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Genomic Profile of Urothelial Carcinoma of the Upper Tract from Ureteroscopic Biopsy: Feasibility and Validation Using Matched Radical Nephroureterectomy Specimens

Aditya Bagrodia[a,†], **Francois Audenet**[b,†], **Eugene J. Pietzak**[b], **Kwanghee Kim**[b], **Katie S. Murray**[b], **Eugene K. Cha**[b], **John P. Sfakianos**[c], **Gopa Iyer**[d], **Nirmish Singla**[a], **Maria Arcila**[e], **Bernard H. Bochner**[b], **Hikmat A. Al-Ahmadie**[e], **David B. Solit**[d], **Jonathan A. Coleman**[b,*]

[a]Department of Urology, UT Southwestern Medical Center, Dallas, TX, USA

[b]Department of Surgery, Urology Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[c]Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY USA

[d]Department of Medicine, Genitourinary Oncology Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[e]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Urothelial carcinoma of the upper tract (UTUC) presents specific challenges regarding accurate staging and tumor sampling. We aimed to assess the feasibility of applying next-generation sequencing to biopsy specimens and gauged the concordance of their genetic profiles with matched radical nephroureterectomy (RNU) specimens. Of the 39 biopsy specimens collected, 36

*Corresponding author. Department of Surgery, Urology Service, Kimmel Center for Prostate and Urologic Cancers, Memorial Sloan Kettering Cancer Center, 353 E. 68th Street, New York, NY 10065, USA. Tel. +1 646 4224432; Fax: +1 212 4523323. colemanj@mskcc.org (J.A. Coleman).

†These authors contributed equally to this manuscript.

Author contributions: Aditya Bagrodia had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Bagrodia, Audenet, Iyer, Solit, Coleman.

Acquisition of data: Bagrodia, Audenet, Pietzak, Kim, Murray, Cha, Arcila, Sfakianos, Iyer, Singla, Al-Ahmadie.

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(92%) had adequate material for sequencing using a hybridization-based exon capture assay (MSK-IMPACT). The most frequently altered genes across the patient cohort were consistent with the urothelial carcinoma-associated alterations identified in a cohort of 130 RNU specimens previously sequenced at our center, including mutations in the *TERT* promoter (64%), hotspot activating mutations in *FGFR3* (64%), and frequent mutations in chromatin remodeling genes. For 12 patients, a matching tumor sample from a subsequent RNU was sequenced. We found a high level of concordance between matched biopsy and RNU specimens, up to 92% for the likely pathogenic alterations.

Patient summary:

We evaluated the feasibility of genomic characterization of tumor tissue collected at the time of ureteroscopic biopsy and found high concordance with subsequent radical nephroureterectomy specimens. Molecular characterization of urothelial carcinoma of the upper tract biopsies could guide treatment decision-making and identify high-risk patients who could benefit from neoadjuvant chemotherapy and low-risk patients who could benefit from conservative or organ-sparing strategies.

Keywords

Upper tract urothelial carcinoma; Genomics; Prediction; Biomarkers; Transitional cell carcinoma

Urothelial carcinoma of the upper tract (UTUC) comprises 5–10% of all urothelial carcinomas and presents distinct challenges compared to bladder cancer [1]. Specifically, obtaining representative diagnostic biopsies and accurate staging are extremely difficult with current endoscopic techniques and cross-sectional imaging. Owing to these limitations, patients typically undergo radical nephroureterectomy (RNU), even though some could probably be managed conservatively if predictive information could be derived from pathological and/or molecular characterization [2]. Furthermore, there is growing evidence of the benefit of perioperative chemotherapy for patients with muscle-invasive UTUC but us use has been limited by the lack of predictive biomarkers for response and treatment benefit [3]. Patients with UTUC are often precluded from receiving adjuvant cisplatin-based chemotherapy because of poor renal function following surgery [4].

We previously identified genetic signatures in RNU specimens that are associated with adverse pathologic features [5]. Applied to diagnostic biopsy specimens patients with UTUC before treatment, such prognostic molecular data may help to identify a subset of high-risk patients most likely to derive benefit from neoadjuvant chemotherapy (NAC). Furthermore, the literature demonstrates that biomarker-based identification of biologic pathways involved in urothelial carcinogenesis can be used to predict response to systemic treatment [6].

In this study we sought to assess the feasibility of next-generation sequencing using the limited tumor material collected at the time of diagnostic ureteroscopic biopsy and to gauge the concordance of genetic profiles with subsequent primary RNU specimens from the same individuals. In the pilot phase of this study, we established that an adequate quantity of high-quality DNA could be extracted from diagnostic ureteroscopic biopsy samples. To do so, we

retrospectively requested formalin-fixed and paraffin-embedded blocks of UTUC biopsies available from our institutions biorepository (n = 18). All were deemed to have adequate tumor content (>40%) on pathologic re-review and were submitted for DNA extraction and sequencing. To augment the 18 patients from this retrospective cohort, we enrolled 21 patients on a prospective tissue procurement protocol and sequenced their UTUC biopsy specimens prospectively.

All biopsies were obtained using ureteroscopic techniques. An effort was made to remove large pieces of interact tissue by performing laser tissue excision or using 3.2F cup biopsy forceps. Hematoxylin and eosin stains were reviewed to confirm the histologic diagnosis and to select the most appropriate sections for molecular characterization before DNA extraction. Matching blood was used as a source of germline DNA. Following extraction, both tumor and germline DNA was sequenced using a hybridization-based exon capture assay (MSK-IMPACT) as previously described [7]. The version of MSK-IMPACT used was capable of identifying missense mutations, small insertions and deletions, copy number alterations (CNAs), and select fusions (including *FGFR3:TACC3*) in 410 cancer-related genes.

In total, 39 samples were identified and 36 (92%) had adequate material for sequencing, with a median DNA yield of 157 ng (interquartile range 31–574; Supplementary Table 1). The mean coverage for all tumors was 749×. A total of 1147 nonsynonymous mutations and 79 CNAs were identified in the cohort. The mean number of somatic alterations (mutations and copy number) per patient was 34 (range 3–416). The most frequently altered genes across the cohort are shown in Figure 1. *TERT* and *FGFR3* were the two most frequently altered genes, with mutations observed in 64% of cases (23 of 36). Consistent with prior studies [8,9], chromatin remodeling genes including *KMT2D* (56%), *KDM6A* (47%), *KMT2C* (33%), *ARID1A* (31%), and *CREBBP* (31%) were commonly mutated, with most mutations in these genes predicted to result in protein inactivation. Alteration of *TP53* was found in 25% of cases.

To confirm that the cohort of ureteroscopic biopsies analyzed was representative of the broader population of patients with UTUC, we compared the molecular landscape of the biopsies to a cohort of 130 RNU specimens previously sequenced at our institution using the same MSK-IMPACT assay. The two cohorts were highly similar. Of the 20 most frequently mutated genes, only five genes exhibited statistically significant differences in alteration prevalence between the ureteroscopic biopsy and RNU cohorts according to univariate analysis: *FGFR3* (64% vs 45%;p = 0.041), *KMT2D* (56% vs 37%; p = 0.044); *KMT2A* (22% vs 6%; p = 0.004), *PTPRS* (19% vs 5%; p = 0.003), and *MSH2* (19% vs 5%; p = 0.007) were all more frequently altered in the biopsy cohort. Some of these differences could be explained by a higher frequency of low-grade tumors in the biopsy cohort (34% vs 17%; p = 0.035).

To determine whether intratumoral heterogeneity or intervening tumor evolution could be a source of molecular discordance between biopsy samples collected at diagnostic ureteroscopy and bulk tumor collected at RNU, we compared the molecular profiles of biopsy specimens and matching tumor samples from RNU for 12 patients with available

material. Of these patients, 7/12 received NAC after the biopsy was performed. For the patients who did not receive NAC, 71% of all mutations and 92% of likely pathogenic mutations were present in both the biopsy and RNU specimens (Fig. 2). We found 100% concordance in 2/5 patients. For the patients who received NAC, the concordance was lower (53% and 62% for all and likely pathogenic mutations, respectively), and may have been the result of outgrowth of a drug-resistant population under the selective pressure of chemotherapy [10]. Nevertheless, 82% of the likely oncogenic mutations present in the RNU or metastasis specimen were found in the prior biopsy (Supplementary Fig 1).

In conclusion, genomic profiling of biopsy specimens is technically feasible via next-generation sequencing for the majority of patients (92%). This study was limited by the number of matched RNU specimen available and the absence of correlation between genomic alterations from the biopsy and the outcomes because of the small number of events. However, it is the first study to evaluate genomic characterization from UTUC biopsy and supports the validity of genomic concordance between biopsy material and primary UTUC tumors, which could be used to guide treatment decisions in the preoperative setting. Evaluation of urinary cell-free DNA could provide similar information using noninvasive collecting techniques and is under investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Take Home Message

Urothelial carcinoma of the upper tract presents specific challenges for diagnosis and treatment strategies. We demonstrated that next-generation sequencing is feasible for ureteroscopic biopsy specimens and concordant with radical nephroureterectomy specimens. This could guide treatment decisions.

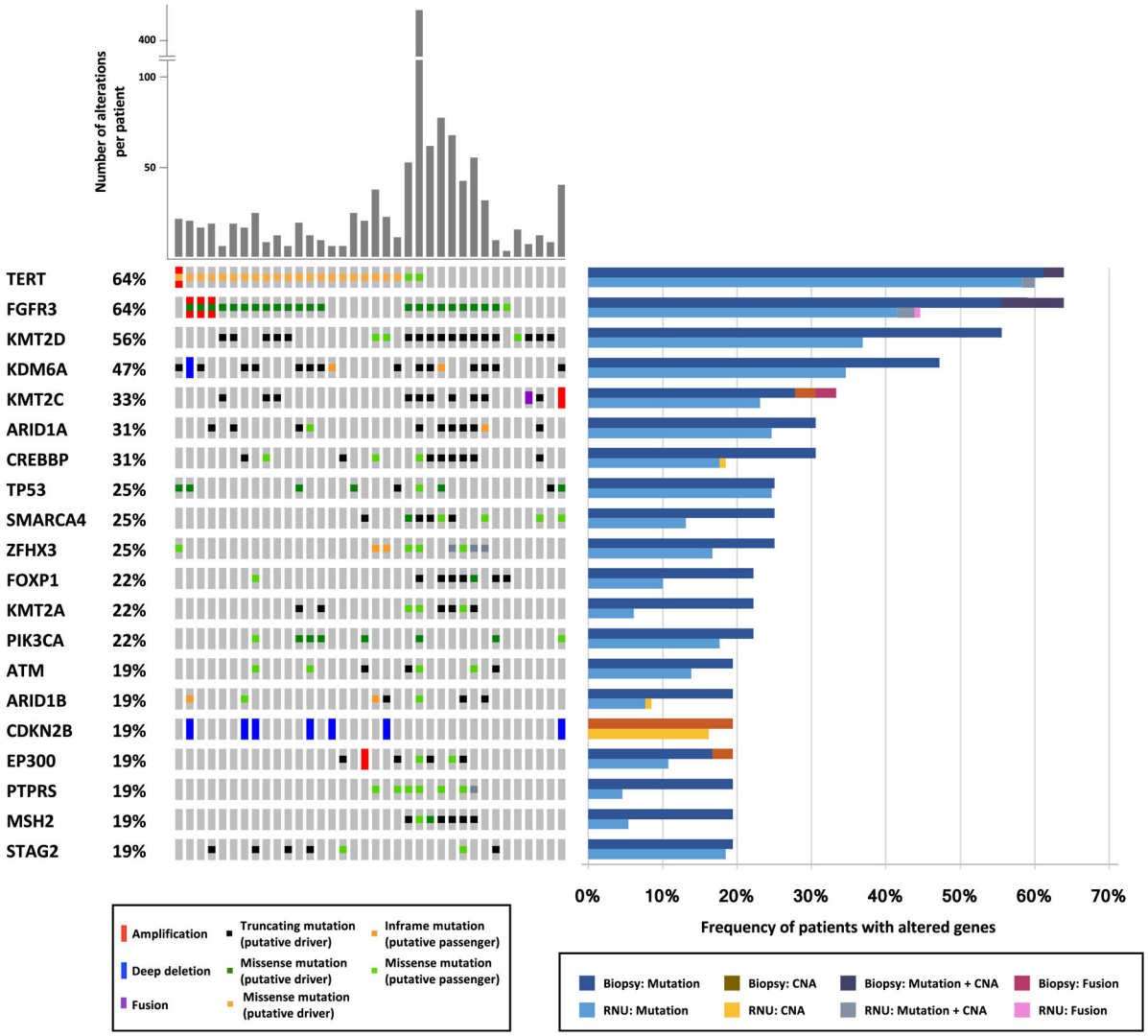

Fig. 1 –.

Oncoprint of the most frequently altered genes for 36 biopsy samples successfully sequenced using MSK-IMPACT for 410 cancer-related genes. The frequency of patients with altered genes is compared to a cohort of 130 radical nephroureterectomy specimens sequenced using the same assay.

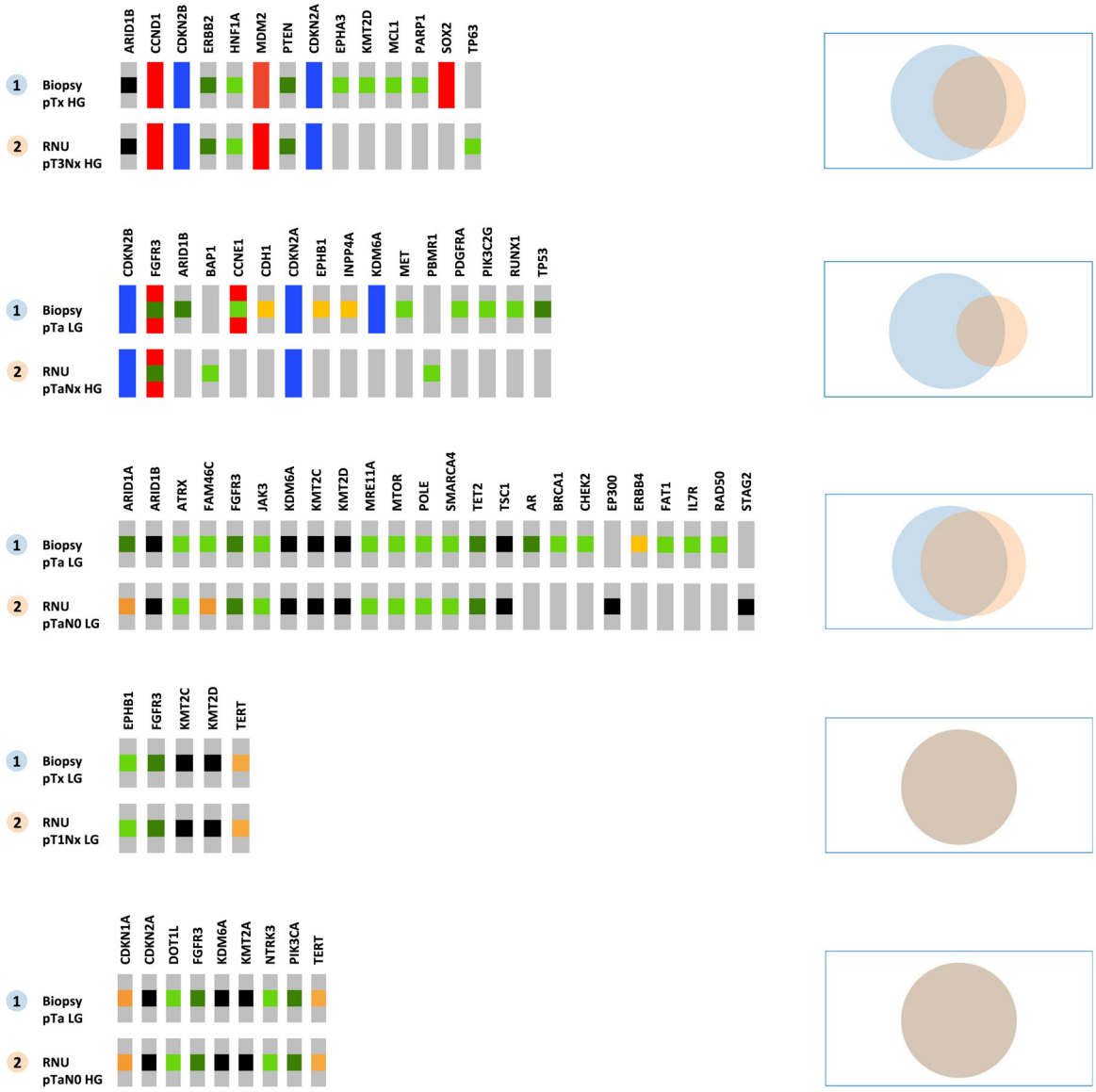

Fig. 2 –.

Oncoprints and Venn diagrams for matched-pair comparison of biopsy and radical nephroureterectomy specimens from patients who did not receive neoadjuvant chemotherapy.