Original Article
Oncocytic papillary renal cell carcinoma: a clinicopathological study emphasizing distinct morphology, extended immunohistochemical profile and cytogenetic features

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Abstract: Papillary renal cell carcinoma (PRCC) is traditionally classified into type 1 and type 2. Recently, an oncocytic variant of PRCC has been described. We report a series of 6 oncocytic renal papillary tumors (OPRCC) which tended to occur in older patients (mean, 56.8 years) with a male preference (male-to-female ratio is 5:1). All 6 patients are alive with no evidence of disease after initial resection, showing an indolent clinical behavior. Histologically, tumors exhibited predominant papillary structure with delicate fibrovascular cores. Papillae were lined by single layers of cells with large, deeply eosinophilic and finely granular cytoplasms and round regular nucleus. The phagocytosis of tumor cells was frequently and evidently seen in our cases that hemosiderin-laden tumor cells and foamy tumor cells were noticed in five and four cases respectively. All tumors were immunoreactive for racemase, vimentin, CD10, and MET and negative for CD117. While E-cadherin, EMA, and cytokeratin 7 exhibited variable immunopositivity. FISH analysis was performed in five of six cases and found heterogeneous results. Trisomy of chromosomes 7 was found in three cases and trisomy of chromosomes 17 in two cases. Loss of chromosome Y was noted in one of four tumors in male patients. MET gene status was also investigated by direct sequencing in all 6 cases and found no distinct mutation in any case. These results suggest that OPRCC shows distinct morphology, indolent clinical behavior, and similar immunohistochemical and cytogenetic features with PRCC, seems to be a variant in the PRCC group. Whether the strong expression of MET indicates a potential therapeutic target is still unknown and requires further investigation in clinical trials.

Keywords: Kidney, papillary renal cell carcinoma, oncocytic tumors, MET

Introduction

Papillary renal cell carcinomas (PRCC) is a well-established subtype of RCC with characteristic gross and histological features and is further subdivided into 2 subtypes, type 1 and 2, for its distinct morphological feature and prognostic implications. Type 1 PRCC consist of small cells with low nuclear grade and a scant amount of cytoplasm arranged in a single layer, whereas type 2 PRCC tumor cells are larger, with abundant eosinophilic cytoplasm, higher nuclear grade, and nuclear pseudostratification. The two types of PRCC also demonstrate different clinical behavior. Patients with type 2 have a poorer prognosis than those with type 1 [1]. Therefore, accurate subtyping of PRCC is important for prognosis and proper patient management.

Recently, a new histopathologic variant of PRCC named oncocytic PRCC (OPRCC) has been described. It was first reported by Lefevre et al. in 2005 that 10 cases of RCC with the features of prominent papillary architecture, abundant granular eosinophilic cytoplasm and low-grade nonoverlapping nuclei [2]. These tumors exhibited histological features overlapping those of type 1 (low nuclear grade and a single layer) and type 2 (abundant eosinophilic cytoplasm)

PRCC, and characterized by strong expression of CD10, vimentin, and AMACR. While none showed the genetic changes of trisomy 7 or 17, which were reported in more than 90% of type 1 and 70% of type 2 PRCC. Lefevre et al. regarded these tumors as an independent subtype of PRCC. After then, a few similar tumors have been reported as OPRCC, but showed heterogeneous clinicopathologic features. Their immunoreactivity seemed conflict and variable. And their cytogenetic data remained controversial that most cases showed trisomy of chromosome 7 and 17, while some cases did not [3-7]. In this article, we reported 6 such oncocytic papillary renal tumors. For these cases, we documented distinct histopathology, immunophenotype, molecular genetic features, and clinical behavior.

Materials and methods

Patients

We retrieved approximate 1500 RCCs between 1997 and 2011 from the files of Departments of Pathology at Nanjing Jingling Hospital (China) and selected 6 cases with the presence of both prominent papillary architecture and abundant oncocytic cytoplasm with low-grade nonoverlapping nuclei. The clinicopathologic features such as age, sex, disease histology, treatment, and the final follow-up dated from the time of initial diagnosis were recorded.

Immunohistochemistry

Tissues were fixed in 10% formalin and embedded in paraffin. Sections of 3-mm thickness were stained for hematoxylin and eosin and Prussian blue. Immunohistochemical analysis used the following antibodies: Vimentin (V9, Zymed, 1:200), racemase (P-504S, Zeta, Sierra Madre, 1:50), EMA (E29, DAKO, Glostrup, 1:1000), CK7 (OV-TL12/30, Zymed, 1:300), CD10 (56C6, Novocastra, 1:100), E-Cadherin (18-0223, Zymed, 1:100), CD117 (Polyclonal, Dako, 1:100), MET (24H2, Cell Signaling Technology, 1:100). Immunoreaction was performed using the labeled streptavidin-biotin method. Diaminobenzidine (3,3'-diaminobenzidine) was used for visualization. The interpretation of immunoreactivity was performed in a semiquantitative manner by analyzing the extent of the staining positivity of the tumor cells. The interpretation score was as follows: 0

or negative ≤ 5% tumor cell positivity; 1+ or focal = 5% to 10% tumor cell positivity; 2+ or moderate = 11% to 50% tumor cell positivity; and 3+ or diffuse > 50% tumor cell positivity.

MET mutation analysis

Genomic DNA were extracted from the formalin-fixed, paraffin-embedded tissue samples of the tumor and nonneoplastic tissue by the DNeasy Blood & Tissue Kit (QIAgen, Hilden, Germany), according to the manufacturer's protocol. In the composite tumors, genomic DNA of different components was extracted respectively from corresponding blocks or areas. The 14-21 exons of MET were amplified by PCR using the previously described primer pairs [8]. For sequence analysis, the PCR products were purified using the Wizard PCR Preps Purification System (Promega Corp.). Sequencing was performed using Big Dye Terminator and an ABI Basecaller (Applied Biosystems).

Fluorescence in situ hybridization

Deparaffinized slides containing tumor tissue were subjected to heat pretreatment (pressure cooking for 10 min at full pressure) in distilled water and then digested using 0.25% pepsin (Sigma, Taufkirchen, Germany) and 0.01 M HCl for 15 min at 37°C. After rinsing twice in 2 × SSC for 5 min, slides were dehydrated by immersing in 70%, 85%, and 100% ethanol for 1 min each at room temperature and then air-dried. Slides containing tissue DNA probe (10 μl/slide) were codenatured in an in situ thermocycler (Perkin Elmer, System 1000, Germany) at 88°C for 9 min, annealed at 37°C and hybridized in a humid chamber at 37°C overnight. After posthybridization washing in 0.4 × SSC (°C 2 min), and 2 × SSC (room temperature 2 min), slides were coverslipped by 10 ml of 4,6 diamino-2-phenylindole counterstain.

Spectrum Green centromeric probes (CEP) for chromosomes 7, 17, and Y (Vysis, Downers Grove, IL) were used. Each probe was diluted 1:30 in tDenHyb1 buffer (Insitus, Albuquerque, NM). Ten microliters of diluted probe was applied to each slide and cover slips were placed over the slides. Denaturation was achieved by incubating the slides at 83°C for 12 minutes in a humidified box, then hybridization was done at 37°C over night. The cover slips were then removed and the slides were

immersed at room temperature in 0.5 × SSC for 2 minutes, in 50% formamide/1 × SSC for 5 minutes, and in 2 × SSC for 2 minutes. The slides were air-dried and counterstained with 10 lL DAPI/Antifade (DAPI in Fluorguard, 0.5 µg/mL, Insitus).

FISH evaluation

For each case, a minimum of 100 tumor nuclei were examined with probes by fluorescence microscopy at × 1000 magnification. Nonneoplastic kidney parenchyma was used as control tissue. Only nonoverlapping tumor nuclei were evaluated. Definitions of chromosomal gains and losses were based on the Gaussian model and related to the nonneoplastic controls. Any tumor with a signal score beyond the cutoff value was considered to have had a gain or loss of the specific chromosome. The cutoff value was set at the mean ± 3 standard deviations (SDs) of the control values [9-13].

Results

Clinical features

The clinical features are summarized in **Table 1**. The 6 cases of OPRCC were diagnosed from 1990 to 2011 at Nanjing Jinling Hospital, accounting for 0.5% of all renal tumors diagnosed during this period. The patients' ages ranged from 40 to 68 years (mean, 56.8; median, 59.5 years). The male-to-female ratio was 5:1, exhibiting a male preference. None of the patients had bilateral or multifocal neoplasms. Nephrectomy was performed at the time of diagnosis for all patients. None of these patients was treated with chemotherapy or radiation therapy after surgery. Follow-up was available for 6 patients (range, 33 to 121; mean, 82; median, 84 months). All 6 patients were alive with no evidence of disease after initial resection.

Morphology

The histological features are summarized in **Table 1**. These tumors ranged from 25 to 65 mm in size (mean, 40; median, 36 mm). All tumors showed a predominant papillary structure with delicate fibrovascular cores, while diffuse growth pattern was also seen in 4 cases. Papillae were lined by single layers of cells with large, deeply eosinophilic and finely granular cytoplasm, a low nucleocytoplasmic ratio, and round to oval nuclei. All tumors were Fuhrman grade 2, except for one tumor that was focally grade 3 due to huge nucleolus. Most of our cases (4/6) showed foamy macrophages within papilla cores. Likewise, hemorrhages were observed in five of six cases. No case had either necrosis or psammoma bodies (**Figure 1A** and **1B**).

Interestingly, the phagocytosis of tumor cells was frequently and evidently seen in our cases. Hemosiderin-laden tumor cells were exhibited in five cases (**Figure 2A**, **2B** and **2D**), while foamy tumor cells were noticed in four cases (**Figure 2C**).

Immunohistochemistry

The results of immunohistochemistry are summarized in **Table 2**. The neoplastic oncocytic cells in all cases exhibited strong, diffuse positivity for vimentin, CD10, racemase and MET. Tumorous cells demonstrated variable positivity for E-cad (5/6, 83%), CK7 (1/6, 17%), and EMA (2/6, 33%). CD117 was negative in all cases (**Figure 1C-F**).

MET mutation analysis

We analyzed the 14-21 exons of MET gene by direct sequencing in all 6 cases and found no distinct mutation in any case.

FISH analysis

The summary of FISH data is presented in **Table 2**. FISH analysis was performed in five of six cases, demonstrating trisomy of chromosomes 7 in three cases and trisomy of chromosomes 17 in two cases. Loss of chromosome Y was noted in one of four tumors in male patients.

Discussion

OPRCC was first reported by Lefevre et al. in 2005 with the histological features overlapping those of type 1 (low nuclear grade and a single layer) and type 2 (abundant eosinophilic cytoplasm) PRCC, and regarded as an independent subtype of PRCC not only for its distinct pathological features but also for its indolent clinical behavior [2]. After then, a few correlational research were published and 41 similar tumors have been reported as OPRCC [3-5, 7].

Table 1. Clinicopathological data

Case No.	Age/ Sex	Size (mm)	TNM Stage	Fuhrman	Hemorrhage	Necrosis	Foamy Macrophages	Foamy Tumor Cells	Hemosiderin-laden tumor cells	Treatment	Outcome
1	67/M	32	pT1N0M0	2	Present	Absent	Present	Present	Present	RN	NED 121 months
2	67/M	28	pT1N0M0	2	Absent	Absent	Absent	Present	Absent	RN	NED 82 months
3	52/F	50	pT1N0M0	3	Present	Absent	Absent	Absent	Present	RN	NED 86 months
4	40/M	25	pT1N0M0	2	Present	Absent	Present	Present	Present	RN	NED 74 months
5	68/M	65	pT1N0M0	2	Present	Absent	Present	Absent	Present	RN	NED 33 months
6	47/M	40	pT1N0M0	2	present	Absent	Present	Present	present	RN	NED 96 months

RN, radical nephrectomy; NED, no evidence of disease.

Table 2. Immunohistochemical Profiles and Fluorescence In Situ Hybridization Analysis

Case No.	Immunohistochemistry								Fluorescence In Situ Hybridization		
	racemase	CD10	vimentin	CK7	MET	CD117	E-cadherin	EMA	CEP 7	CEP 17	CEP Y
1	++	+	+++	0	+++	0	+	0	—	—	—
2	++	++	++	0	+++	0	++	0	Disomic	Disomic	Lost
3	++	+++	+++	0	+++	0	+	0	Trisomic	Trisomic	Female
4	++	+	+++	0	+++	0	+++	0	Trisomic	Disomic	Not Lost
5	++	++	++	++	+++	0	+	+	Disomic	Trisomic	Not Lost
6	+++	+	++	0	+++	0	0	+	Trisomic	Disomic	Not Lost

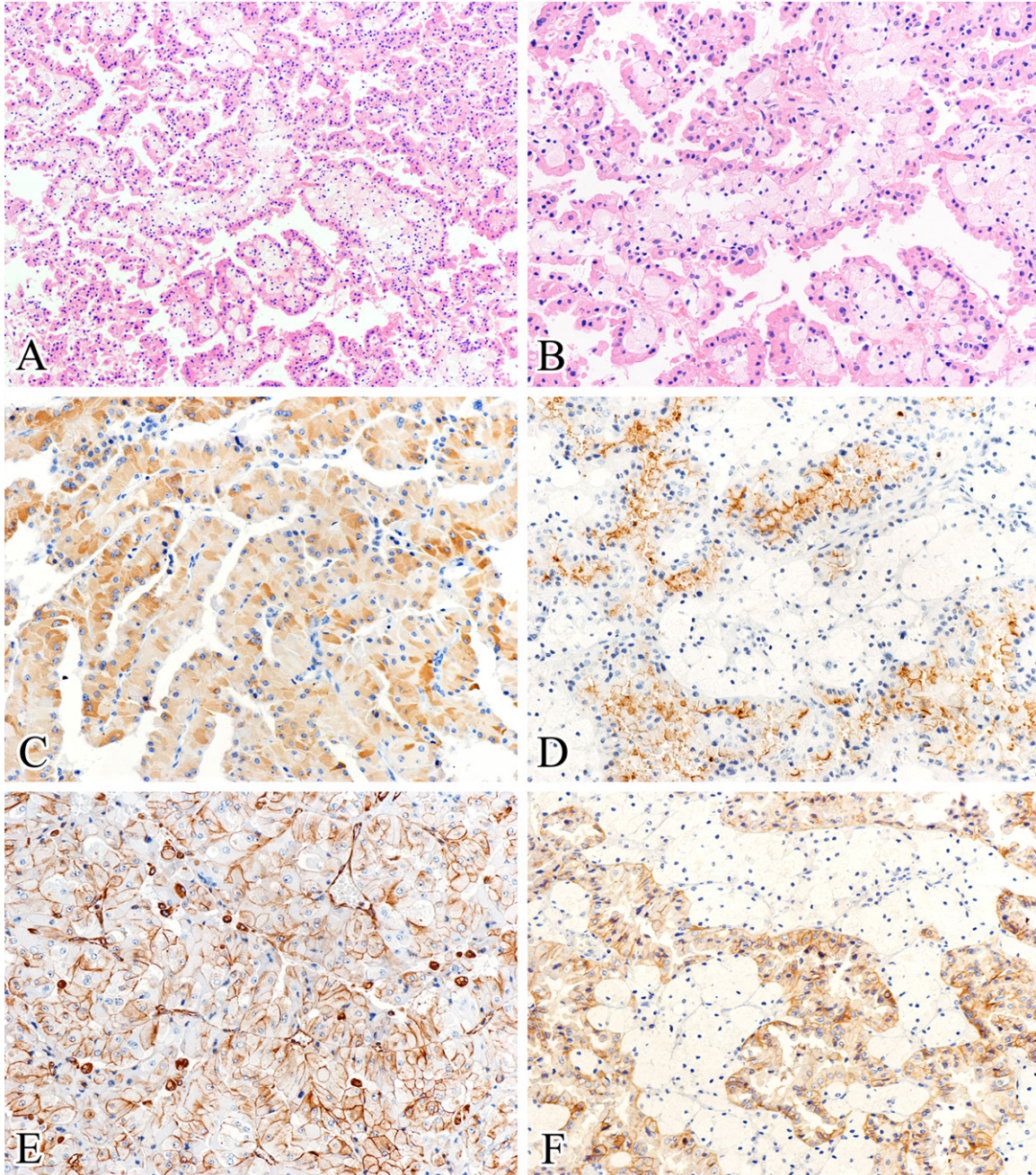0, null; +, mild; ++, moderate; +++, intense staining.

Figure 1. Histological and immunohistochemical features. A: All tumors showed a predominant papillary structure and delicate fibrovascular cores, with foamy macrophages usually seen in the papilla cores (hematoxylin and eosin). B: Papillae were lined by single layers of tumor cells with large, deeply eosinophilic and finely granular cytoplasm, and low grade nuclei (hematoxylin and eosin). C-F: All cases exhibited strong, diffuse positive for racemase (C), CD10 (D), vimentin (E), and MET (F) staining (immunohistochemical staining). (Original magnifications: A × 100; B-F × 200).

Herein, we describe 6 patients with OPRCC, accounting for 0.5% of RCCs in our renal tumor series. The clinical and histological features are consistent with previous studies. Patients in our study tend to be older in age (mean, 56.8; median, 59.5 years) with a male preference (male-to-female ratio is 5:1). All 6 patients are alive with no evidence of disease after initial resection, showing an indolent clinical behavior. Histologically, tumors exhibit predominant papillary structure with delicate fibrovascular cores. Papillae are lined by single layers of cells
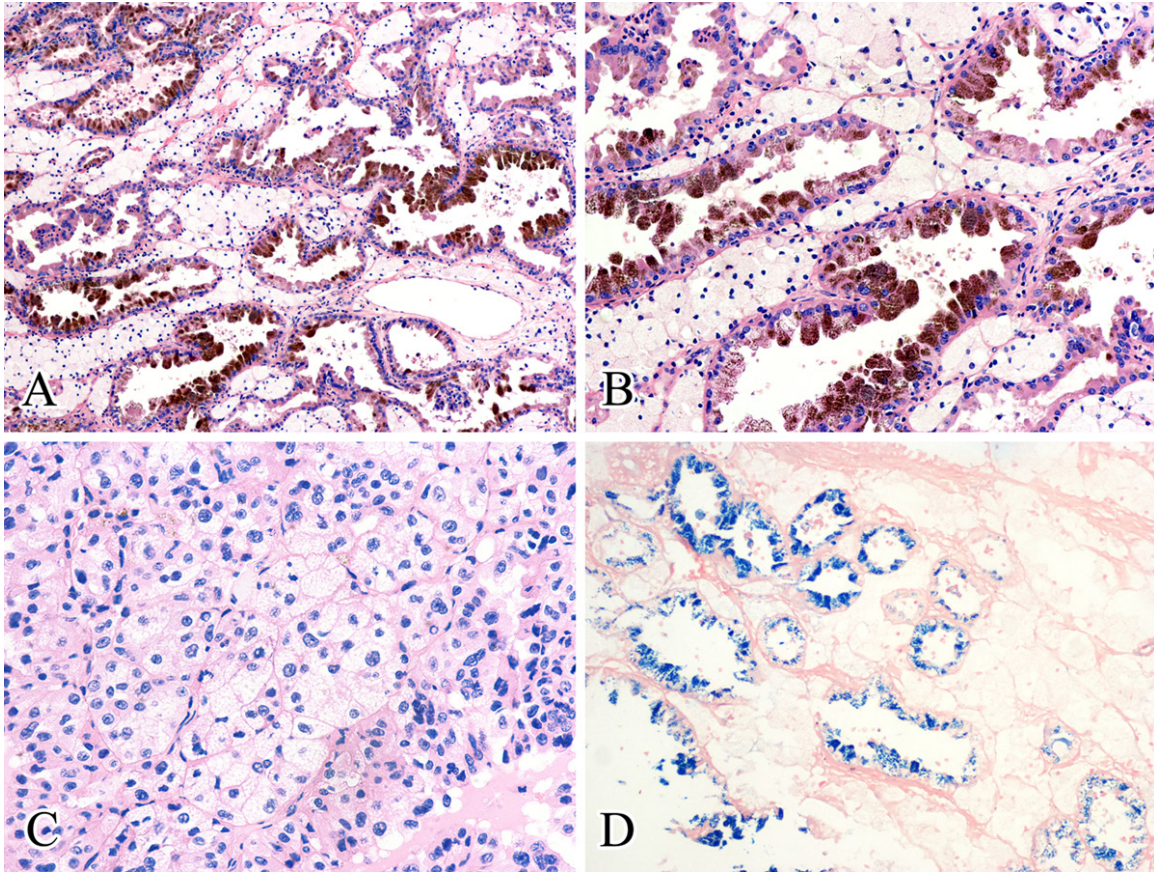
Figure 2. The phagocytosis of tumor cells. A, B: Hemosiderin-laden tumor cells were frequently and evidently seen in our cases (hematoxylin and eosin). C: Foamy tumor cells were noticed in four cases (hematoxylin and eosin). D: Prussian blue staining was used to reveal intracellular hemosiderin (Prussian blue staining). (Original magnifications: A, D × 100; B × 200; C × 400).

with large, deeply eosinophilic and finely granular cytoplasm. In some studies, it was described that the nuclei of OPRCC tend to be at the periphery rather than at the center of the cells, suggesting an inverted nuclear pattern [7]. But in our series, such phenomenon is not frequently seen. It is of interest that the phagocytosis of tumor cells is frequently and evidently seen in our cases, which has not been described previously. Hemosiderin-laden tumor cells were exhibited in five cases, while foamy tumor cells were noticed in four cases. In our experience, it is not usually found in the majority of RCCs, such as conventional RCC or typical PRCC. These clues may be of some help in pointing to the correct diagnosis.

The immunoprofiles of OPRCC are generally similar to typical PRCC. Of all the reported cases of OPRCC, including the six described here, 94% (45/48), 85% (35/41) and 79% (38/48) of the cases showed diffuse positivity

for AMACR, vimentin and CD10, respectively. CD117 is always negative in such tumors although one study found that seven of seven tumors exhibited CD117 expression. But CK7 is variable expressed among reports, ranging from 17% to 100% [2-5, 7]. The present study find CK7 inmmunopositivity in only one of the six tumors (17%), exhibiting a slight difference with typical PRCC, which is always diffusely positive for CK7 [1]. These conflicting results can be explained because OPRCC represents a heterogeneous group.

Otherwise, we find uniform positive immunostaining for MET in all cases, indicating MET overexpression contrast to adjacent normal tissue. MET is the receptor for the hepatocyte growth factor (HGF), belongs to the family of tyrosine kinase (TK). The HGF/MET signaling pathway is involved in proliferation, survival, cell growth, differentiation, and cell migration. MET was reported to be overexpression in

80-90% of sporadic RCC by the immunohisto-chemistry. A germline mutation of MET gene of 7q31 has been detected in patients with hereditary PRCC as well as a small portion (5-13%) of sporadic PRCC. Most sporadic PRCCs show trisomy of chromosome 7 without MET mutation, and the increased MET gene copy number may also lead to MET overexpression in protein level. Most MET mutations that correlate to PRCC are missense, located within the TK domain [8, 14-16]. We performed the DNA mutation analysis by direct sequencing of exon 14-21 of MET gene which included the TK domain, and found no mutation in any case. We also performed FISH analysis of chromosome 7, 17 and Y in five of six cases. The results demonstrate trisomy of chromosomes 7 in three cases and trisomy of chromosomes 17 in two cases. Loss of chromosome Y was noted in one of four tumors in male patients. The cytogenetic features of OPRCC are consistent with that of typical PRCC, supporting the diagnosis of OPRCC as a subtype of PRCC. In present study, gains of chromosomes 7 can partly explain the overexpression of MET. Whereas, there are two cases showed high level of MET protein but were absent for chromosomes 7 gain or MET gene mutation. Their mechanisms of MET overexpression remain unexplained. It may be because of other events such as upstream activation and translational control deregulation. Recently, considerable attention has been focused on the role of MET overexpression and amplification in tumor genesis. MET-inhibitors have been in trial and demonstrated antitumor activity for papillary RCC [17, 18]. For MET is high expressed in OPRCC, it may be a potential therapeutic target for this tumor.

In conclusion, we reported a distinct subset of PRCC with oncocytic features, strong diffuse expression of racemase, vimentin, CD10, and MET, but negative for CK7 in most cases. Trisomy for chromosome 7 and 17 and loss of chromosome Y exhibited variable results. All cases have an indolent clinical behavior. Whether the strong expression of MET indicates a potential therapeutic target is still unknown and requires further investigation in clinical trials.

Disclosure of conflict of interest

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