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Original Article

Renal cell carcinoma with unusual morphological features: the clinical utility of next-generation sequencing in distinguishing renal cell tumors

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Abstract: Objectives: Clear cell and papillary renal cell carcinomas (RCC) are the two most common RCC subtypes, accounting for approximately 70% and 15% of kidney cancers, respectively. Clear cell RCC is commonly associated with *VHL* alterations, while papillary RCC typically exhibits chromosomal abnormalities such as +7, +17, and -Y. Furthermore, clear cell RCCs are less likely to exhibit *PBRM1* and *SETD2* alterations. This study aims to improve the accuracy of RCC diagnosis by investigating molecular alterations in RCC cases with clear cells, papillary structures, and other atypical histological features. Methods: Nine RCC cases were retrospectively selected and analyzed using histologic slides and immunohistochemical staining for CAIX, RCC, CD10, CK7, P504S, Vimentin, and EMA. Next-generation sequencing was performed on all cases to identify genetic mutations, and cytogenetic analysis was conducted on one case. Results: The cohort consisted of nine male patients aged 49 to 68 years (mean 61.4). Surgical specimens included six radical and three partial nephrectomies; seven tumors were located in the left kidney and two in the right. Tumor sizes ranged from 0.8 to 15.2 cm. Immunohistochemical analysis revealed positive staining for RCC (6/9), CAIX (3/4), CD10 (6/6), and CK7 (5/9). In six clear cell RCCs, next-generation sequencing identified *VHL* mutations in four tumors, *PBRM1* alterations in three, and *SETD2* mutations in one. Five tumors with papillary fronds, sarcomatous components, or unclassified features harboring *VHL*, *PBRM1*, and/or *SETD2* mutations were reclassified as clear cell RCC. One clear cell RCC with leiomyomatous stroma showed *mTOR* mutations. A case of clear cell papillary renal cell neoplasm showed no reportable gene mutations. The role of a *FANCA* mutation in one papillary RCC remains uncertain. Cytogenetic analysis of one case (Case #5) revealed 50, X, -Y, +3, +7, +16, +17, +20, consistent with papillary RCC. Conclusions: Next-generation sequencing is a useful method for categorizing RCCs with clear cells, papillary features, and unusual histology. Additionally, *VHL* mutations could be a promising target for personalized treatment in clear cell RCCs and their histologic variants.

Keywords: Renal cell carcinoma, clear cells, papillary, next-generation sequencing, *VHL* alterations

Introduction

Kidney and renal pelvic cancers are the sixth most common cancer in American men and the ninth most common in American women [1]. Renal cell carcinoma (RCC) is the most common primary malignancy of the kidney. It occurs more often in men than in women, and the ratio of men to women is approximately 2:1 [2]. Approximately 81,600 new cases of kidney cancer are diagnosed each year. Most people diagnosed with RCC are older and are between 55 and 74 years of age [2]. The incidence of kidney cancers has been increasing for many years.

According to the WHO 2022 Classification of Tumors of the Kidneys, tumors are classified based on their histological features and unique genetic alterations [2]. Clear cell RCC is the most common type of renal cancer and accounts for 65-70% of the cases. It is charac-

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terized by nests of tumor cells with clear to eosinophilic cytoplasm surrounded by a delicate network of blood vessels [2]. It has been shown that inactivation of *VHL* (located on 3p15) as a result of deletion, inactivating mutations, or promoter region methylation occurred in more than 90% of sporadic clear cell RCC [2-4]. Papillary RCC is the second most common RCC and accounts for approximately 19% of the kidney cancers. Papillary RCCs are typically composed of papillae with fibrovascular cores covered by eosinophilic epithelial cells. Foamy macrophages and calcifications are sometimes noted [2, 3]. Cytogenetically, trisomy and tetrasomy of chromosomes 7 and 17 and loss of the Y chromosome occur more frequently in low-grade papillary RCCs [2, 3, 5]. Immunohistochemistry, in addition to its characteristic histological features, can distinguish these two types in many cases.

Occasionally, we encounter RCCs with clear cell and papillary characteristics. These tumors are typically composed of papillary fronds covered by varying amounts of clear cells. These tumors have been initially diagnosed as clear cell RCC with papillary features or unclassified RCC [6]. Immunohistochemical stains usually demonstrate ambiguous results. Next-generation sequencing is increasingly utilized in the diagnosis and management of renal cell carcinoma, enabling the identification of genetic mutations that guide treatment decisions and improve patient outcomes [3, 4]. In this study, we have performed next-generation sequencing and recategorized clear cell RCCs with papillary features, unclassified RCCs with sarcomatous components, and RCCs with unusual histology.

Materials and methods

Subjects

Nine RCCs with unique histopathological characteristics, such as papillary RCC with clear cell change and unclassified RCC, were retrieved from the slide archives in Pathology and Laboratory Medicine Service, Overton Brooks VA Medical Center, Shreveport, LA. H&E and immunohistochemically stained slides were examined.

RCC cases included in the study exhibited clear cells, papillary architecture, sarcomatoid components, leiomyomatous stroma, or unclassified features such as focal clear cells, solid tumor nests with arborizing vasculature, or a mixture of clear cell and papillary histology. Cases of chromophobe RCC, collecting duct carcinoma, mucinous tubular and spindle cell carcinoma, renal medullary carcinoma, and oncocytic renal tumors were excluded. Clinical data and molecular reports were electronically collected by using Vista-R2 and the Computerized Patient Record System (CPRS) Chart.

Immunohistochemistry

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were sectioned at 4 μm thickness using a microtome and mounted on positively charged microscope slides. The slides were then dried on a hot plate to remove moisture and enhance tissue adherence. Immunochemistry was performed on the Leica Biosystems BOND-III fully automated IHC/ISH staining system (Deer Park, IL). A compact Polymer™ detection system with ancillary reagents was used. BOND-PRIME Epitope Retrieval Solution 1 (Leica Biosystems, Deer Park, IL) was used for the antigen retrieval process. Endogenous peroxidase activity was quenched by treating the sections with the BOND-PRIME Polymer DAB Detection System (Leica Biosystems, Deer Park, IL).

The following monoclonal antibodies were applied: carbonic anhydrase IX (CA-IX) (1:200 dilution) (Cell Marque, Rocklin, CA), renal cell carcinoma marker (RCC) (1:200 dilution) (Cell Marque, Rocklin, CA), cluster of differentiation 10 (CD10) (1:280 dilution) (Leica Biosystems, Deer Park, IL), cytokeratin 7 (CK7) (1:280 dilution) (Leica Microsystems, Deer Park, IL), alpha-methylacyl-CoA racemase (AMACR) (1:280 dilution) (Leica Microsystems, Deer Park, IL), vimentin (Vim) (1:280 dilution) (Leica Microsystems, Deer Park, IL), and epithelial membrane antigen (EMA) (1:280 dilution) (Leica Microsystems, Deer Park, IL) (**Table 1**). Slides were incubated with the primary antibodies for 2 hours. Immunoreactivity was visualized using 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 5 minutes, producing a brown chromogenic signal. Sections were counterstained with hematoxylin and mounted in Leica Mounting Media (Leica Biosystems, Deer Park, IL).

Table 1. Immunohistochemical stain

Antibody	Clone	Manufacturer
Carbonic anhydrase IX (CA-IX)	MRQ-54	Cell Marque
Renal cell carcinoma (RCC)	PN-15 Mab	Cell Marque
Cluster of differentiation 10 (CD10)	56C6	Leica Microsystems
Cytokeratin 7 (CK7)	RN7	Leica Microsystems
Alpha methylacyl CoA racemase (AMACR)	P504s	BioCare
Vimentin (Vim)	V9	Leica Microsystems
Epithelial membrane antigen (EMA)	GP1.4	Leica Microsystems

Next-generation sequencing

Molecular studies were conducted by using the FoundationOne® CDx test (Foundation Medicine, Cambridge, MA), a next-generation sequencing (NGS)-based comprehensive genomic profiling assay. The next-generation technology used the Illumina® HiSeq 4000 platform to analyze 324 cancer-related genes, including coding exons and non-coding introns, as well as specific introns of 34 related genes. The generated sequence data were analyzed to detect various types of genomic alterations, including substitutions, insertions/deletions, copy number variations (amplifications and homozygous deletions), and select gene fusions/rearrangements [7]. The report also includes details about microsatellite instability (MSI), tumor mutational burden (TMB), and genomic loss of heterozygosity (LOH), as well as suggestions for possible targeted treatments, immunotherapies, and related clinical trials.

Results

Clinical characteristics

The cohort consisted entirely of male patients, with ages ranging from 49 to 68 years (**Table 2**).

During the evaluation of other medical conditions, all patients initially presented with renal masses on CT imaging. Tumor sizes varied widely, ranging from 0.8 cm to 15.2 cm, with an average size of 5.7 cm (**Table 2**). The majority of the tumors were located in the left kidney (7 cases), while 2 cases were in the right kidney. Pathological staging showed 4 tumors classified as pT1a, 2 as pT1b, and 3 as pT3a (**Table 2**). In terms of survival outcomes, 3 patients remained alive, with follow-up periods ranging from 3 years and 7 months to 7 years and 4 months. Five patients did not survive, with follow-up durations spanning from 1 year and 1 month to 10 years and 4 months (**Table 2**).

Clear cell RCC with papillary features (cases #1, #4, and #8)

Out of the nine clear cell RCC cases with papillary features, three tumors (cases #1, #4, and #8) exhibited varying amounts of clear cells (>80%) and papillary structures (0 to >80%) (**Table 3**, **Figure 1**). All the tumors showed nuclear grade 3. Immunohistochemical analysis revealed that CAIX immunostaining was positive in case #4, while RCC immunomarker was positive in all three cases. CD10 was expressed in two of the three cases (cases #1 and #8), and CK7 was positive in cases #1 and #4 (**Table 3**). Genomic analysis showed distinct mutation profiles. Case #1 harbored mutations in *VHL, APC, ERBB3 (EGFR), mTOR, NTRK*, and *PTPN11*, while case #8 exhibited mutations in *VHL, PBRM1, SF3B1, TERT, and TP53* (**Table 4**). These two cases were confirmed as clear cell RCC based on the presence of *VHL* mutations in case #1 and both *VHL* and *PBRM1* mutations in case #8 (**Table 4**). In contrast, case #4 displayed only a single mutation in *FANCA* (**Table 4**).

Unclassified RCC (cases #2 and #5)

Microscopically, case #2 exhibited 50-80% clear cells without papillary structures (**Table 3**, **Figure 1**). The nuclear grade was determined to be grade 4. Immunohistochemically, RCC marker and CD10 were positive, while CK7 was negative (**Table 3**). Next-generation sequencing revealed mutations in *VHL*, *PTEN*, and *MSH6*, leading to the diagnosis of clear cell RCC (**Table 4**). Case #5 tumor was composed of nests of eosinophilic cells, approximately 30% of which

Table 2. Clinical characteristics of patients

Case #	Age (y)	Gender	Clinical Presentation	Surgical Procedure	Tumor Size (cm)	pT	Follow-up (y/m)
1	67	Male	Polycythemia, renal mass	Left partial nephrectomy	2.8	pT1a	Died, 10y4m
2	63	Male	Left lower pole renal mass on CT scan for status post open cholecystectomy	Left radical nephretomy	15.2	pT3a	Died, 1y1m
3	65	Male	Left renal mass on status post right renal clear cell carcinoma	Left radical nephrectomy	0.8	pT1a	Died, 8y7m
4	56	Male	Renal cysts bilateral CT scan for thrombocytosis	Left partial nephrectomy	5.0	pT1b	Alive, 7y4m
5	49	Male	Hematuria, renal mass on CT scan for urethral diverticulum	Left partial nephrectomy	3.3	pT1a	Alive, 6y3m
6	68	Male	Renal mass on staging CT scan for prostate cancer	Right radical nephrectomy	7.0	pT3a	Died, 3y2m
7	57	Male	Renal mass on staging CT scan for prostate cancer	Right partial nephrectomy	4.0	pT1a	Alive, 4y0m
8	62	Male	Renal mass on CT scan for metastatic renal cell carcinoma of the bladder	Left radical nephrectomy	5.8	pT1b	Died, 1y8m
9	66	Male	Renal mass on CT scan screening for abdominal aortic aneurysm	Left radical nephrectomy	7.8	pT3a	Alive, 3y7m

y: year; m: month.

Table 3. Pathological characterization of renal cell neoplasm with clear cells, papillary, and unusual features

Case #	Histopathology			Immunohistochemical stain							Diagnosis
	% Clear	% Papillary	Nuclear grade	CAIX	RCC	CD10	CK7	P504S	Vim	EMA	
1	>80	30-50	3	n/a	+	+	+	+	n/a	n/a	Clear cell RCC with papillary features
2	50-80	0	4	n/a	+	+	-	n/a	-	n/a	Unclassified RCC
3	>80	0	3	n/a	-	-	+	n/a	-	n/a	Clear cell RCC with leiomyomatous stroma
4	>80	>80	3	+/-	+	-	+	n/a	n/a	n/a	Papillary RCC with clear cell features
5	0	<30	3	-	+	+	+	n/a	n/a	+	Unclassified RCC
6	50-80	0	4	+	+	-	-	n/a	n/a	n/a	Clear cell RCC with sarcomatous features
7	>80	30-50	2	+	-	+	+	+/-	n/a	+	Clear cell papillary renal cell tumor
8	>80	0	3	n/a	+	+	-	n/a	+	n/a	Clear cell RCC
9	0	0	4	n/a	-	+	-	+	n/a	+	Clear cell RCC with sarcomatous features

RCC: renal cell carcinoma.

had papillary configurations (**Table 3**, **Figure 1**). The nuclear grade was grade 3. Immunohistochemical analysis showed positive staining for RCC, CD10, and CK7 (**Table 3**). Next-generation sequencing identified mutations in *JAK2, MLH3,* and *TET2*, with no alterations in *VHL* (**Table 4**). Additionally, cytogenetic analysis revealed the following karyotype: 50, X, -Y, +3, +7, +16, +17, +20 (**Table 4**). Based on these findings, a diagnosis of papillary RCC was suggested.

Clear cell RCC with sarcomatous components (cases #6 and #9)

Two cases of clear cell RCC with sarcomatous components (cases #6 and #9) were examined (**Table 3**, **Figure 1**). Case #6 displayed nests of atypical epithelial cells interspersed with spindle-shaped tumor cells possessing hyperchromatic nuclei. Clear cells composed 50-80% of the tumor, with no papillary structures observed (**Table 3**, **Figure 1**). The nuclei were graded as grade 4. Immunohistochemical staining showed tumor cell positivity for CAIX and RCC, while CD10 and CK7 were negative (**Table 3**). Next-generation sequencing identified mutations in *PBRM1, SETD2*, *NFE2L2,* and *TP53* (**Table 4**). Case #9 revealed solid nests of tumor cells without clear cells or papillary structures (**Figure 1**). Nuclear grade was also grade 4. Immunostains demonstrated positivity for CD10 and P504S, with negative results for RCC and CK7 (**Table 3**). Molecular studies identified alterations in *VHL, PBRM1, SET2,* and *TP53* (**Table 4**). These findings confirmed both tumors as clear cell RCC with sarcomatous stroma.

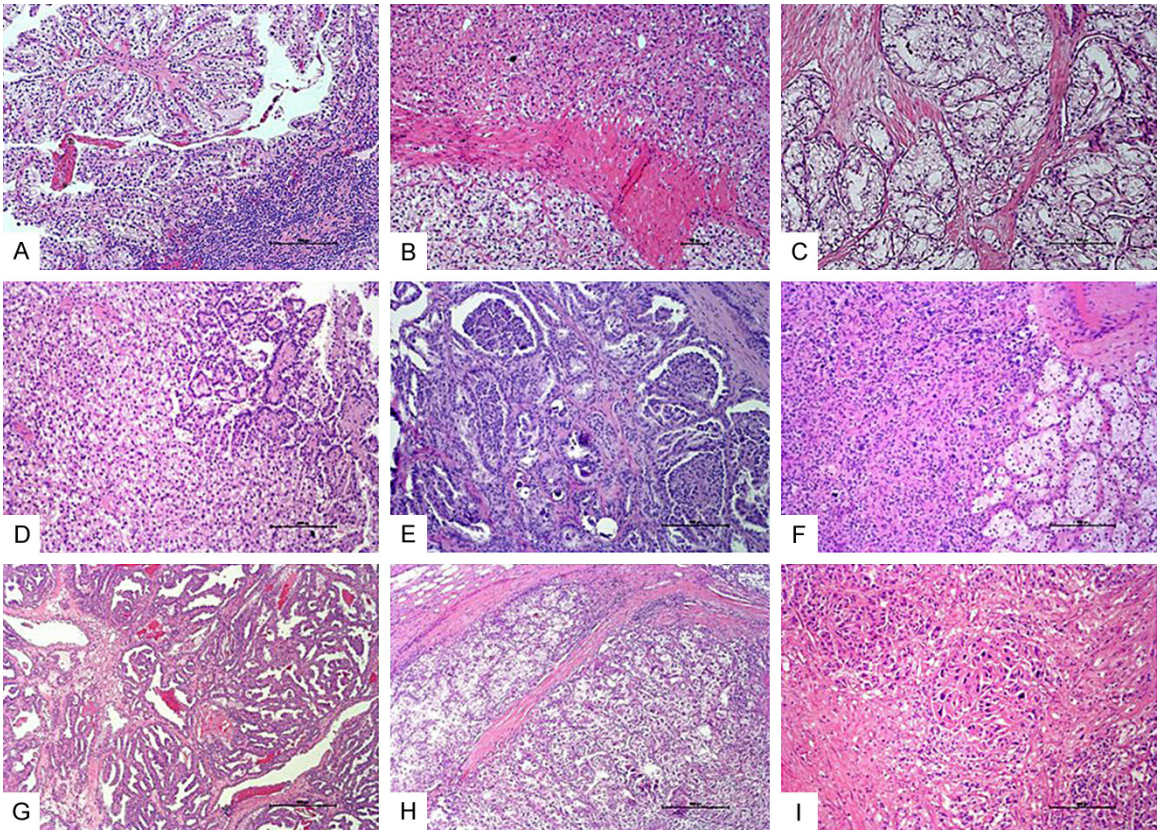

Figure 1. (A) Clear cell renal cell carcinoma (RCC) with papillary features. (B) Unclassified RCC. (C) Clear cell RCC with leiomyomatous stroma. (D) Papillary RCC with clear cell changes. (E) Unclassified RCC. (F) Clear cell RCC with sarcomatous features. (G) Clear cell papillary renal cell tumor. (H) Clear cell RCC. (I) Clear cell RCC with sarcomatous features. Scale bar: 100 μm (A, C-I), 10 μm (B) (Hematoxylin and eosin, 100×).

Table 4. Genetic alterations in renal cell neoplasm with clear cells, papillary, and unusual features

Case #	Genetic alteration	Suggested diagnosis after next- generation sequencing
1	*VHL, APC, ERBB3 (EGFR), mTOR, NTRK, PTPN11*	Clear cell RCC
2	*VHL, PTEN, MSH6*	Clear cell RCC
3	*mTOR*	Clear cell RCC with leiomyomatous stroma
4	*FANCA*	Papillary RCC
5	*JAK2, MLH3, TET2*; (50, X, -Y, +3, +7, +16, +17, +20)	Papillary RCC
6	*PBRM1, SETD2, NFE2L2, TP53*	Clear cell RCC with sarcomatous component
7	No reportable gene mutations	Clear cell papillary renal cell tumor
8	*VHL, PBRM1, SF3B1, TERT, TP53*	Clear cell RCC
9	*VHL, PBRM1, SET2, TP53*	Clear cell RCC with sarcomatous component

RCC: Renal cell carcinoma.

Clear cell RCC with leiomyomatous stroma (case #3)

Microscopic examination of case #3 revealed a well-circumscribed tumor composed of small nests and nodules of renal epithelial cells surrounded by a capillary network (**Figure 1**). These structures were embedded within a cellular stroma of intertwining bundles of spindle cells, identified as benign smooth muscle (**Figure 1**). The renal epithelial cells exhibited clear cytoplasm, and the nuclei were graded as grade 3 according to the WHO/ISUP histologic grading system for clear cell RCC (**Table 3**). No

hyaline globules were observed in the cytoplasm of the tumor cells. Fascicles of criss-crossed smooth muscle fibers were most prominent at the tumor margins. Immunohistochemical staining demonstrated positivity for CK7 and negativity for RCC, CD10, and vimentin (**Table 3**). Next-generation sequencing identified an mTOR mutation (**Table 4**). Based on these findings, the tumor was classified as clear cell RCC with leiomyomatous stroma.

Clear cell papillary renal cell tumor (case #7)

Case #7 featured a tumor composed of nests, tubules, and papillae in varying proportions (**Figure 1**). More than 80% of the tumor consisted of clear cells, with 30-50% of them forming papillary structures (**Table 3**). The tumor cells were cuboidal to low columnar with small nucleoli, corresponding to histologic grade 2. Tumor cells with uniform nuclei, inconspicuous nucleoli, and alignment toward the luminal aspect of the papillae were observed. Immunohistochemical staining showed positivity for CAIX, CD10, CK7, and EMA, while RCC was negative. P504S staining was equivocal (**Table 3**). Next-generation sequencing revealed no reportable gene mutations (**Table 4**). According to the WHO 2022 Classification of Renal Tumors, this case was diagnosed as a clear cell papillary renal cell tumor.

Discussion

Clear cell RCC typically consists of tumor cells arranged in acinar structures with epithelial cells exhibiting clear cytoplasm. The stroma is composed of a delicate vascular network [8]. Tumor cells stain positive for CAIX, RCC, CD10, and vimentin [9, 10]. In contrast, papillary RCC consists of papillary fronds with fibrovascular cores, which are covered by eosinophilic cells. These cells are positive for RCC, CD10, P504S, and CK7 [11-13]. However, it is quite uncommon to encounter RCC cases that have a mix of clear cells and papillary configurations in different amounts, which makes diagnosis difficult [14-17].

For many years, clear cell RCC has been strongly associated with alterations in the *VHL* gene [18]. These alterations lead to increased levels of the transcription factor hypoxia-inducible factor (HIF)-1α, which subsequently upregulates downstream vascular endothelial growth factor (*VEGF*), contributing to tumor survival and cellular growth [18]. Advances in next-generation sequencing have expanded our understanding of clear cell RCC genetics, revealing mutations in additional genes such as *PBRM1*, *SETD2*, and *BAP1* [19-23]. In clear cell RCC, *PBRM1* mutations occur in approximately 40-50% of cases, while *SETD2* mutations are found in about 12% [23]. In this study, the presence of *VHL* gene mutations (in cases #1, #2, #8, and #9), *PBRM1* alterations (in cases #6, #8, and #9), and *SETD2* mutations (in case #6) confirmed the diagnosis of clear cell RCC. Notably, cases #6 and #9 displayed sarcomatous differentiation, which emphasizes the complexity of clear cell RCC pathology. Genetic mutations have a significant impact on clear cell RCC prognosis. *PBRM1* mutations, initially identified in breast cancer, are mostly linked to the clear cell type of RCC and are rarely observed in papillary RCC [23, 24]. Although reports are sometimes contradictory, *PBRM1* alterations are generally linked to higher tumor grade, advanced clinical stage, and worse outcomes [24-26]. Similarly, *SETD2* mutations are strongly associated with poor prognosis [27]. In this study, two of the three cases with *PBRM1* mutations (with or without accompanying *SETD2* mutations) experienced poor clinical outcomes. Case #6 died 3 years and 2 months after diagnosis, and case #8 died 1 year and 8 months after diagnosis (**Table 2**). In contrast, case #9, who also exhibited a sarcomatous component, remains alive at 3 years and 7 months of follow-up (**Table 2**).

Papillary RCC is characterized by unique cytogenetic alterations. Although molecular markers for diagnosing papillary RCC are limited, common genetic changes involve trisomy or tetrasomy of chromosomes 7 and 17, as well as loss of the Y chromosome [28-30]. Low-grade papillary RCC is frequently associated with mutations in the *MET* gene. In this study, case #5, initially classified as unclassified RCC, exhibited a cytogenetic profile of 50, X, -Y, +3, +7, +16, +17, +20. This pattern, characteristic of papillary RCC, supported its reclassification into this subtype. For case #4 (papillary RCC with clear cell features), the tumor exhibited a *FANCA* mutation. While *FANCA* mutations have been implicated in certain cancers, their role in RCC remains unclear and warrants further investigation.

Renal neoplasms featuring clear cells and smooth muscle differentiation can be observed in different types of RCC, such as clear cell conventional RCC, papillary RCC, clear cell papillary RCC, and translocation-associated RCC [31]. These kidney tumors can usually be identified by the morphology and organization of clear cells, as well as the amount of intertumoral and intracapsular smooth muscle [31]. Tumors with alterations in *TCEB1, TSC1, TSC2,* or *mTOR* genes are often linked to clear cells and smooth muscle stroma [32, 33]. In our present case (#3), we have identified *mTOR* mutations. Emerging studies indicate that RCC with leiomyomatous stroma with *mTOR* mutations may constitute a distinct pathological entity [34]. However, the biological and clinical implications of this finding remain unclear. Further research is essential to elucidate its role in tumor pathogenesis and its potential significance in diagnosis, prognosis, and treatment.

Clear cell RCCs with papillary features and other unusual morphological changes are very rare. Among genetic alterations, *VHL* mutations are the most commonly identified in clear cell RCC, followed by less common mutations in *PBRM1* and *SETD2* [35].

Previous studies have reported that *VHL* mutations occur in about 60% and *PBRM1* mutations in about 13.3% of clear cell RCCs with papillary features [36]. In our study, we found *VHL* mutations in 4 out of 9 cases (44.4%), *PBRM1* mutations in 3 out of 9 cases (33.3%), and *SETD2* mutations in 2 out of 9 cases (22.2%) of clear cell RCCs with papillary features and other unusual morphological characteristics. However, among the 4 cases of clear cell RCCs with 30-80% papillary features, only 1 case (25%) harbored a *VHL* mutation. Notably, none of these 4 cases showed mutations in *PBRM1* or *SETD2*. Compared to previous studies, the frequencies of *VHL* and *PBRM1* mutations observed in our cohort appear lower [35, 36].

According to the NCCN guidelines, immune checkpoint inhibitors are recommended for the treatment of relapsed or stage IV RCC [37]. Pembrolizumab, when combined with VEGF receptor-targeting tyrosine kinase inhibitors, serves as a cornerstone of first-line therapy for advanced disease, offering synergistic efficacy in managing tumor progression [37]. However, in our cases, none exhibited a high tumor mutation burden (<10 mutations/Mb), a biomarker that can enhance the efficacy of immunotherapy.

Also, belzutifan, a new HIF-2α inhibitor, has been approved by the Food and Drug Administration to treat tumors related to *VHL*, including RCCs [38].

Conclusion

RCC with unusual morphological changes, particularly papillary features, is uncommon. Mutations in the *VHL* gene are the most commonly observed alterations in clear cell RCC. Apart from *VHL* mutations, clear cell RCC also exhibits recurrent alterations in *PBRM1* and *SETD2* [3-6]. Histomorphology and immunohistochemistry remain foundational methodologies for diagnosing the majority of renal tumors. However, in ambiguous or challenging cases, next-generation sequencing is a useful method for categorizing RCCs with clear cells, papillary features, and other uncommon histomorphological structures. Next-generation sequencing offers high sensitivity and specificity for detecting a broad spectrum of genetic alterations. Furthermore, in advanced or high-grade RCCs, molecular profiling plays a pivotal role in guiding personalized treatment strategies, embodying the principles of precision medicine.

However, its application in RCC remains an evolving field, with ongoing efforts to refine its use for more precise diagnostic and therapeutic purposes.

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Disclosure of conflict of interest

None.

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