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ORIGINAL ARTICLE

Molecular profiling of clear cell adenocarcinoma of the urinary tract

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Abstract

Clear cell adenocarcinoma (CCA) of the urinary tract is a rare type of malignancy whose molecular profiles remain undefined. Here we reported an integrated clinicopathologic and molecular profiling analysis of four cases of clear cell adenocarcinoma arising in the urethra or the bladder. Utilizing a clinically validated 130-gene exon-sequencing assay, we identified recurrent pathogenic *PIK3CA* (p. E545K) and *KRAS* (p.G12D) variants in three of four (75%) of the cases. In addition, an *APC* variant (P.S2310X), a *TP53* variant (p.R273C), and a MYC amplification event were identified. The only CCA case without either *PIK3CA* or *KRAS* variants has a distinct pathogenesis through BK virus, demonstrated by positive BK virus PCR and SV40 immunohistochemistry. The novel finding of recurrent variants in the PI3K/AKT/mTOR pathway provides not only insights into oncogenesis but also potential clinical therapeutic targets for patients with clear cell adenocarcinoma of the urinary tract.

Keywords Clear cell adenocarcinoma · Urinary tract · Molecular profiling · *PIK3CA* · *KRAS*

Introduction

Clear cell adenocarcinoma (CCA) of the urinary tract is a rare genitourinary malignancy occurring predominantly in the bladder or urethra, with about 100 cases reported in the literature [1]. There is a female predominance, with initial clinical manifestations of hematuria, dysuria, or obstructive symptoms. This malignancy shares histological similarities to the Müllerian-type clear cell carcinoma (CCC) arising in the gynecologic tract, characterized by cuboidal neoplastic cells with pleomorphic nuclei and abundant clear cytoplasm, forming solid, tubulocystic and/or papillary architectures [2]. Urinary CCA also exhibits overlapping immunophenotypes with Müllerian CCC, conventional urothelial carcinoma, and nephrogenic adenoma. Expressions of EMA, CA-125, CK7, CK20, PAX-2, PAX-8, uroplakin III, and CD10 have been reported [3–6], making this entity a diagnostic challenge.

Several theories have been proposed regarding the pathogenesis of urinary CCA. The presence of adjacent endometriosis or Müllerian-driven structures in some cases led to the hypothesis that this malignancy could develop from Müllerian elements in the genitourinary system [7, 8]. Similarly, adjacent nephrogenic metaplasia has been reported with subsequent CCA with shared molecular signatures, suggesting a nephrogenic origin [9]. In other series, urothelial lesions (urothelial carcinoma, urothelial carcinoma in situ, cystitis glandularis) were associated with bladder CCA, and X-chromosome inactivation analysis suggested a clonal relationship between CCA and adjacent urothelial lesions [10].

Limited by the number of the cases in the literature, the prognosis of urinary CCA remains unclear: local recurrence, lymph node metastasis, distant metastasis, and disease-related mortality have been reported [8, 11]. The treatment includes surgical resection with or without adjuvant chemotherapy or radiation therapy [11]. Brachytherapy has been utilized as well [12]. No known targeted therapy has been reported due to a lack of recurrent somatic mutations.

By UroVysion fluorescent in situ hybridization (FISH), a case series of 12 urinary tract CCA reported copy number gains of chromosome 3, 7, and 17, as well as copy number loss of 9q21 in a subset of cases [10]. The copy number loss of chromosomes 9 and 17, along with a *TP53* missense mutation

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(p.H178N), has been documented in one case of bladder CCA [9]. It is known that urinary tract CCA usually show strong TP53 staining [13]. One study interrogated urethral CCA molecular perturbations by next-generation sequencing and demonstrated copy number gains (chromosome 7q, 8q, 20q, and 22q), copy number losses (chromosome 12q and 18q), fusion products (*ANKRD28-FNDC3B*, *RIPK2-OSGIN2*, *PDLIM1-LOC728558*, *HMGA2-EML5*, and *RB1-SLC15A2*), and a nonsense variant of *ATM* (p.Q1537X) [14]. Given the paucity of cases in which recurrent genetic alteration were described, the molecular signature of urinary CCA remains largely unknown.

To address the lack of data on the molecular landscape of urinary CCA, we sought to molecularly profile these tumors using a clinically validated targeted exon-sequencing assay, with the aim to provide insights into the possible pathogenesis and potential targeted therapy of CCC of the urinary tract.

Materials and methods

Case selection and review of clinicopathologic features

This study was approved by the Institutional Review Board. A retrospective review of the institutional pathology database (PowerPath) was executed and searched for any diagnosis of clear cell carcinoma from the urinary tract from 1980 to 2017. Cases from outside institutions for consultation or review for which archival tissue was unavailable were excluded. Pertinent clinical and pathologic features were extracted from electronic medical record systems. We identified a total of 4 patients by the aforementioned criteria. Hematoxylin and eosin–stained sections and associated immunohistochemical stains for each case were procured and reviewed by two pathologists, including one genitourinary pathologist who confirmed the diagnosis. TNM staging was performed according to the 2017 edition of the American Joint Committee on Cancer (AJCC) TNM staging system for the urinary bladder or urethra. In addition, urothelial carcinoma cases analyzed with Solid Tumor Actionable Mutation Panel, version 2 (STAMP v.2) for clinical reasons were identified in the database, and their mutational profiles were collected.

Targeted exon sequencing

A clinically validated targeted exon-sequencing assay (STAMP v.2) with a 130 cancer-associated gene panel was performed on urinary CCA cases after the diagnosis was confirmed by reviewing hematoxylin and eosin–stained slides. Primary tumor samples were used for cases #1, #3, and #4; both primary tumor sample and lung metastasis samples were tested for case #2. However, the DNA quality for the primary tumor from case #2 was too poor to perform the analysis. Detailed methods have been described previously [15]. Briefly, sequencing libraries were prepared using DNA extracted from archived formalin-fixed paraffin-embedded blocks. For cases with low tumor cellularity (< 10% tumor cellularity), enrichment by coring was performed. The 130 genes included in this panel are *ABL1*, *AKT1*, *ALK*, *APC*, *AR*, *ARAF*, *ARID1A*, *AURKA*, *BAP1*, *BCL2*, *BCR*, *BRAF*, *BRCA1*, *BRCA2*, *CASP8*, *CCND1*, *CCND2*, *CCND3*, *CCNE1*, *CDH1*, *CDK12*, *CDK4*, *CDK6*, *CDKN1B*, *CDKN2A*, *CDKN2B*, *CHEK2*, *CREBBP*, *CTNNB1*, *CUL3*, *DDR2*, *DNMT3*, *DPH3*, *EGFR*, *EP300*, *EPHA2*, *EPHA3*, *ERBB2*, *ERBB3*, *ERBB4*, *ESR1*, *EZH2*, *FBXW7*, *FGF3*, *FGF4*, *FGFR1*, *FGFR2*, *FGFR3*, *FLT3*, *FOXO1*, *GATA3*, *GNA11*, *GNAQ*, *GNAS*, *HGF*, *HNF1A*, *HRAS*, *IDH1*, *IDH2*, *IGF1R*, *JAK2*, *JAK3*, *KDR*, *KEAP1*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MDM2*, *MDM4*, *MED12*, *MET*, *MLH1*, *MPL*, *MSH2*, *MTOR*, *MYC*, *MYCL*, *MYCN*, *MYD88*, *NF1*, *NF2*, *NFE2L2*, *NKX2-1*, *NOTCH1*, *NRAS*, *NTRK1*, *NTRK2*, *NTRK3*, *PALB2*, *PCBP1*, *PDGFRA*, *PDGFRB*, *PIK3CA*, *PIK3R1*, *PLEKHS1*, *POLD1*, *POLE*, *PPP2R1A*, *PTCH1*, *PTEN*, *PTPN11*, *RAC1*, *RAF1*, *RB1*, *RET*, *RHEB*, *RHOA*, *RIT1*, *ROS1*, *SDHD*, *SETBP1*, *SETD2*, *SF3B1*, *SMAD4*, *SMO*, *SOX2*, *SPOP*, *SRC*, *SRSF2*, *STK11*, *TERT*, *TP53*, *TP63*, *TSC1*, *TSC2*, *U2AF1*, *VEGFA*, *VHL*, and *YAP1*. The libraries were sequenced using multiplexed, paired-end runs on the Illumina MiSeq platform (Illumina, San Diego, CA). All sequence reads were mapped to the hg19 reference genome, after which single nucleotide variants (SNV) were called and annotated by an in-house pipeline. Based on publically available resources and the primary literature, the variants were categorized into five pathogenicity groups: pathogenic variants, likely pathogenic variants, variants of unknown significance (VUS), likely benign variants, and benign variants. A normalized read depth-based approach was applied for copy number analysis.

BK virus PCR assay

Polymerase chain reaction (PCR) for the detection of BK polyomavirus has been reported previously [16]. The Altona RealStar BKV assay (Hamburg, Germany) was performed according to the manufacturer's recommendations on a Rotor-Gene Q (Qiagen, Germantown, MD) real-time PCR instrument. Cycling conditions were as follows: 95 °C for 10 min, and 45 cycles of 95 °C for 15 s and 58 °C for 60 s. The extracted DNA for targeted exon sequencing was used as input.

Immunohistochemistry stain for D2-40

Immunohistochemistry stain for D2-40 was performed using Leica Bond III Autostainer, with the D2-40 antibody from

Table 1 Clinicopathological features of urinary CCAs

	Case #1	Case #2	Case #3	Case #4
Demographics				
Age	52	31	8	39
Gender	Female	Male	Female	Female
Clinical features				
Site	Urethra	Urethra	Bladder	Urethra
Size	5.9 × 5.8 × 4.9 cm	3.0 cm	6.5 × 5.5 × 4.5 cm	6.0 × 5.4 × 5.0 cm
Gynecologic or genitourinary history	Urothelial diverticulum	None	None	Endometriosis, adenomyosis, and leiomyomata
Treatment	Neoadjuvant CT/RT(cisplatin + capecitabine with 70 Gy radiation to the mass and 50 Gy radiation to pelvic lymph nodes) followed by pelvic exenteration	Cystoprostatectomy and total urethrectomy	Partial cystectomy with en bloc resection of the umbilicus and urachus	Neoadjuvant CT (carboplatin AUC6/paciltaxel) followed by pelvic exenteration and adjuvant RT
Outcome (F/U time)	NED (52 months)	Metastasis to lung (29 months)	NED (7 months)	NED (9 months)
Pathologic features				
Specimen type	Pelvic exenteration	Radical cystectomy and urethrectomy	Partial cystectomy	Pelvic exenteration
Grade	High grade	Moderately differentiated	High grade	Moderately to poorly differentiated
Adjacent structures involvement	Bladder	Not identified	Not identified	Bladder and anterior vaginal wall
LVI	Not identified	Not identified	Not identified	Not identified
Lymph node involvement	Negative	Negative (0/4)	Negative (0/6)	Positive (2/78)
Immunohistochemistry	Positive for PAX-8 and CK7; negative for CK20 and GATA-3.	Positive for PAX-8 and negative for TTF-1. (lung metastasis)	Positive for PAX-8, SV40, CK7 (variable), SALL-4 (variable); negative for WT-1, ER and OCT 3/4.	Positive for PAX-8, CK7, AMACR, p16 (variable) and vimentin (variable); negative for GATA3, uroplakin III, CDX2, WT1, napsin, ER, PR, p40, CK20, calretinin, CD10, CD15 and CEA; wild type expression of p53.
Pathologic TNM classification	ypT4N0Mx	pT1N0M1	pT2aN0Mx	ypT4N2Mx

CT, chemotherapy; *RT*, radiation therapy; *F/U time*, follow-up length after initial diagnosis (in months); *NED*, no evidence of disease; *LVI*, lymphovascular invasion

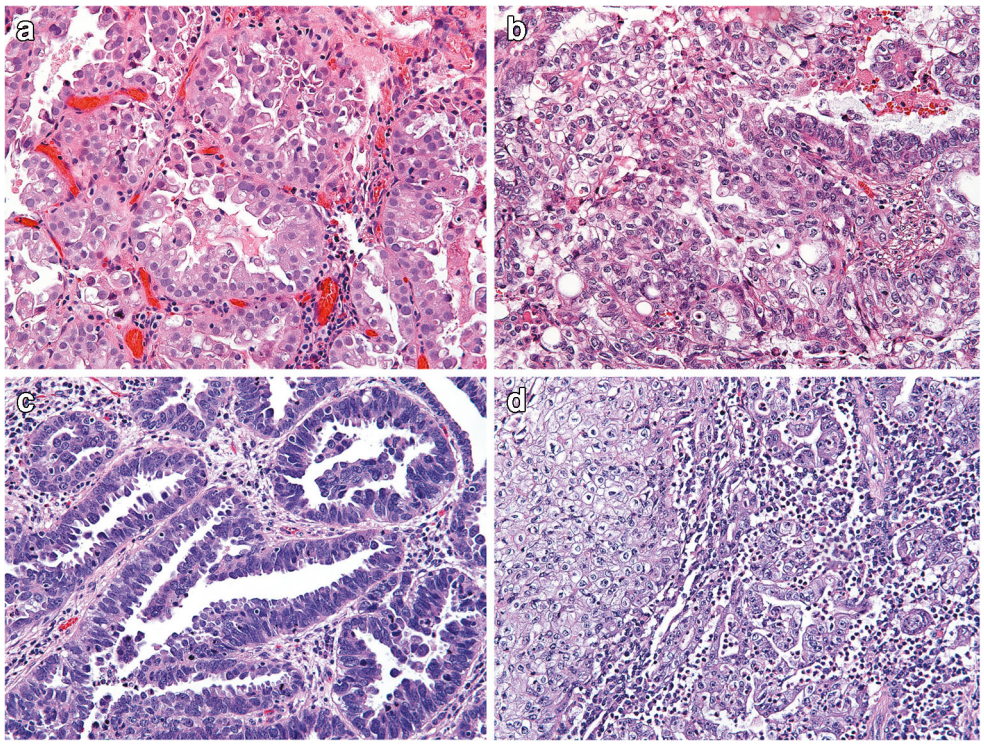

Fig. 1 Microscopic findings of the urinary tract clear cell adenocarcinoma. Representative images of hematoxylin and eosin–stained slides from case #1 (Fig. 1a), #2 (Fig. 1b), #3 (Fig. 1c), and #4 (Fig. 1d) are shown. Variable papillary and tubular architectures were present, lined by cuboidal epithelial cells with moderate to severe cytologic atypia and clear to eosinophilic cytoplasm. A hobnail appearance is readily seen. In case #4 (Fig. 1d), focal solid area is noted

Biocare Medical (Pacheco, CA), per manufacture protocol. HE-stained slides of all urinary CCA cases are reviewed, and areas suspicious for lymphovascular invasion are subjected to D2-40 immunochemistry stains.

Results

Clinical parameters and histopathological features

We identified four cases of urinary CCA fitting the inclusion criteria for the study. Details regarding the patient demographics, clinicopathologic features, treatment, and follow-ups are listed in Table 1. The median age at diagnosis was 35 years (range 8–52 years). Three patients (75%) were female. The primary anatomic site was urethra in three cases (75%), and bladder in one case (25%). All patients underwent surgical intervention. There was no evidence of recurrent disease in three of four patients (75%) at the most recent follow-up visit (median follow-up after initial diagnosis 22 months, range 7–52 months) and one patient (25%) developed metastatic disease to the lung (28 months after initial diagnosis).

By histologic examination, two cases (50%) showed microscopic involvement by tumor of adjacent structures (bladder and/or vagina) and were suspicious for lymphovascular invasion, and one case (25%) exhibited regional lymph node metastases. All cases were composed of heterogeneous morphologic patterns including tubulocystic, papillary and diffuse arrangements of flat to cuboidal- and columnar-shaped tumor cells with clear to eosinophilic cytoplasm. All cases demonstrated at least focal hobnail cells (Fig. 1a–d). By immunohistochemistry, the tumor cells expressed PAX-8 and CK7 in all cases; differential expression of other markers by immunohistochemistry is detailed in Table 1.

Molecular profiling of urinary CCA

STAMP v.2 assay was performed on the initial biopsy specimen for case #1, metastatic carcinoma in the lung for case #2, and surgical specimens for cases #3 and #4. Twenty-one single nucleotide variants (SNVs) and small insertion-deletion variants (< 50 bp) were identified: 17 missense variants, 2 nonsense variants, 1 splice site variant, and 1 5′UTR variant. Among these variants, we identified two recurrent pathogenic variants: a *PIK3CA* p.Glu545Lys (c.1633G>A, p.E545K) variant present in three cases (#1, #2, and #4) and a *KRAS* p.Gly12Asp (c.35G>A, p.G12D) variant in two cases (#2 and #4) (Table 2).

Three additional pathogenic variants were detected: a nonsense *SMAD4* variant (c.431C>G, p.S144X), a *TP53* missense variant (c.817C>T, p.R273C), and a nonsense *APC* variant (c.6929C>A, p.S2310X). The variants of unknown significance (VUS) detected are listed in Table 2. In addition, high-level *MYC* amplification was noted in case #2, a finding confirmed by fluorescence in situ hybridization (data not shown).

Case #3 was the only case that carried neither *PIK3CA* nor *KRAS* pathogenic variants. Compared with other three cases, case #3 is a bladder CCA rather than a urethra CCA. This

Table 2 Single nucleotide variants and short insertion-deletion variants identified in urinary CCA

	Gene	Transcript ID	Variant Description Pathogenicity
Case #1			
PIK3CA	NM_006218.2	c.1633G>A, p.E545K	Pathogenic
SMAD4	NM_005359.5	c.431C>G, p.S144X	Pathogenic
ERBB2	NM_004448.2	c.1370C>T, p.S457L	VUS
SOX2	NM_003106.3	c.695C>T, p.T232I	VUS
Case #2			
PIK3CA	NM_006218.2	c.1633G>A, E545K	Pathogenic
KRAS	NM_033360.2	c.35G>A, p.G12D	Pathogenic
TP53	NM_000546.5	c.817C>T, p.R273C	Pathogenic
ARID1A	NM_006015.4	c.1636C>T, p.Q546T	VUS
PIK3CA	NM_006218.2	c.865G>A, p.A289T	VUS
PIK3CA	NM_006218.2	c.556G>A, p.D186N	VUS
APC	NM_000038.5	c.379A>G, p.S127G	VUS
CCNE1	NM_001238.2	c.779A>T, p.N260I	VUS
Case #3			
APC	NM_000038.5	c.6929C>A, p.S2310X	Pathogenic
AR	NM_000044.3	c.1967C>G, p.T656R	VUS
MSH2	NM_000251.2	c.1995C>A, p.H665Q	VUS
Case #4			
PIK3CA	NM_006218.2	c.1633G>A, p.E545K	Pathogenic
KRAS	NM_033360.2	c.35G>A, p.G12D	Pathogenic
EP300	NM_001429.3	c. 4230G>T, pR1410S	
EPHA2	NM_004431.3	2054-11_2056del, p.?	VUS
KDR	NM_002253.2	c.2863A>G, p.I955V	VUS
PLEKHS1	NM_024889.4	c.-20+70G>A, p.?	VUS

VUS: variant of unknown significance

patient had a prior clinical history of stem cell transplant with a complicated post-transplant clinical course. She had received various immunosuppressant drugs [17]. This case demonstrated diffuse and strong Simian virus 40(SV40) expressivity in tumor cells by immunohistochemistry, whereas adjacent non-neoplastic tissue was negative for the stain. To confirm the presence of BK virus DNA, real-time PCR was performed on the extracted nucleic acids from all four cases. Only case #3 contained detectable BK virus DNA, while the other three cases were negative. There is no clinical history suggestive of an immunocompromised status for cases #1, #2, and #4.

Comparison with genomic landscape of urothelial carcinomas

In a retrospective review of our institutional target exon-sequencing database, 12 urothelial carcinoma cases have been submitted for STAMP v.2 testing for clinical purposes. These 12 patients had high-grade urothelial carcinoma. No *KRAS*, *NRAS*, or *HRAS* variants were identified in these cases. Two pathogenic *PIK3CA* variants were identified in this patient population (2/12 cases, 17%): a p.E726K variant and a p.E81K variant. In addition, a canonical *TERT* promoter variant (c.124C>T) was identified in 9 of 12 cases (75%), a prevalence similar to what has been reported in the literature [18]. *TP53* pathogenic/likely pathogenic variants were identified in 10/12 cases (83%).

Discussion

In our case series of urinary tract CCA, all but one case contained either a *PIK3CA* or *KRAS* pathogenic variant. Our findings possibly suggest a role of PI3K/AKT/mTOR pathway in the pathogenesis of urinary tract CCA.

The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene (*PIK3CA)* encodes the catalytic subunit p110α of phosphatidylinositol 3-kinase (PI3K). The activation of PI3K drives downstream signaling pathways that govern cell survival, cell migration, and other oncogenic processes [19]. Gain-of-function *PIK3CA* variants or *PIK3CA* amplifications have been reported in solid tumors, such as breast cancer, gynecologic malignancies, gastrointestinal tumors, bladder cancer, and non-small cell lung cancer [20]. The

PIK3CA p.E545K variant resides in the helical domain of the PIK3CA subunit, and is one of the most common gain-of-function, hot-spot *PIK3CA* variants in solid tumors [21]. This variant has been shown to be oncogenic in that it produces ligand-independent activation of the PI3K/AKT/mTOR signaling pathway [22]. *KRAS*, a member of the RAS kinase family, encodes a GTPase that upregulates multiple downstream signaling pathways, including MAPK/ERK and PI3K/AKT/mTOR pathways [23]. Activating *KRAS* variants, the p.G12D variant being one of the most common hotspots, has been identified in multiple types of solid tumors, with the highest prevalence in pancreatobiliary, colorectal, endometrial, and non-small cell lung carcinoma [24].

Having identified two recurrent pathogenic variants in the PI3K/AKT/mTOR pathway, we postulate that this pathway is critical for the oncogenesis of urinary tract clear cell adenocarcinoma, and thus might serve as a therapeutic target. Multiple types of small molecular inhibitors targeting the PI3K/AKT/mTOR pathway have been developed, with clinical trials demonstrating antitumor activity in breast cancer, gynecologic malignancies, recurrent head and neck squamous cell carcinoma, EGFR TKI-resistant non-small cell lung carcinoma, and lymphoma [25–27]. Ongoing biomarker-based clinical trials would provide more insights into the therapeutic, predictive, and prognostic values of *PIK3CA* and *KRAS* variants in solid tumors [21].

One patient in our series (case #3) did not harbor PIK3CA or KRAS variants. This pediatric patient was in an immunocompromised state due to prior stem cell transplant for refractory infantile Crohn's disease. During an imaging study to workup gastrointestinal discomfort, a heterogeneous mass arising from the bladder was identified. The histologic examination of the tumor demonstrated an adenocarcinoma with predominately papillary/glandular/tubulocystic architecture and prominent hobnailing. The histologic and immunophenotypic features (summarized in Table 1) are consistent with clear cell adenocarcinoma. Multiple genitourinary pathologists have reviewed this case and concurred. Despite the unique expression of SV40 and lack of PIK3CA/KRAS variants, the histologic features seen in this case were not distinct from those expected for a urinary CCA. We hypothesize that the oncogenesis of this case was mediated by BK-polyomavirus. The involvement of BK virus was confirmed by both SV40 immunohistochemistry and BK virus DNA real-time PCR assay. The presence of polyomavirus in malignant urothelial carcinomas has been described and postulated to be pertinent to tumorigenesis in the literature [28]. However, additional studies are needed to further establish the detailed pathogenesis of BKV-related cancers and whether there are any distinct clinicopathologic features.

Papillary urothelial carcinoma is among the differential diagnoses for urinary CCA, given their overlapping morphologic features. Based on our findings and reports in the literature, there is also a certain degree of overlap in their molecular signatures. In a series of 131 urothelial carcinoma cases from The Cancer Genome Atlas [29], *PIK3CA* variants were detected in 20% of cases, and were predominantly located in the helical domain. *KRAS* was not one of the recurrently mutated genes; however, variants in *HRAS* were identified in 5% of the cases. In another study of high-stage urothelial carcinoma [30], *PIK3CA* mutations were identified in 26% of the cases and *KRAS* mutations in 2.8%; *MYC* amplification was detected in 8.5% of the cases. In our single institution retrospective review of urothelial carcinoma cases, we identified *PIK3CA* variants in 17% of the cases, compatible with what has been reported in the literature. The prevalence of *TP53* pathogenic/likely pathogenic variants (83%) was higher than reported in other studies, which is to be expected as our data, based on clinical testing, is heavily biased towards patients with advanced disease [29, 30].

Urinary tract CCA also bears morphological similarities to gynecological clear cell carcinoma (CCC), raising the possibility of a shared molecular signature or pathogenic mechanism. Several studies have demonstrated recurrent *PIK3CA* and *KRAS* variants in ovarian CCC [31–33]. Up to 50% of ovarian CCC harbors *PIK3CA* variants, a prevalence higher than other types of epithelial ovarian malignancy [31]. *KRAS* variants are less commonly identified in ovarian CCC, in up to 17%. As *KRAS* and *PIK3CA* variants are frequently found in ovarian CCC, a recent literature demonstrated the feasibility of detecting such variants in cell-free circulating DNA for disease diagnosis and monitoring [34], a modality that has potential clinical implication for urinary CCA.

The molecular profiling urachal adenocarcinoma, another rare entity in this anatomic location, has been recently reported [35]. The most common genes harboring mutations was *TP53* (66%). *KRAS* variants were identified in 21% of the cases and *PIK3CA* variants were identified in 4% of the cases. The urachal adenocarcinoma usually exhibits mucinous differentiation, a useful morphological feature to distinguish from CCA. However, the similarities of morphological signatures might suggest common, or at least overlapping, pathogenesis of these two rare cancer entities in the genitourinary tract.

There are some limitations of the current study. First of all, the case number is relatively small. Urinary CCA is a rare disease entity. A multi-institute effort to study additional cases from different anatomical locations (urethra, urinary bladder, ureter, etc.) might provide further insights. In addition, the temporal and spatial heterogeneity of urinary CCA was not addressed in the current study. For case #2, the metastatic carcinoma was used for analysis instead of the primary tumor, due to the poor DNA quality of the primary tumor specimen. Future studies on serial specimens would be able to address this agenda.

In summary, we investigated the molecular landscape of clear cell adenocarcinomas of the urinary tract using a

clinically validated targeted exon-sequencing technology. We identified two distinct oncogenic pathways: BK virus–mediated oncogenesis (1/4 cases), and PI3K/AKT/mTOR pathway activation (3/4 cases) demonstrated by *PIK3CA* and *KRAS* gain-of-function variants. With independent validation cohort in the future, this novel finding of recurrent molecular alterations not only sheds light on the pathogenetic features of this tumor, but provides support for the consideration of small-molecule inhibitors for this rare urothelial malignancy in the era of precision oncology.

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Contributions C.-Y. Lin, the first author and corresponding author, designed the study, collected and analyzed the molecular profiling data, and prepared the manuscript and figures. A. Saleem collected the clinicopathologic data and prepared the manuscript and figures. H. Stehr and J. Zehnder contributed to the molecular data analysis and provided inputs for manuscript preparation. B. Pinsky assisted with BK virus analysis and provided inputs for manuscript preparation. C. Kunder reviewed the histologic sections as well as the molecular data, and provided inputs for manuscript preparation.

Compliance with ethical standards

Conflict of interest Author C. A. Kunder's spouse is an employee of Genentech. Other authors declare that they have no conflict of interest.

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