the data collection. A genitourinary pathologist assessed the pathological characteristics (confirmation of transitional cell carcinoma histology; confirmation of high grade, stage, lymphovascular invasion; presence of carcinoma in situ and other tumor characteristics) using the American Joint Committee on Cancer 2010 TNM staging system.[6]

Immunohistochemical staining of serial sections from the same paraffin embedded tumor block was performed for p21, p27, p53, cyclin E and Ki-67 using a Dako Autostainer (Dako North America Inc., Carpinteria, California) as previously described.[5,7] Bright-field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System, Clarient, Aliso Viejo, California) was used for automated scoring. Cutoff points to determine alteration of the specified markers were determined previously.[5] p21 was considered altered if immunoreactivity of staining was less than 10%, p27 and cyclin E were considered altered when nuclear staining was less than 30%, p53 was considered altered if nuclear activity demonstrated 10% or greater staining and Ki-67 was considered altered if samples showed more than 10% staining. Based on the number of altered markers, a prognostic biomarker score was calculated as reported before.[5] The prognostic MS was considered favorable if 2 or fewer markers were altered and was considered unfavorable if more than 2 markers were altered.[7]

Subgroup analyses were conducted in a stage for stage manner between patients with UTUC and those with BC. Fisher's exact test and the chi-square test were used to assess the association among expression of markers, number of altered markers, MS, and UTUC and BC. Kaplan-Meier analysis was performed to calculate survival functions and survival estimates. Differences were assessed using the log rank statistic. Significance was defined as $p \leq 0.05$. All reported p values are 2 sided. Analyses were conducted with SPSS® (version 19).

RESULTS

Patient demographics are shown in table 1. Of the patients with UTUC 52 (54.2%) underwent LND during surgery whereas 159 (100%) patients with BC underwent LND ($p < 0.001$). The mean number of lymph nodes removed was 6.8 (range 0 to 33) in