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Case Report

Clear cell renal cell carcinoma with *BAP1* mutation: a report of two cases

Jennifer Lee[1,2], Moe Thuzar[1,2], Angela C DiPoto-Brahmbhatt[1,2], Catherine Chaudoir[1,2], Anthony Tanner[1], Tajammul Fazili[3,4], Xiuping Yu[4,5], Michael Constantinescu[1,2], Y Albert Yeh[1,2,4]

[1]Pathology and Laboratory Medicine Service, Overton Brooks VA Medical Center, Shreveport, LA, USA; [2]Department of Pathology and Translational Pathobiology, Louisiana State University Health Shreveport, Shreveport, LA, USA; [3]Department of Urology, Overton Brooks VA Medical Center, Shreveport, LA, USA; [4]Department of Urology, Louisiana State University Health Shreveport, Shreveport, LA, USA; [5]Department of Biochemistry and Molecular Biology, Louisiana State University Health Shreveport, Shreveport, LA, USA

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Abstract: Clear cell renal cell carcinoma is the most common subtype of renal cell carcinomas (RCCs) and accounts for 60%-70% of all RCCs cases in adults. Aberrations in the von Hippel-Lindau (*VHL*) gene on chromosome 3p occurred in > 90% of clear cell RCCs. Other tumor suppressor genes located on chromosome 3p, such as *BAP1*, *PBRM1,* and *SETD2*, also contribute to tumorigenesis. Clear cell RCCs with both *BAP1* and *VHL* mutations may display distinctive histopathological features. Here, we report two cases of clear cell RCCs with *BAP1* mutation. One tumor had *VHL*, *BAP-1*, and *RAF1* mutations and the tumor nests and alveoli of tumor cells were surrounded by proliferative vessels and the optically clear cytoplasm contained numerous eosinophilic granules and hyaline globules of varying sizes. The other tumor had *BAP1* and *ATM* mutations, and demonstrated clear cells with numerous eosinophilic granules and other typical histopathological features of conventional clear cell RCC. Furthermore, many tumor nodules with dense peripheral lymphocytic infiltrates contained rhabdoid cells. Sarcomatoid cells were also observed. Both tumor cells showed high-grade nuclei. Clear cell RCCs with *BAP1* mutation exhibit aggressive clinical behaviors.

Keywords: Clear cell renal cell carcinoma, *BAP1* mutation, eosinophilic granules, hyaline globules

Introduction

Clear cell renal cell carcinoma (RCC) is the most common subtype of RCCs and accounts for 60%-70% of all RCCs cases in adults [1]. Aberrations in the von Hippel-Lindau (*VHL*) gene and loss of chromosome 3p occur in > 90% of clear cell RCCs [2, 3]. *VHL* is a tumor suppressor located on chromosome 3p25, and its inactivation through mutations, promotor hypermethylation, or loss of the chromosome 3p arm results in the accumulation of the alpha-subunit of hypoxia-inducible factor (HIF-1α), which subsequently upregulates downstream target genes to promote angiogenesis and tumor growth [4, 5]. Furthermore, mutations in three chromatin-remodeling genes located on chromosome 3p - *BRCA1*-associated protein 1 (*BAP1)*, *PBRM1*, and *SETD2* - also significantly contribute to the oncogenesis of clear cell RCCs [5]. *BAP1* mutations occur in approximately 10%-12% of clear cell RCCs cases [6, 7]. Tumors with *BAP1* mutation are more likely to possess a high tumor grade and *BAP1* mutations are usually associated with aggressive clinical behavior and adverse outcomes with a median overall survival of 4.6 years [8]. Standard treatments did not differ between clear cell RCCs with and without VHL mutations and with or without *BAP1* mutations. Patients with localized disease are treated with partial or radical nephrectomy, while those with metastatic disease are treated with systemic therapy [9]. Several studies on clear cell RCCs with *BAP1* and *VHL* or *PBRM1* mutations have revealed distinct histomorphological features [6-9]. However, clear cell RCCs with *BAP1* mutation alone have not been thoroughly described.

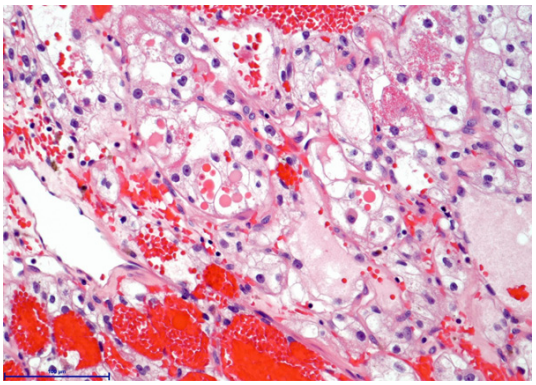

Figure 1. Case 1. Nests and alveoli are composed of renal carcinoma cells with clear cytoplasm and numerous eosinophilic granules. Hyaline globules of varying sizes are also present in many carcinoma cells (Hematoxylin and eosin, 200 ×).

Herein, we report two cases of clear cell RCCs with *BAP1* mutation and without *VHL* alterations. Neither of the two cases had a *PBRM1* mutation. Clinical and histomorphological features that correlated with genetic changes have also been described.

Case report

Case 1

A 68-year-old male patient presented with lower back pain for many years. His medical history included aortic aneurysm, diabetes mellitus, and hyperlipemia. He denied having hematuria or lower urinary tract obstructive symptoms. Magnetic resonance imaging of the lumbar region showed a left renal heterogeneous mass measuring 11.1 × 13.3 × 9.6 cm. The tumor margins were not well defined. Non-contrast computed tomography (CT) of the abdominal pelvis revealed a 13-cm lobulated mass encompassing the majority of left kidney. Intratumoral calcification was also observed. A left radical nephrectomy was performed.

Macroscopically, the left kidney measured 18.5 × 14 × 9.0 cm and weighed 1084 g. The anterior aspect of the left kidney appeared to be irregular. The attached 7.3-cm long ureter was unremarkable. No lymph nodes were observed. The mid kidney showed a well-circumscribed tumor measuring 12.3 × 11.0 × 9.0 cm. The tumor was 6.7 cm from the ureter margin and 0.3 cm from the hilar region. The cut surface of the tumor revealed a golden yellow-to-red, heterogeneous mass with irregular borders and cystic degeneration. The percentage of necrotic tissue was approximately 30%. This mass encroached the renal capsule and focal extension of the fatty tissue of the renal sinus was observed.

Histopathological examination of the kidney tumor revealed a well-circumscribed tumor composed of nested and microcystic structures surrounded by small, delicate blood vessels with a “chicken-wire-like” network. Some alveoli had lumens filled with hemorrhage. The tumor nests and microcysts consisted of epithelial cells with grade 3 (ISUP/WHO) nuclei and an optically clear cytoplasm. Furthermore, tumor cells containing “dust-like” eosinophilic cytoplasm were also observed. Moreover, some tumor cells diffusely contained fine-to-coarse eosinophilic granules (**Figure 1**). Numerous eosinophilic cytoplasmic inclusions and hyaline globules were also observed (**Figure 1**). Tumor necrosis was also seen. Immunohistochemically, the tumor cells expressed RCC, PAX-8, CAIX, and CD10 proteins (**Figure 2**). The immunomarkers CK7 and CD117 were negative. The patient was diagnosed with clear cell RCC (eosinophilic variant).

Next generation sequencing was performed at the Foundation Medicine (Cambridge, MA). The tumor was microsatellite stable and had a tumor mutational burden of 3 mutations/Mb. The gene mutations included *VHL* (S68fs*91), *BAP1* (M1I), and *RAF1* (V263G - subclonal).

Case 2

A 54-year-old male patient presented with hypercalcemia. His medical history included diabetes mellitus, hypertension, sleep apnea, and urolithiasis. The patient received umbilical hernia repair and appendectomy when he was a child. He had a weight loss of 50 lb over the course of 18 months. He denied having any gross hematuria or flank pain. Abdominal and pelvic CT scan revealed a 10-cm kidney mass.

Laboratory tests showed normochromic normocytic anemia and leukocytosis. Hypercalcemia was also observed and was treated with furosemide. Right radical nephrectomy was performed.

Grossly, the right kidney measured 22.5 × 16.6 × 7.8 cm and weighed 1599 g. Segments of the renal artery, renal vein, and 3.1-cm ureter

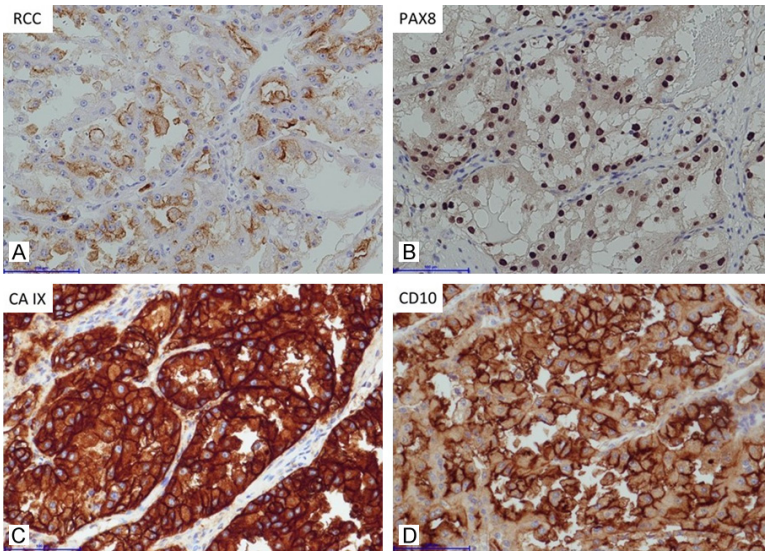

Figure 2. Case 1. Renal cell carcinoma cells show positive staining for RCC (A), PAX8 (B), CAIX (C), and CD10 (D) immunomarkers (Immunoperoxidase stain, 200 ×).

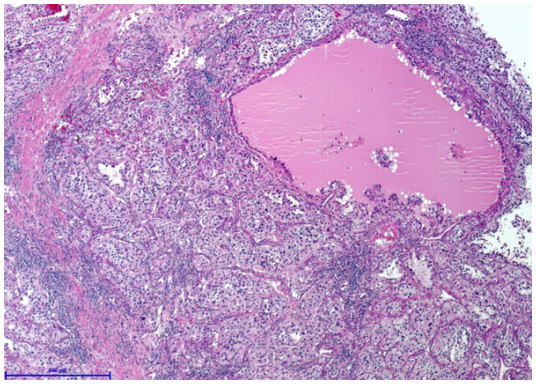

Figure 3. Case 2. The renal cell carcinoma is separated by fibrous septa and reveals many nodules of tumors with dense peripheral infiltrates. Occasional medium-sized cysts filled with eosinophilic serous fluid are observed (Hematoxylin and eosin, 200 ×).

extended from the hilum. A 1.1 × 0.6 × 0.5 cm tan-brown nodule adherent to the vascular wall was also noted within the renal margin. The nodule was not connected to the primary tumor. A cut surface of the tumor revealed a 11.5 × 7.8 × 7.1 cm white-pink, firm, lobulated and well-circumscribed mass replacing the majority of the upper pole of the renal parenchyma. The tumor involved the renal sinus and appeared poorly demarcated because it extended grossly into the hilar fat.

Microscopic examination of the kidney tumor revealed a well-circumscribed tumor separated by fibrous septa and composed of nodules with different morphological features. Medium-sized cysts filled with serous fluid were found elsewhere in the tumor and dense lymphocytic infiltrates encircling the tumor nodules were observed (**Figure 3**). Small tumor nodules composed of acini and microcysts lined by epithelial cells with optically clear cytoplasm and filled with hemorrhage were found in the lumen. Numerous eosinophilic granules were observed in the cytoplasm (**Figure 4**). A proliferation of branching vessels constructing a "chicken-wire" network was evident. These characteristic features are indicative of clear cell RCC. Furthermore, many large nodules composed of rhabdoid cells characterized by eccentric grade 4 (ISUP/WHO) nuclei and abundant eosinophilic cytoplasm were found (**Figure 5**). Tumor cells with sarcomatoid features were also observed. Immunohistochemical staining revealed that the tumor cells were stained positive for CAIX and CD10 immunomarkers (**Figure 6**). The immunohistochemistry staining was negative for CK7 and CD117.

Next generation sequencing was performed at the Foundation Medicine (Cambridge, MA). The tumor was microsatellite stable and had a tumor mutational burden of 1 mutation/Mb. The gene mutations included *ATM* (P2775fs*33) and *BAP1* (L429fs*14).

Discussion

RCCs with a clear cytoplasm have been observed in several types of renal tumors. The differential diagnoses of clear cell RCC include papillary RCC, clear cell papillary RCC, MiTF family translocation-associated RCC, clear cell RCC with fibromyomatous stroma, and chromophobe RCC [10]. However, the diagnosis of clear cell RCC is usually not difficult. The histopathological appearance of clear cell RCC typically shows tumor nests with and without lumens embedded in a stroma with delicate and arborizing vessels. The acinar, alveolar and microcystic structures are lined with clear cells

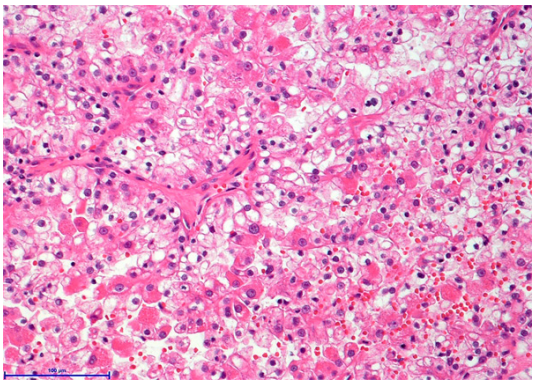

Figure 4. Case 2. Classical features of clear cell renal cell carcinoma are characterized by nests of clear cells with many eosinophilic cytoplasmic granules. The tumor nests are surrounded by a proliferative vascular network (Hematoxylin and eosin, 200 ×).

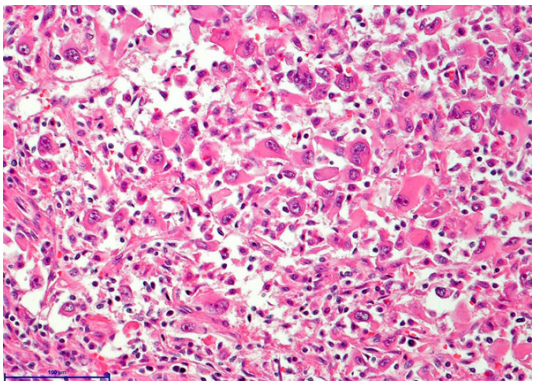

Figure 5. Case 2. Many solid tumor nodules are composed of rhabdoid-like cells with eccentric high-grade nuclei and abundant eosinophilic cytoplasm (Hematoxylin and eosin, 200 ×).

and the lumens are filled with proteinaceous fluid and fresh hemorrhage [4]. The lining cells of the acini and microcysts are comprised of cells with an optically clear cytoplasm and occasional eosinophilic granules or globules. Clear cell RCC typically demonstrates positive staining for CAIX, RCC, CD10, and PAX8 immunomarkers. The immunohistochemical stains CK7, AMACR, CD117, TFE3, and TFEB are usually negative [11-13]. The tumor in Case 1 and the focal areas of the tumor in Case 2 presented typical features of a clear cell RCC. Furthermore, the positivity of the immunomarkers CAIX and CD10 and the negativity of CK7 and CD117 also supported this diagnosis. The ultimate diagnosis of clear cell RCC with *BAP1* mutation is defined by next-generation sequencing, which demonstrated a *BAP1* mutation with or without a *VHL* mutation.

For many years, the VHL-HIF canonical signaling axis has been the main pathway involved in the tumorigenesis of clear cell RCC [14]. VHL encodes the VHL protein, which after binding to elongin B and C, ubiquitinates and subsequently degrades HIF-1α [14]. Therefore, a decrease in functional VHL increases HIF-1α, which upregulates downstream molecules including vascular endothelial growth factor (VEGF) and promotes vascular and cellular proliferation [14]. Interestingly, chromosome 3p also harbors three tumor suppressor genes - *PBRM1*, *SETD2*, and *BAP1*. These mutations are thought to contribute to renal tumorigenesis [14]. It has been proposed that *VHL* deletion alone may be insufficient for renal oncogenesis based on the observation that co-deletions of one or more of the three genes, *PBRM1*, *SETD2*, and *BAP1,* are frequently found in *VHL*-mutated RCCs [15]. In addition to co-opting for *VHL* in renal tumorigenesis, *BAP1* exerts its tumor suppressor function through multiple complex processes, including DNA repair. transcription, and mitochondrial metabolism [15]. Germline *BAP1* mutations lead to *BAP1* tumor predisposition syndrome, an inherited disorder of developing tumors, including uveal melanoma, malignant mesothelioma, atypical Spitz tumor, cutaneous melanoma, RCC, and basal cell carcinoma [16]. Somatic *BAP1* mutations occur in approximately 15% of patients with clear cell RCC [17]. With both *VHL* and *BAP1* mutations, the tumor in Case 1 displayed unique histopathological features. Classical "*VHL* mutation-like" changes including arborizing vascular proliferation, acini, and microcysts lined by clear cells were noted. Glycogen and lipid droplets were found in the cytoplasm. Furthermore, most lining renal cells with optically clear cytoplasm were voluminous and contained numerous eosinophilic granules. Many hyaline and eosinophilic cytoplasmic globules of varying sizes were also observed. However, these hyaline globules were much larger than the cytoplasmic eosinophilic granules. These features of clear cell RCC are consistent with the distinct morphology of *BAP1*-mutated clear cell RCC reported in the literature [7, 8]. The diffuse involvement of clear cells by numerous eosinophilic granules suggests a synergistic interaction of dysregulated mitochondrial metabolic function between *VHL* and *BAP1* genes.

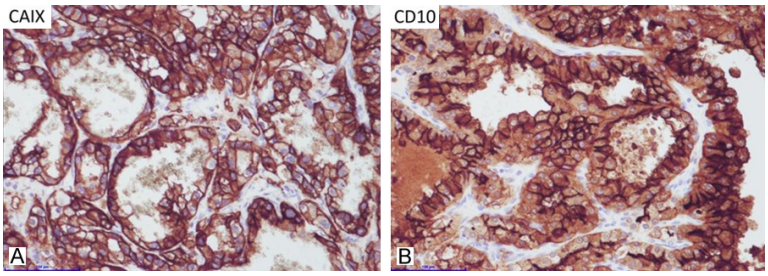

Figure 6. Case 2. The tumor cells stain positive for CAIX (A) and CD10 (B) immunomarkers (Immunoperoxidase stain, 200 ×).

Combined inactivation of *BAP1* and *PBRM1* has been reported in a few RCCs with rhabdoid features [6]. Interestingly, typical mutations in *BAP1* gene, but not in *PBRM1* are associated with high tumor grade [6]. *BAP1* and *ATM* mutations were detected in Case 2 in this study. No *VHL* or *PBRM1* mutation was detected. Nevertheless, in addition to the focal areas of typical "*VHL* mutation-like" appearance, Case 2 displayed distinct histopathological features of solid nodules of high-grade renal cells with rhabdoid features. Furthermore, eosinophilic granules and hyalinized material were observed in the cytoplasm. Although *ATM* inactivation may contribute to high tumor grade, we propose that *BAP1* mutations in RCC, without *VHL* or *PBRM1* involvement may be associated with rhabdoid and sarcomatoid features. However, we cannot exclude the possibility of intratumor heterogeneity, from which tissues with VHL alterations have not been thoroughly sampled. Thus, more cases of RCCs with only *BAP1* mutations are required to further support this hypothesis.

Currently, there are no separate guidelines or recommendations for the management of *BAP1*-mutated clear cell RCC. Cryosurgery or thermal ablation can be performed for tumors smaller than 3 cm. Radical and partial nephrectomy (nephron-sparing surgery) remains the standard treatment for clinically localized RCC [9]. Partial nephrectomy is the treatment of choice for clinically staged cT1a tumors (< 4 cm). It has been shown that the local recurrence rate was up to 3.6% and overall cancer-free survival ranged from 86.4%-98.4% [18]. It should be considered in treating patients with T1b or T2 tumors, and is indicated in clinical settings for bilateral renal masses, solitary kidneys, and chronic kidney diseases [9]. Radical nephrectomy is indicated to manage more advanced tumors (> 4 cm, cT1b through cT4) [9]. Metastatic renal cell carcinomas are treated with systemic therapies. VEGF inhibitors including sunitinib, sorafenib, bevacizumab, pazopanib, cabozantinib, nivolumab, and lenvatinib have shown efficacy for advanced tumors. In a phase 3 trial, sunitinib-alone group and nephrectomy-sunitinib group displayed median overall survival of 18.4 months and 13.9 months, respectively [19]. Adjuvant pembrolizumab (anti-programmed death 1 antibodies) after nephrectomy has revealed a higher percentage of patients with disease free survival at 24 months [20].

In conclusion, we report two cases of *BAP1*-mutated clear cell RCC with distinct histopathological features. *BAP1*-mutated clear cell RCCs are associated with aggressive clinical behavior and poor clinical outcome. The follow-up visits 18 months after surgery were uneventful in Case 1; however, Case 2 was diagnosed with lung metastasis 14 months after radical nephrectomy. Pembrolizumab immunotherapy was recommended for further treatment, but it was denied by the patient.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Y Albert Yeh, Pathology and Laboratory Medicine Service, Overton Brooks VA Medical Center, No. 510 East Stoner Avenue, Shreveport, LA 71101, USA. Tel: 318-221-8411 Ext. 16259; Fax: 318-990-4834; E-mail: Yunshin.Yeh@va.gov

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