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Urothelial Cancer

Comprehensive Genomic Characterization of Upper Tract Urothelial Carcinoma

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Abstract

Background: Upper urinary tract urothelial cancer (UTUC) may have unique etiologic and genomic factors compared to bladder cancer.
Objective: To characterize the genomic landscape of UTUC and provide insights into its biology using comprehensive integrated genomic analyses.
Design, setting, and participants: We collected 31 untreated snap-frozen UTUC samples from two institutions and carried out whole-exome sequencing (WES) of DNA, RNA sequencing (RNAseq), and protein analysis.
Outcome measurements and statistical analysis: Adjusting for batch effects, consensus mutation calls from independent pipelines identified DNA mutations, gene expression clusters using unsupervised consensus hierarchical clustering (UCHC), and protein expression levels that were correlated with relevant clinical variables, The Cancer Genome Atlas, and other published data.
Results and limitations: WES identified mutations in *FGFR3* (74.1%; 92% low-grade, 60% high-grade), *KMT2D* (44.4%), *PIK3CA* (25.9%), and *TP53* (22.2%). APOBEC and CpG were the most common mutational signatures. UCHC of RNAseq data segregated samples into four molecular subtypes with the following characteristics. Cluster 1: no *PIK3CA* mutations, nonsmokers, high-grade <pT2 tumors, high recurrences. Cluster 2: 100% *FGFR3* mutations, low-grade tumors, tobacco use, noninvasive disease, no bladder recurrences. Cluster 3: 100% *FGFR3* mutations, 71% *PIK3CA*, no *TP53* mutations, five bladder recurrences, tobacco use, tumors all <pT2. Cluster 4: *KMT2D* (62.5%), *FGFR3* (50%), *TP53* (50%) mutations, no *PIK3CA* mutations, high-grade pT2+ disease, tobacco use, carcinoma in situ, shorter survival. We identified a novel *SH3KBP1-CNTNAP5* fusion.
Conclusions: Mutations in UTUC occur at differing frequencies from bladder cancer, with four unique molecular and clinical subtypes. A novel SH3KBP1 fusion regulates RTK signaling. Further studies are needed to validate the described subtypes, explore their responses to therapy, and better define the novel fusion mutation.

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Patient summary: We conducted a comprehensive study of the genetics of upper urinary tract urothelial cancer by evaluating DNA, RNA and protein expression in 31 tumors. We identified four molecular subtypes with distinct behaviors. Future studies will determine if these subtypes appear to have different responses to treatments.

1. Introduction

Upper tract urothelial carcinoma (UTUC) is histologically similar to urothelial bladder cancer yet several clinical, biological and molecular features are unique to UTUC, prompting the term *disparate twins* when considering the similarities and differences between bladder urothelial cancer and UTUC [1]. Major knowledge gaps remain in our understanding of the biology and genomic landscape of UTUC, a rare disease in Western countries but of potentially epidemic proportions in the Far East [2]. Biologically interesting features along the environmental-genetic spectrum include the strong association between known exposure to agents such as tobacco [3] and aristolochic acid [2] and genetic predisposition in patients with Lynch syndrome [4].

The Cancer Genome Atlas (TGCA) reported the mutation landscape in muscle-invasive bladder cancer, which has a high somatic mutation frequency among adult solid tumors, similar to melanoma and lung adenomas and squamous carcinomas [5]. The most common mutation was *TP53*, with frequent alterations in chromatin modifier genes (MLL2, ARID1A, KDM6A) [6,7]. Four expression-based subtypes were described, and were shown to be associated with overall survival and response to immune checkpoint inhibition and potentially cisplatin-based chemotherapy [8–10].

The largest targeted genomic study of UTUC to date evaluated 300 cancer-associated genes in 83 patients using next-generation sequencing [11]. Mutations of *FGFR3*, *CREBBP*, and *STAG2* were commonly found in low-grade tumors, while *TP53* mutations were more common in high-grade tumors. *FGFR3* mutations were observed at a similar rate in high- and low-grade tumors [6].

In this study we report the first integrated comprehensive genomic analysis of UTUC using whole exome sequencing (WES), gene expression profiling, and protein expression analysis to further characterize the genomic landscape of UTUC and provide deeper insights into the biology of this rare cancer.

2. Materials and methods

UTUC samples were obtained from 31 patients under protocols approved by institutional review boards using endoscopic biopsy or surgical resection, and were stored frozen at −80 °C. Ten samples were primary ureter and 21 were renal pelvis in origin. Histology slides were reviewed by genitourinary pathology experts at each respective institution (M.I., C.G.). All tumors were composed of conventional urothelial carcinoma, and no variant histology was present. Microdissection was not performed, as all specimens were enriched with tumor cells. Patients were excluded if they had inadequate clinical data or prior treatment, or if the histological tumor purity was <30% tumor cells. White blood cells from peripheral blood were used as a normal control for somatic mutation discovery.

Of the 31 samples, DNA was purified from 27 tumor and matched normal tissues and used for WES. RNA was purified from 28 tumors, and the polyA+ mRNA fraction was used to generate stranded cDNA libraries. Protein was extracted from 20 tumors and used in analysis via reverse-phase protein array (RPPA). Supplementary Table 1 summarizes the molecular data available for each sample.

Somatic mutations were called via a standard cancer analysis pipeline at the Baylor College of Medicine Human Genome Sequencing Center [12] and by using VARSCAN2 [13] (Supplementary methods). Copy number alterations were assessed using VARSCAN2. Microsatellite instability was evaluated in all WES samples using our previously published method, involving evaluation of insertions and deletions in sequencing reads coving regions of homopolymer, for a length of 6–10 bp [14,15]. Somatic mutation data were also used to evaluate mutation signatures in all patients [16,17].

Expression levels were computed for all genes from RNA sequencing (RNAseq) data, and consensus clustering was used to classify patients into groups according to expression patterns. Gene fusions were detected in the RNAseq data using deFuse [18] and SOAPfuse [19]. Identified fusions were validated by reverse transcriptase–polymerase chain reaction (RT-PCR).

RPPA was performed by the Functional Proteomics RPPA core facility at MD Anderson Cancer Center using standardized protocols as previously described [20]. The Supplementary material provides further details.

3. Results

3.1. Patient demographic and clinical data

Patient demographic and clinical data are shown in Table 1. The male/female ratio of 2:1 is similar to that in previous reports for UTUC [21]. The majority of patients were white and former or current smokers. The majority had high-grade tumors and 32.3% had muscle-invasive or higher-stage disease (pT2+). Recurrences that were local, distant, or in the bladder were detected in approximately half of patients during median follow-up of 20 mo (range 3–66) for living patients. Median overall survival was 18 mo (range 1–66). There was no significant difference in overall patient survival between the two institutions (log-rank test $p > 0.9$).

3.2. Genomic alterations in UTUC

WES for samples from 27 patients identified 2784 somatic mutations. Three patients exhibited a high mutation frequency. Among these, one patient had more than 750 mutations, including an *MSH2* frame-shift deletion, and mild microsatellite instability (MSI) was identified by mutational analysis. Two other patients each had more than 300 mutations, including mutations in the helicase ATP-binding domain of *ERCC2* (Supplementary Fig. 1).

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