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Original article

Feasibility of obtaining biomarker profiles from endoscopic biopsy specimens in upper tract urothelial carcinoma: Preliminary results

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Abstract

Objective: To prospectively evaluate the feasibility of obtaining a reliable histochemical assessment of cell cycle biomarkers from endoscopic biopsy specimens of patients with upper tract urothelial cancer.

Methods: Overall, 17 patients were identified who had an available biopsy as well as those who underwent subsequent radical nephroureterectomy (RNU) or segmental ureterectomy (SU) for clinically localized high-grade upper tract urothelial cancer of the renal pelvis or ureter. Of those 17 patients, 15 (88%) had sufficient tissue to undergo immunohistochemical staining. Biopsies were obtained using various endoscopic techniques. Tumor characteristics were recorded and prospectively evaluated for immunohistochemical expression of 5 biomarkers: p21, p27, p53, cyclin E, and Ki67/pRb. Unfavorable prognostic score (PS) was defined as >2 altered markers.

Results: The median age of the patients was 68 years (range: 53–82 y) with 87% being males. Of the 15 specimens, 9 (60%) tumors were organ confined ($T \leq 2$ and N0), and all were high grade. Of the 15 patients, 4 (27%), 7 (46.6%), 3 (20%), and 1 (6.7%) individuals had 1, 2, 3, and 5 markers altered on biopsy marker profiling, respectively, with Ki67 being the most frequent alteration (13/15; 87.7%).

An overall concordance rate of 60% (9/15) was seen between biopsy and RNU/SU PS. Those patients with favorable biopsy biomarker PS were less likely to display adverse pathological features, with organ-confined disease in 7/11 (63.6%) patients and 9/11 (81.8%) being free of carcinoma in situ in the final specimen. Additionally, 10/11 (91%) had no evidence of necrosis and 7/11 (64%) had no evidence of lymphovascular invasion on final pathologic evaluation.

Conclusions: Preliminary results suggest that obtaining interpretable biomarker profile of ureteroscopic biopsy specimens is feasible. Tumor heterogeneity and limited biopsy material may account for the discordance between biopsy and RNU/SU specimens. Meaningful biopsy biomarker profiling could serve as a powerful tool for individualizing treatment regimens and augmenting current predictive variables. Further studies are needed to evaluate clinical applicability.

Keywords: Upper tract urothelial carcinoma; Cell cycle; Biomarkers

1. Introduction

Upper tract urothelial cancer (UTUC) comprises roughly 5% of all urothelial cancers [1]. At presentation, 30% of patients will demonstrate invasive disease or locally advanced disease, or both, and up to 20% will harbor metastatic disease [1–3]. Nearly 45% to 60% of patients with advanced disease will develop disease progression following surgery [2,4]. Despite available advancements in the treatment of UTUC, 5-year cancer-specific survival (CSS) rates for patients with advanced UTUC continue to be poor, ranging from 12.2% to 74.7% [2].

Current pathological prognostic factors such as stage, grade, tumor location, and lymphovascular invasion (LVI) provide important prognostic information but are not reliably ascertainable before extirpative surgery and fail to capture individual biological variability of tumors [5].

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Over the past few years, the importance of elucidating molecular markers that have the ability to probe the biological heterogeneity of tumors has gained tremendous momentum. Several studies by Shariat and Lotan have described the utility of a panel of cell cycle regulators (p53, p21, p27, and cyclin E) and proliferative molecular markers (Ki67) in accurately predicting disease-free survival and CSS in patients with urothelial bladder cancer [6–9]. Furthermore, a similar panel of tissue biomarkers obtained from transurethral resection specimens was shown to correlate with non–organ-confined disease at the time of radical cystectomy [10]. One of the main problems in appropriately counseling patients with UTUC is the inadequacy of clinical staging owing to small amounts of tissue obtained at diagnosis and the inability to completely resect the tumor endoscopically. As such, useful pathological features such as depth of penetration, concomitant carcinoma in situ (CIS), necrosis, and presence of LVI are difficult to assess before extirpative surgery. Consequently, appropriate selection of patients for multimodal therapy is difficult until after surgical extirpation.

In this pilot study, we sought to evaluate the feasibility of obtaining an interpretable biomarker panel in the ureteroscopic biopsy specimens of patients with UTUC. In addition, correlation between pathological features and biopsy marker panel prognostic score (PS) was assessed.

2. Material and methods

2.1. Patient population

In total, 17 patients were identified between January 2006 to February 2012 who had an available biopsy as well as who also underwent subsequent open or laparoscopic radical nephroureterectomy (RNU) or segmental ureterectomy (SU) with or without regional lymphadenectomy for clinically localized biopsy-proven high-grade UTUC of the renal pelvis or ureter.

Of those 17 patients, 15 (88%) had sufficient tissue to undergo immunohistochemical staining. Recurrences in the bladder were coded as second primaries and not as local or distant recurrences.

Biopsy specimens were obtained using endoscopic techniques. Patient and tumor characteristics were recorded into an institutional review board–approved database; primary tumors and biopsy specimens were prospectively evaluated for immunohistochemical expression of a panel of cell cycle and proliferative markers such as p21, p27, p53, cyclin E, and Ki67/pRb. In general, our pathologists require at least enough biopsy tissue to view tumor occupying at least 5 cells per high power field.

2.2. Immunohistochemistry and scoring

Immunohistochemical staining from paraffin-embedded tumor blocks were performed for cyclin E1, Tp53, CDKN1A, p27, and MKI67 employing the Dako Autostainer (Dako North America Inc, Carpinteria, CA, USA). The staining and scoring protocols for all antibodies were previously described in detail [9,11,12].

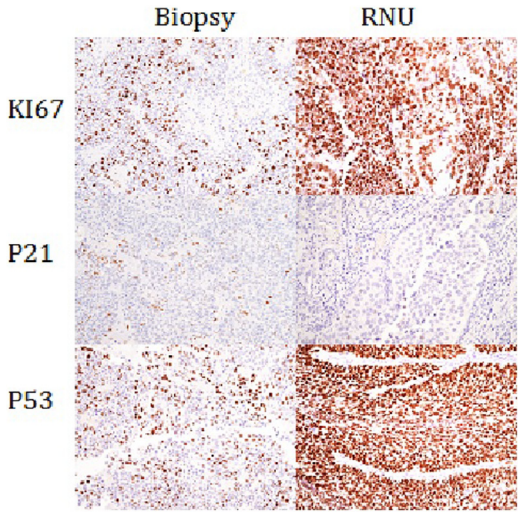

Fig. 1. Representative immunohistochemical assessment of endoscopic biopsies and surgical specimens from patients with UTUC utilizing cell cycle and proliferative biomarkers.

Briefly, specimens of normal urothelium from patients undergoing RNU/SU served as internal controls. Bright-field microscopy imaging along with an advanced color detection software (Automated Cellular Imaging System, Clarient, CA, USA) was used for semiquantitative scoring. Staining intensity and percentage of positive nuclei per area measurements were evaluated using 10 random spots within each specimen. Staining was done on tissue from the renal biopsy specimen performed as well as the RNU/SU specimens. Nuclear Tp53 immunoreactivity was altered when samples revealed >10% nuclear reactivity. CDKN1A immunoreactivity was altered when samples demonstrated <10% staining. Nuclear p27 and cyclin E were considered altered when samples demonstrated <30% nuclear reactivity. MKI67 immunoreactivity was altered when samples showed >20% nuclear reactivity (Fig. 1). Based on the number of altered biomarkers, we used a previously established PS such that altered expression of 0 to 2 biomarkers was considered favorable PS, whereas an alteration of at least 3 biomarkers signified an unfavorable PS [6,13].

3. Results

Clinicopathological data are shown in Table 1. The median age of the patients was 68 years (range: 53–82 y) with 87% of the cohort being males. Median time to recurrence was 17 months (1–58), and median follow-up was 20 months (range: 3–58).