Molecular Characterization of Upper Tract Urothelial Carcinoma for Precision Therapeutics and Non-invasive Diagnostics

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Newly developed high-throughput techniques have enabled us to classify various types of cancer into biologically and clinically meaningful subtypes. Fujii et al. recently conducted a comprehensive genomic characterization of 199 samples of upper urinary tract urothelial carcinomas (UTUCs) and provided new insights into the pathogenesis and heterogeneous nature of this largely unexplored cancer [1].

UTUCs are cancers that arise from the epithelial cells lining the renal pelvis and ureter, comprising 5%-10% of all urothelial malignancies. They are less prevalent than urothelial bladder carcinomas (UBCs) and are considered more clinically aggressive; two-thirds of UTUC patients present an invasive disease as compared to 15%-25% of UBC patients [2]. UTUCs are frequently associated with poor prognosis, which may be attributable not only to biological aggressiveness but also to the difficulty in early detection. Because minimally- or non-invasive urinary cytology is less sensitive to establishing a conclusive diagnosis for UTUCs (41% vs. 86% for UBCs), UTUCs require more invasive procedures for definitive diagnosis [3, 4]. Therefore, there is a pressing need to develop non-invasive techniques that allow for the early detection of UTUCs. Recently, sequencing of urine-derived DNA has emerged as a promising non-invasive approach for diagnosing urothelial cancer; however, limited studies have explored its potential regarding molecular diagnostics and prognostication of UTUCs [5, 6].

In 2021, Fujii et al. performed comprehensive molecular analyses of UTUCs, based on which they delineated the molecular pathogenesis of UTUCs in terms of gene mutations, copy number alterations, DNA methylation, and gene expression [1]. In addition to the comprehensive molecular characterization of UTUCs, they shed light on the diagnostic value of sequencing urine-derived DNA for non-invasive detection and molecular classification of UTUCs. The comprehensive molecular examination classified UTUCs into five molecular subtypes: TP53/MDM2-mutated, RAS gene-mutated, FGFR3-mutated, triple-negative, and hypermutated subtypes. The TP53/MDM2-mutated subtype was the largest UTUC subtype (37.7%; 75/199), characterized by TP53 mutations or MDM2 amplification, and typically enriched in invasive tumors. In accordance with a prior report [7], this subtype displayed the most aggressive phenotype, having a high frequency of metastasis (40.0%) and the shortest disease-specific survival. The FGFR3-mutated subtype, constituting 35.2% (70/199) of the cohort samples, was defined by FGFR3-hotspot mutations and characterized by a high frequency of co-occurring mutations in the TERT promoter, STAG2, PIK3CA, and KDM6A. Clinically and pathologically, this subtype was enriched for early-stage disease, low-grade histology with papillary morphology, and a favorable prognosis. The RAS gene-mutated subtype, constituting 15.1% (30/199) of the cohort, was defined by hotspot mutations in RAS family genes (i.e., HRAS, KRAS, and NRAS). This subtype harbored the lowest mutational burden and exhibited frequent mutations of TERT promoter (70.0%) and DDX17 (26.7%), along with copy number alterations involving chromosomes 3, 8, 9, 19, and 20. This subtype was characterized by patients’ smoking history, young age at diagnosis, renal pelvis localization, and squamous cell differentiation. The survival rate of the RAS gene-mutated subtype was intermediate, that is, better than that of TP53/MDM2-mutated or triple-negative subtypes and worse than that of FGFR3-mutated subtypes. The hypermutated subtype, which was defined by extremely large numbers of mutations, constituted 5.5% (11/199) of the cohort samples. This subtype was characterized by a high frequency of FGFR3 mutations and excellent disease-specific survival. Among the 11 cases of the hypermutated subtype, 8 showed biallelic defects in mismatch repair genes, and 6 of the 8 cases had a prior history of cancer, implying Lynch syndrome. The triple-negative subtype, which accounted for 6.5% (13/199) of the cohort, lacked any specific subtype-defining gene mutations (i.e., TP53/MDM2, FGFR3, and RAS genes) or hypermutations. Patients with this subtype showed a low disease-specific survival comparable to that of the TP53/MDM2-mutated subtype.

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These DNA-based molecular subtypes were highly correlated with clinicopathological features, such as tumor localization/histology, patient profile, and survival [1]. Furthermore, this novel molecular classification has clinical implications that might aid in making better therapeutic choices. With the advent of immune checkpoint inhibitors and targeted therapy, pharmaceutical intervention for urothelial carcinoma has evolved drastically. FGFR inhibitors may be effective for patients with FGFR3-mutated and hypermutated subtypes. The hypermutated and TP53/MDM2-mutated subtypes are characterized by high tumor mutation loads; therefore, patients with these subtypes may benefit from immune checkpoint inhibitors.

The cohort samples were also subclassified based on gene expression or DNA methylation signatures [1]. In line with a previous study [8], a substantial portion of the UTUC cases was characterized by increased expressions of “luminal” markers (e.g., UPIK2 and GATA3), while “basal” and “squamous” markers (e.g., KRT5 and TP63) were upregulated in the others [1]. Unsupervised clustering was performed on the basis of the DNA methylation status of tumor-specific CpG islands; however, there existed no significant association between the methylation signatures and patient survival [1].

Overall, UTUCs and UBCs have a similar mutational signature in driver genes; however, the mutation frequency of some genes, such as KMT2D, varies [1, 9]. Interestingly, the mutation frequency of KMT2D is higher in the phenotypically normal epithelium of the ureter (33%) than in that of the bladder (9%), which corresponds to the difference in the mutation frequency of KMT2D between ureter and bladder carcinomas (85% vs. 25%) [10, 11]. The shared mutation frequency between phenotypically normal ureteral and urothelial carcinoma suggests differences in field carcinization of the urothelium.

Importantly, this study revealed the feasibility of urine-derived DNA sequencing to identify molecular subtypes in UTUCs [1]. Overall, 67% of mutations and 96% of focal copy number alterations observed in primary tumors were also detected in preoperative urine samples, but none were found in postoperative urine samples from the same patients. When comparing the sensitivity and specificity for cancer detection, urine-derived DNA sequencing showed superiority (82% sensitivity and 100% specificity) over urinary cytology (33% and 89%, respectively). Due to the anatomical location of UTUCs, it is sometimes challenging to obtain adequate UTUC tissue through biopsy, contributing to the worse survival rate of UTUC patients than that of UBC patients. This urine-based “liquid biopsy” may enable us to categorize UTUC patients according to tumor molecular characteristics and provide them with the most effective therapeutic intervention. Furthermore, this non-invasive technique would assist in the therapeutic decision for inoperable UTUC patients and allow longitudinal follow-up of these patients [1], thus, helping the expansion of the frontiers of precision therapeutics and non-invasive diagnostics for UTUCs.

CONFLICT OF INTEREST

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