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Case Report

Mutations in a High-Grade Micropapillary Urothelial Carcinoma of the Renal Pelvis: A Case Report

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Abstract
Micropapillary urothelial carcinoma (MPUC) of the renal pelvis is an upper tract urothelial carcinoma originating in the renal pelvis region. Few genetic studies are available, and the mechanism of pathogenesis of genetically driven models is unclear. We report a case of genomic alterations in MPUC of the renal pelvis and compare the results with existing literature. DNA was extracted, followed by the next-generation sequencing of 351 oncogenes and tumor suppressor genes. Targeted gene sequencing analysis revealed somatic variants in *ERBB2*, *KMT2C*, *FOXA1*, and germline variants in *CDKN1B*, *ELF3*, *TP53*, and *RB1* genes. The present case study sheds light on recognizing genetic variants in high-grade MPUC of the renal pelvis. Understanding molecular mechanisms helps with better prognostication and development of more effective therapeutics and treatment.

Introduction

The micropapillary urothelial carcinoma (MPUC) of renal pelvis origin is a rare upper tract urothelial malignancy and an aggressive type of urothelial carcinoma [1, 2]. Urothelial carcinomas are the fourth most common tumor, where upper tract urothelial carcinomas (UTUCs) represent 5–10% of all urothelial carcinomas [3, 4]. Urothelial carcinomas of the renal pelvis account for approximately 7% of all renal neoplasms, and the mechanism of

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pathogenesis of the genes involved is unclear. MPUC of the renal pelvis has an intrusive clinical course with early nodal/distant metastases. Here, we report a case of MPUC with mutations in seven genes.

Case Presentation

We present a 59-year-old woman diagnosed with an advanced metastatic high-grade MPUC of the renal pelvis origin. She has been diagnosed with preexisting clinical conditions such as hypertension and hypothyroidism problems. A scan of the renal area revealed a right renal fossa lesion in the right kidney. Her blood work reported an elevated level of adrenocorticotropic hormone and serum cortisol. Histochemical studies are positive for CD10, HMWCK, and GATA-3 antibodies. Histological analyses revealed poorly differentiated carcinoma favoring the MPUC. The tumor cells showed a high nucleus-to-cytoplasmic (N:C) ratio with large irregular hyperchromatic nuclei and focal cytoplasmic vacuolations. Mitotic activity was brisk, with many atypical forms. The patient's mother had leukemia, according to the family history. The patient was started on first-line treatment with immune checkpoint inhibitor (ICI) therapy and chemotherapy later (online suppl. Fig. 1 – case report timeline; for all online suppl. material, see https://doi.org/10.1159/000530710). The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (online suppl. Table 2).

The attending physician sought tumor profiling and multigene panel testing to identify gene variations that may have diagnostic or prognostic importance and potential therapeutic implications. After written informed consent from the patient, DNA was extracted from FFPE blocks using the MN NucleoSpin DNA FFPE XS kit, followed by preparation of the NGS library using the SureSelect XT HS2 DNA system. This hybrid capture-based technology includes 351 genes (Agilent Cancer Core Panel; online suppl. Table 1) subjected to paired-end sequencing on the Illumina NovaSeq 6000 platform. A total of 9.5 GB of raw data were generated, followed by the quality screening of FASTQ files, adapter trimming, mapping of data to the hg38 reference genome, and generation of Sam/Bam files. VCF file was generated using GATK 4.2.2 pipeline. Ensembl VEP and Oncotator performed annotation of the VCF file. g:Profiler, a web server, was used for functional enrichment analysis of the resulting genes [5]. The interaction map was fashioned with the String 10.5 program (http://string-db.org).

Results

Analysis of targeted sequencing data revealed mutations in the *ERBB2*, *KMT2C*, *FOXA1*, *CDKN1B*, *ELF3*, *TP53*, and *RB1* genes. We found a somatic missense variant in *ERBB2* (c.929C>T; p.Ser310Phe), nonsense variants in *KMT2C* (c.10771C>T; p.Gln3591Ter), and FOXA1 (c.1064C>A; p.Ser355Ter); a germline nonsense variant in *CDKN1B* (c.268A>T; p.Arg90Ter) and *ELF3* (c.820del; p.His274ThrfsTer20); missense variant in the *TP53* (c.644G>T; p.Ser215Ile) genes; a frameshift variant in *RB1* (c.2057_2058dupCACC; p.Leu688HisfsTer5) (Table 1). The quality of the variants identified in the current sample was observed in the bam file using Integrative Genomics Viewer (IGV) (Fig. 1).

A preliminary analysis of the gene enrichment and pathway of the genes found in the present study in g:Profiler revealed various functions of the *CDKN1B*, *TP53*, *RB1*, and *ERBB2* genes in the multiple stages of cell cycle and growth (Fig. 2). The *KMT2C* gene is essential in

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Table 1. Variants detected in high-grade metastatic micropapillary urothelial carcinoma of the renal pelvis

Gene (transcript)	Position	Exon/ intron	Variant details	Mutation impact (oncogenic/ biological)	Reference (PubMed ID) ID
ERBB2 (NM_004448.4)	chr17:39711955C>T	Exon 8	c.929C>T (p.Ser310Phe) VAF: 9%	Oncogenic/ gain-of-function	[6]
KMT2C (NM_170606.3)	chr7:152162806 G/A	Exon 43	c.10771C>T p.Gln3591Ter VAF: 8%	Likely oncogenic/ likely loss-of-function	[7]
FOXA1 (NM_004496.5)	chr14:37591720G>T	Exon 2	c.1064C>A p.Ser355Ter VAF: 20%	Likely oncogenic/ likely loss-of-function	[8]
RB1 (NM_000321.3)	chr13:48459785-48459788dupCACC	Exon 20	c.2057_2058dupCACC (p.Leu688HisfsTer5) VAF: 54%	Likely oncogenic/ likely loss-of-function	Novel
ELF3 (NM_004433.5)	chr1:202013841delC	Exon 11	c.820del (p.His274ThrfsTer20) VAF: 69%	Likely oncogenic/ likely loss-of-function	Novel
CDKN1B (NM_004064.5)	chr12:12718107A>T	Exon 1	c.268A>T p.Arg90Ter VAF: 29%	Likely oncogenic/ likely loss-of-function	Novel
TP53 (NM_000546.6)	chr17:7674887C>A	Exon 6	c.644G>T p.Ser215Ile VAF: 69%	Likely oncogenic/ likely loss-of-function	[9]

Zygosity column has been removed, as we provided similar information in the form of variant allele fraction.

Mutation impact column is renamed as mutation impact (oncogenic/biological).

VAF, variant allele fraction.

RNA polymerase II transcription. *ELF3*, found in the Notch expression and processing pathway, is known to convert tumor suppressor genes to oncogenes, while *FOXA1* is seen in the ESR-mediated signaling pathway.

Discussion

Micropapillary urothelial carcinomas of the renal pelvis are a rare type of urothelial carcinoma, and very few genomic studies have been reported to date. In the present case, we found gene mutations consistent with other studies on various types of urothelial carcinoma.

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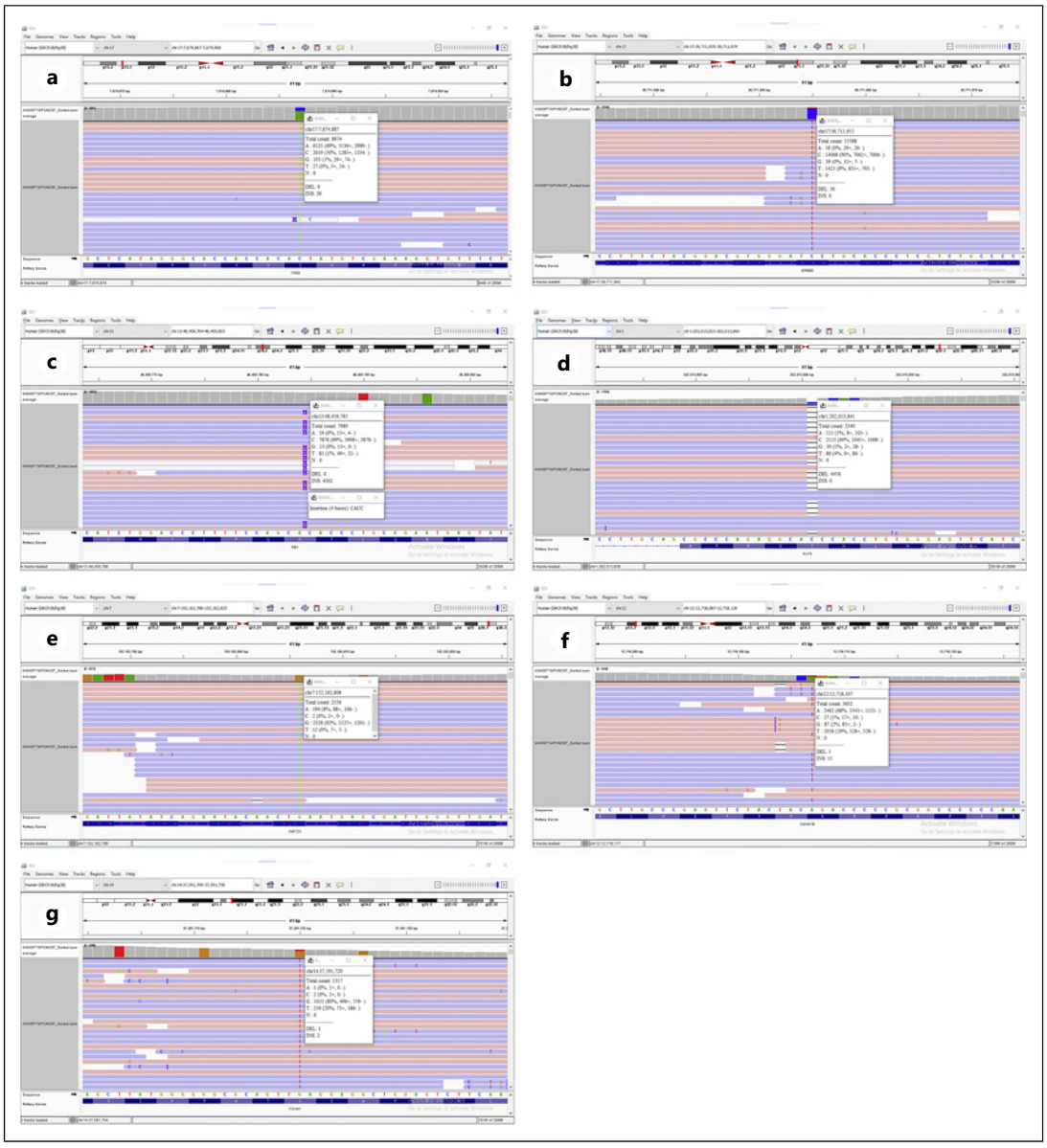

Fig. 1. Integrative Genomics Viewer images showing the mutations present in *ERBB2* (**a**), *TP53* (**b**), RB1 (**c**), *ELF3* (**d**), *KMT2C* (**e**), *CDKN1B* (**f**), *FOXA1* (**g**).

We identified a well-described gain-of-function mutation in the *ERBB2* gene (p.Ser310Phe) that lies in the furin-like cysteine-rich region domain of the protein. This variation is one of the most common pathological hotspots for ERBB2 mutation-driven cancers of the urinary, breast, skin, cervix, and stomach. Ross et al. [6] described the *ERBB2* p.Ser310Phe variant in MPUC. In vitro testing of targeted therapies showed better efficacy of trastuzumab deruxtecan, a HER2 antibody topoisomerase I inhibitor drug that showed inhibitory activity compared to neratinib treatment in conjugate cell lines of both mutant and wild-type *ERBB2* [10].

KMT2C is a tumor suppressor gene encoding histone lysine methyltransferase. Pathogenic variations in this gene confer a predisposition to many types of cancer. Somatic mutations in *KMT2C* had previously been reported in renal pelvic urothelial cancers [7, 9]. A

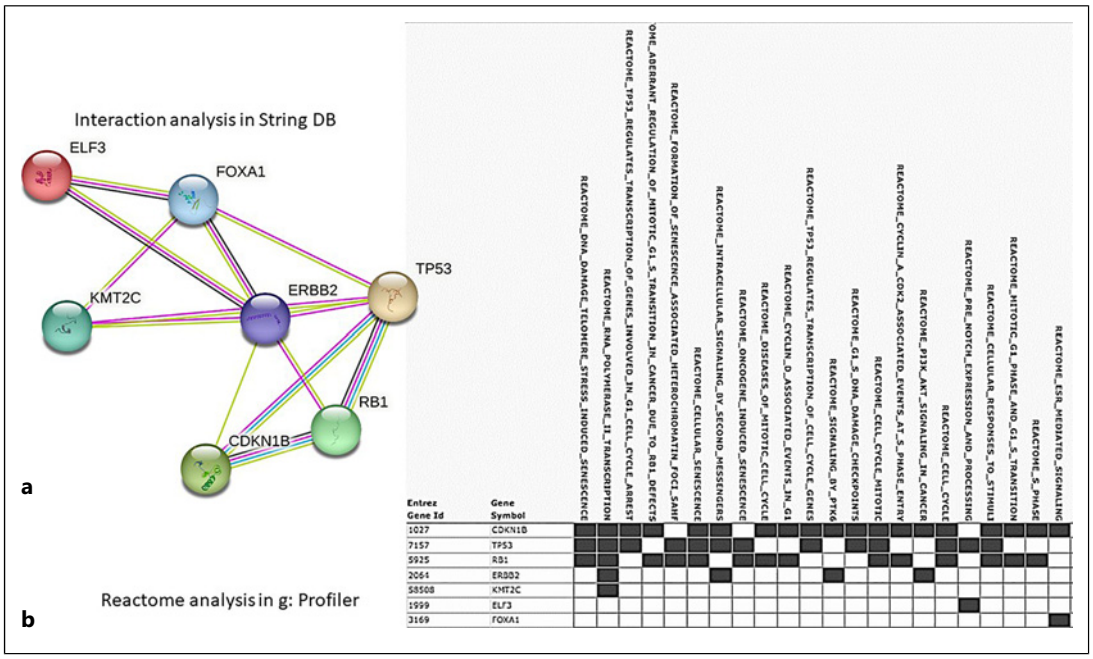

Fig. 2. a, **b** Pathway analysis of the genes in the present case study.

study by Xie et al. [11] highlighted the use of ICI as an emerging therapy for metastatic melanoma, which brings support for using the same medication to treat MPUC, as indicated by the study by Rizzo et al. [12].

FOXA1, a nuclear transcription factor belonging to the forkhead box family, is expressed in the urothelium of the bladder and renal pelvis and maintains normal urothelial differentiation. *FOXA1* gene mutations are predominantly identified in prostate cancer, followed by Hodgkin lymphoma, breast cancer, non-Hodgkin lymphoma, and bladder cancer. Histochemical studies reported *FOXA1* expression in luminal cell types. Low levels of *FOXA1* expression are associated with an increase in cytokeratin 14 expression [13] and aggressive pathological features in UTUC and the basal molecular subtype of bladder urothelial carcinoma [14]. Some functional studies indicated that immune cell heterogeneity dysregulates the expression of *FOXA1*, which induces programmed death-ligand 1 (PD-L1) expression in tumor cells. Anti-PDL-1 antibodies can be adopted as standard therapy for patients with MPUC [15].

This study identified a new heterozygous germline nonsense variant in exon 11 of the *CDKN1B* gene. The loss of *CDKN1B* heterozygosity may result in the loss of tumor suppressor functions, leading to various cancers. Mutations in *CDKN1B* were reported in both Lynch syndrome-associated UTUC and sporadic UTUC [16]. The mutational status of *CDKN1B* could be used as a biomarker to detect MPUC that might need closer follow-up or specific targeted treatments.

ELF3 functions as an epithelial transcription factor and is mainly involved in the development of the urothelium. The clinical impact of haploinsufficiency in *ELF3* is unknown. However, publicly available cancer genomics data suggests that the mutant *ELF3* gene is widely reported in UTUC and carcinoma of the bladder, metastatic melanoma, colorectal, and gall bladder cancers, including clear renal carcinoma and papillary renal cell carcinoma [17]. In this patient, a single nucleotide deletion was found in exon 11 of the *ELF3* gene (chr1: 202013841delC; c.820del; p.His274ThrfsTer20). The observed *ELF3* variant has not been reported in the studies deposited in the TCGA, COSMIC, and cBioPortal databases; however, a single nucleotide duplication (chr1:202013841dupC) resulting in a frameshift variant has

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previously been reported in patients affected by cholangiosarcoma, urothelial bladder carcinoma, UTUC, and muscle-invasive bladder cancer [8]. Developing therapeutic strategies to inhibit oncogenic transcription factor *ELF3* is an area of active research.

TP53 is the most frequently involved gene in many cancers [9], including urothelial carcinoma. In this patient, the substitution of the amino acid isoleucine for the serine codon at 215 (c.644G>T; p.Ser215Ile), a hotspot region in *TP53* is observed; however, the clinical significance of this variant is unknown. *RB1* is also a well-known tumor suppressor gene. To our knowledge, no mutations were reported in the *RB1* gene in all high-grade papillary and non-papillary urothelial carcinomas. Co-mutations in *RB1* and *TP53* are strongly correlated with genomic biomarkers of immune-point inhibitory response in urothelial bladder cancer [18, 19].

Genomic profiling studies highlight that the prevalence of *TP53*, *RB1*, and *ERBB2* mutations is higher in bladder urothelial carcinoma than in UTUC [9]. Recent genomic analyzes of UTUC [17] have highlighted the presence of potential genomic alterations in receptor tyrosine kinases (*FGFR3*, *ERBB2*), *PIK3CA*, *HRAS*, and *TSC1*. These molecular profiling studies have noted genetic heterogeneity among UTUC patients by reporting many genomic variants of unknown significance. Pathway analysis also showed that all genes are involved in various stages of cancer initiation and progression, supporting Knudson's "two-hit" theory of cancer causation.

Many reports on micropapillary urothelial carcinoma of the renal pelvis focus on the histological profiles [1, 20–22]. Even though we observed an association between these genes in this study, uncertainty still exists regarding the functional significance of the identified gene variants and the underlying molecular mechanisms in the development and progression of MPUC of the renal pelvis. Hence, there is a need to uncover the clinical implications and potential therapeutic targets for the remaining variants in MPUC. Functional studies and large-scale genomic and transcriptomic analyses of a larger cohort of patients with MPUC would help elucidate their role in disease pathogenesis. Conducting preclinical and clinical studies on MPUC helps evaluate the therapeutic targets of the detected gene variations and test the effectiveness of targeted treatment. Viscardi et al. [23] and Rizzo et al. [24], in metanalysis studies, concluded that there is a lack of predictive markers for ICI therapy as this treatment fared better among MPUC than conventional chemotherapy [12].

The coming years will bring significant progress in understanding the genomic landscape of the MPUC of the renal pelvis and identifying new therapeutic targets. This leads to better outcomes by integrating genomic data with clinical and pathological data to identify patient subgroups most likely to benefit from specific therapies [25].

Conclusions

Identifying novel variants in a patient can provide valuable clinical insights into the disease and aid in developing more effective cancer prevention strategies. The present case study sheds light on recognizing genetic variants in high-grade micropapillary urothelial carcinoma of the renal pelvis. It may assist researchers in identifying new biomarkers, therapeutic targets, and treatment strategies that may benefit other patients with MPUC in the renal pelvis.

Statement of Ethics

This retrospective review of patient data did not require ethical approval under local guidelines. Written informed consent was obtained from the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

All authors declare no conflicts of interest.

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Author Contributions

Kalyan Ram Uppaluri and Hima Jyothi Challa supervised the project. Ramya Gadicherla and Srinivas Ketavath carried out the experiment. K. Sri Manjari wrote the manuscript with support from Himavanth Reddy Kambalachenu and Saadvik Raghuram. Himavanth Reddy Kambalachenu aided in interpreting the results and analyzed the sample. All authors offered timely feedback and facilitated the manuscript preparation.

Data Availability Statement

All data generated or analyzed in this study are included in this article and its online supplementary material files. Further inquiries can be directed to the corresponding authors.

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