Original Article
Clear cell papillary renal cell carcinoma: a clinicopathological study emphasizing ultrastructural features and cytogenetic heterogeneity

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Abstract: Clear cell papillary renal cell carcinoma (CCPRCC) is a recently recognized renal neoplasm, which was initially described in end-stage renal disease (ESRD), but some cases have been reported in otherwise normal kidneys. We report a series of 11 CCPRCC (age range, 33-72 years; male-to-female ratio, 8:3). Follow-up was available for 8 patients. No patients developed local recurrence, distant or lymph-node metastasis, or cancer death. Histologically, all tumors exhibit morphologic features typical of CCPRCC including a mixture of cystic and papillary components, covered by small to medium-sized cuboidal cells with abundant clear cytoplasm. All 11 cases exhibited moderate to strong positivity for CK7, CA9, Vim, and HIF-1α, coupled with negative reactions for CD10, P504S, and RCC. We did not find any *VHL* gene mutations in all 11 cases. Losses of chromosomes 3 (monoploid chromosome 3) was detected in 3 cases. Ultrastructurally, the tumor cells composed of numerous glycogens with scanty cell organelles, reminiscent of clear cell renal cell carcinoma (CCRCC). In conclusion, the coexpression of CA9 and HIF-1α in the absence of *VHL* gene abnormalities in CCPRCC suggests activation of the HIF pathway by mechanisms independent of *VHL* gene mutation. Losses of chromosomes 3 (monosomies chromosome 3) was detected in 3 cases suggesting that at least some of these lesions have demonstrated abnormalities of chromosomes 3. Ultrastructurally, CCPRCC composed of numerous glycogens with scanty cell organelles, reminiscent of CCRCC suggesting the close pathogenesis relationship of CCPRCC with CCRCC.

Keywords: Renal cell carcinoma, clear cell papillary renal cell carcinoma, *VHL*, immunohistochemistry, fluorescence in situ hybridization, ultrastructure

Introduction

Clear cell papillary renal cell carcinoma (CCPRCC) also known as clear cell tubulopapillary renal cell carcinomas is a recently recognized renal neoplasm, composed of cells with clear cytoplasm lining cystic, tubular, and papillary structures [1-3]. Clinically, it was initially described in end-stage renal disease (ESRD), but some cases have been reported in otherwise normal kidneys [1-3]. These tumors have specific immunohistochemical and genetic profiles distinct from clear cell renal cell carcinoma (CCRCC) and papillary renal cell carcinoma (PRCC) with constant expression of CK7 and carbonic anhydrase IX (CA9), as well as negative reactivity for alpha-methylacyl-CoA racemase (AMACR) and CD10 [1-4]. Alterations of

chromosome 3p and the *VHL* gene, a finding seen in CCRCC, are lacking. None had gains of chromosome 7 and 17 or loss of Y chromosome, typical of PRCC [1-4].

In this article, we reported 11 such clear cell papillary renal tumors, arising in kidneys unaffected by ESRD. For these cases, we documented ultrastructural features and cytogenetic heterogeneity of CCPRCC.

Materials and methods

Cases

Eleven cases of CCPRCC, dating from 2002 to 2012, were retrieved from the archives of the Department of Pathology at Nanjing Jinling Hospital, Nanjing University School of Medicine,

Table 1. Clinicopathological features

Case No.	Age/Sex	Size (cm)	Stage	Fuhrman	Treatment	Outcome
1	72/M	3.6	T1N0M0 (I)	2	RN	NA
2	37/M	1.3	T1N0M0 (I)	2	RN	NED 98 months
3	41/F	1.0	T1N0M0 (I)	2	RN	NED 35 months
4	33/M	3.0	T1N0M0 (I)	2	RN	NA
5	50/F	2.0	T1N0M0 (I)	2	RN	NED 132 months
6	69/M	2.2	T1N0M0 (I)	2	RN	NED 16 months
7	44/M	2.6	T1N0M0 (I)	2	RN	NED 67 months
8	36/M	4.0	T1N0M0 (I)	2	RN	NED 63 months
9	43/M	2.0	T1N0M0 (I)	2	RN	NED 120 months
10	40/M	1.6	T1N0M0 (I)	2	RN	NA
11	34/F	3.4	T1N0M0 (I)	2	RN	NED 43 months

NED, no evidence of disease; RN, radical nephrectomy; NA, not available.

Nanjing, China. The clinicopathologic features, treatments, and follow-up data were recorded.

Light microscopy

Tissues were fixed in 10% formalin and embedded in paraffin. Sections of 3 mm thickness were stained with hematoxylin and eosin (HE), and immunohistochemistry. The following antibodies were used: CK7 (OV-TL 12/30, Zymed, 1:300); CD10 (56C6, Novocastra, 1:100); vimentin (V9, Zymed, 1:200); P504S (AMACR) (13H4, ZETA, 1:60); CA9 (Polyclonal, Abcam, 1:200); hypoxia inducible factor-1α (HIF-1α) (Novus Biologicals, 1:500); Anti-RCC marker (SPM314, Dako, 1:100). Immunoreaction was performed using the labelled streptavidin-biotin method and overnight incubation and evaluated in a semiquantitative way assessing both staining intensity and percentage of positive cells as previously described [5, 6]. For all antibodies, the resulting score was calculated by multiplying the staining intensity (0 = no staining, 1 = mild staining, 2 = moderate staining, and 3 = strong staining) by the percentage of immunoreactive tumor cells (0 to 100). The immunostaining was considered 0 or negative when the score was <25; 1+ or weak, 26 to 100; 2+ or moderate, 101 to 200; and 3+ or strong, 201 to 300.

VHL 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. *VHL* sequence analysis of all three exons was performed as recently described [7]. Six sets of PCR primers covering exon l, exon 2, and the translated region of exon 3 were as follows: 1.1-forward AGGTCGACTCGGGAGC-GC, 1.1-reverse CGTCTTCTTCAGGGCCGTAC, 1.2-forward AGGCAGGCGTCGAAGAGTAC, 1.2-reverse ACGCGCGGACTGCGATTGC, 1.3-forward CA-GGTCATCTTCTGCAATCGC, 1.3-reverse CTTCAG-ACCGTGCTATCGGTC; 2.0-forward GTGGCTCTT-TAACAACCT TTGC, 2.0-reverse CCTGTACTTA-CCACA ACA ACCTTA TC; 3.1-forward TGTACTG-AGACCCTAGTCTGC, 3.1-reverse GCGCTCCTGTG-TCAGCCGCTC, 3.2-forward TGGAAGACCACCCAA ATGTGC, 3.2-reverse GACTCATCAGTACCATCA AAAGC.

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 (FISH) detection for chromosome 3p deletion

FISH detection for chromosome 3p deletion were performed as recently described [2, 4].

Ultrastructural analysis

Tissues were fixed in 2.5% glutaraldehyde and routinely processed for electron microscopic examination as recently described [8].
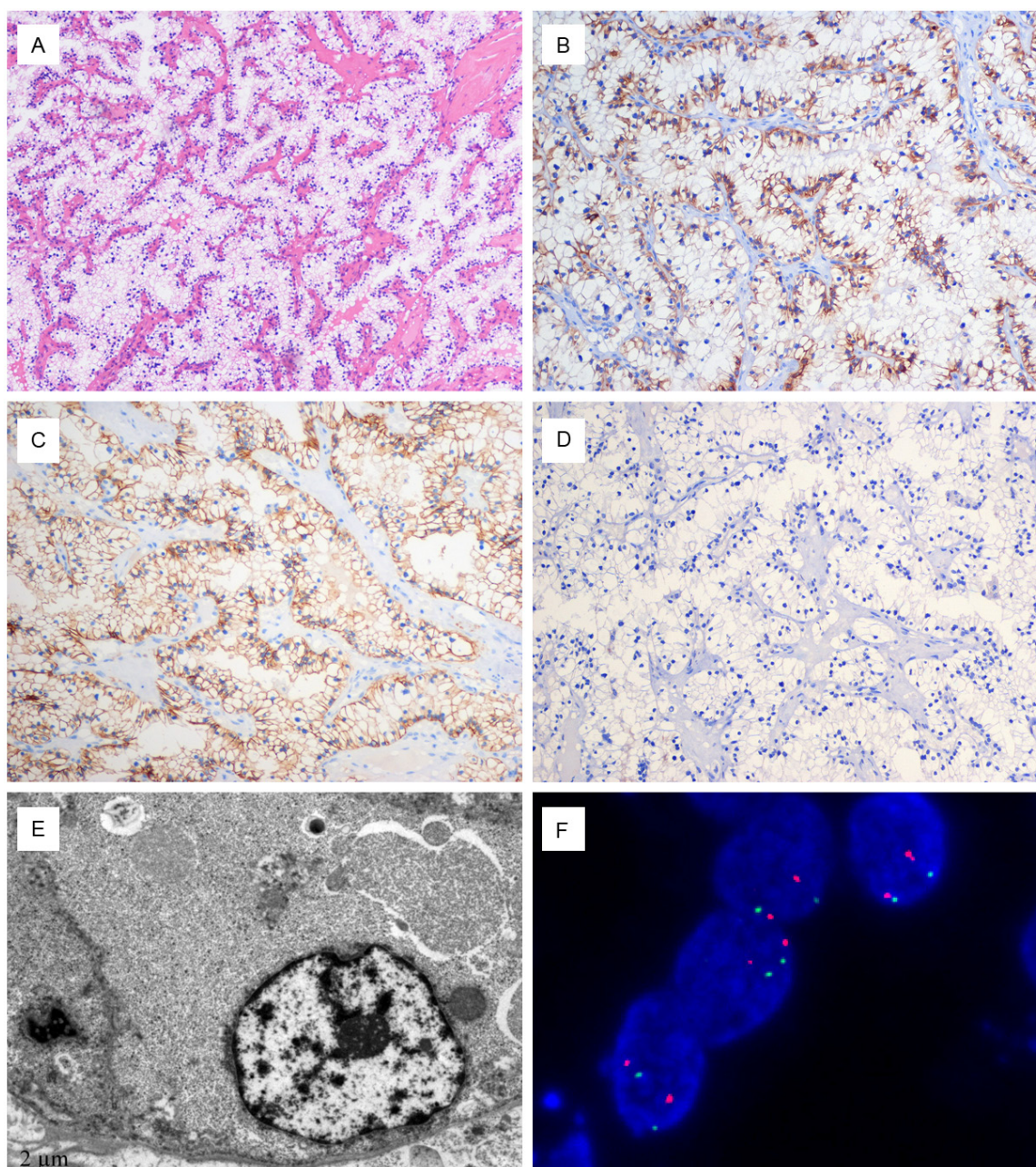
Results

Clinical features

The clinical features are summarized in **Table 1**. The 11 cases of CCPRCC were diagnosed from 2000 to 2012 at Nanjing Jinling Hospital,

Figure 1. Clear cell papillary renal cell carcinoma. (A) The tumor is predominantly papillary with fibrovascular cores of variable thickness. Papillae are covered by small to medium-sized cuboidal cells with abundant clear cytoplasm (original magnification, ×100). The tumor moderately to strongly expresses CK7 (B), and CA9 (C), coupled with negative reaction for CD10 (D) (original magnification, ×200). (E) Ultrastructurally, the tumor cells (case 11) composed of numerous glycogens with scanty cell organelles, reminiscent of CCRCC. (F) FISH with centromeric probe for chromosome 3 (Spectrum Orange) and with subtelomeric probe for 3p25 (Spectrum Green) showing nuclei with 2 hybridization signals for both the probes.

accounting for 1.2% of all renal cell carcinomas diagnosed during this period. The patients' ages ranged from 33 to 72 years (mean, 52.5; median, 45.4 years). The male-to-female ratio was 8:3, exhibiting a male preference. None of the patients had bilateral or multifocal neo-plasms. Nephrectomy was performed at the time of diagnosis for all patients. These tumors ranged from 1.0 to 4.0 cm in size (mean, 2.5; median, 2.4 cm). The Fuhrman nuclear grade of 2 was observed in all 11 tumors. Pathological tumor stage according to the World Health
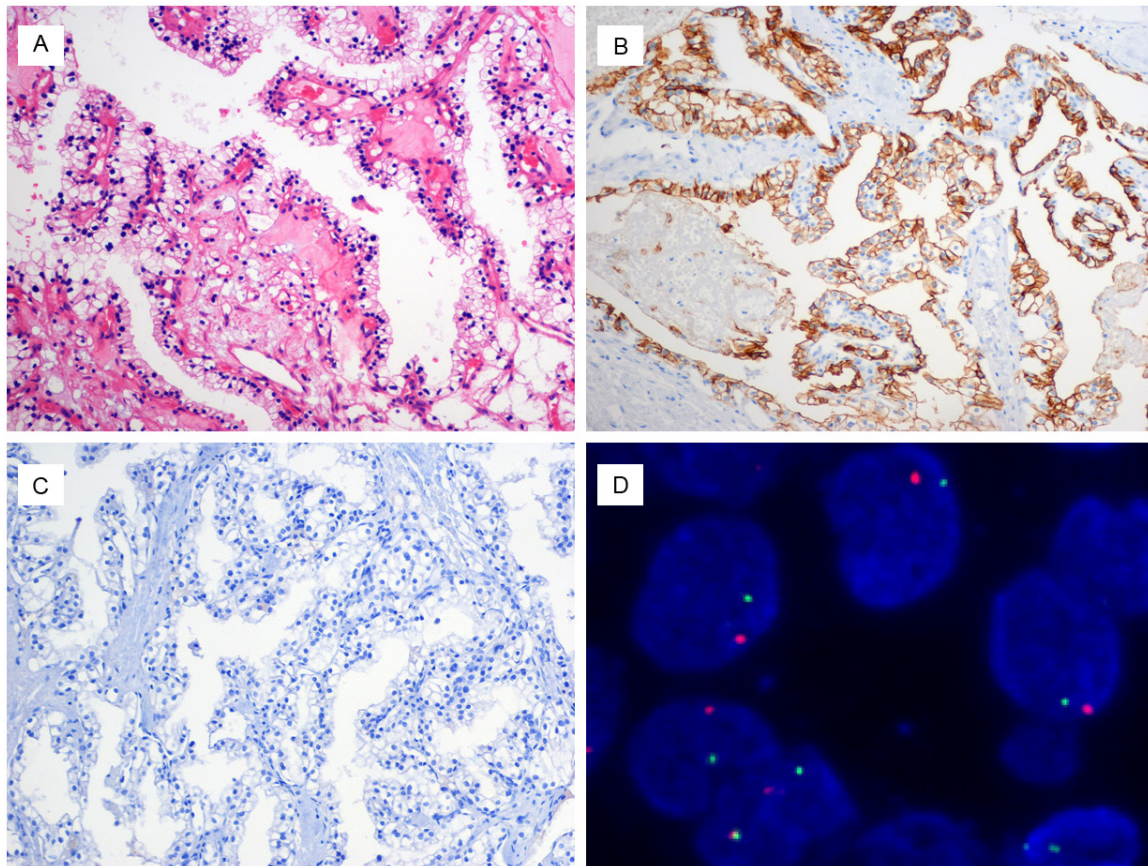
Figure 2. In case 2 (A) the tumor is composed of papillary structures lined by cells with clear cytoplasm (original magnification, ×200). (B) CK7 is diffusely expressed (original magnification, ×200). (C) The expression of CD10 is negative (original magnification, ×200). (D) The tumor showed monoploid chromosome 3.

Table 2. Immunohistochemical profiles and fluorescence in situ hybridization analysis

Case No.	Immunohistochemical Profiles							FISH	
	CA9	CD10	P504S	CK7	RCC	Vim	HIF-1α	3p25	CEP 3
1	+++	0	0	+++	0	+	++	Not loss	Disomic
2	+++	0	0	++	0	+++	++	monoploid	monoploid
3	++	0	0	+++	0	+	+++	Not loss	Disomic
4	+++	0	0	+++	0	+++	++	Not loss	Disomic
5	+++	0	0	++	0	+++	+++	monoploid	monoploid
6	++	0	0	+++	0	+++	++	Not loss	Disomic
7	+++	0	0	++	0	++	++	monoploid	monoploid
8	++	0	0	+++	0	+++	+++	Not loss	Disomic
9	+++	0	0	++	0	++	++	Not loss	Disomic
10	+++	0	0	+++	0	++	+++	Not loss	Disomic
11	+++	0	0	++	0	++	++	Not loss	Disomic

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

Organization (WHO) 2004 classification was pT1 in all cases. None of these patients was treated with chemotherapy or radiation therapy after surgery. Follow-up was available for 8 patients (range, 16 to 132; mean, 74; median, 71.7 months). No patients developed local recurrence, distant or lymph-node metastasis, or cancer death.

Morphology

All tumors were well circumscribed from the renal parenchyma by a fibrous capsule and demonstrated morphologic features typical of CCPRCC as previously described [1-3]. Papillary and cystic architecture were present at least focally in all tumors. The papillae were covered by small to medium-sized cuboidal cells with abundant clear cytoplasm and often showed extensive secondary branching, which were often folded and densely packed, resulting in a solid appearance. The nuclei were round and uniform in shape; nucleoli were not prominent (Fuhrman grade 2). Neither mitotic figures nor necrosis was present (**Figures 1** and **2A**).

Immunohistochemistry

The results of immunohistochemistry are summarized in **Table 2**. The neoplastic cells in all 11 cases exhibited moderate to strong positivity for CK7, CA9, Vim, and HIF-1α. Immunostaining for CD10, P504S, and RCC was negative in all cases (**Figures 1B-D**, **2B** and **2C**).

Molecular analysis

We analyzed the 1-3 exons of *VHL* gene by direct sequencing in all 11 cases and found no distinct mutation in any case.

FISH analysis

The summary of FISH data is presented in **Table 2**. Chromosome 3p deletion were not detected in 8 tumors. Except for monoploid chromosome 3 in 3 cases, none of the other neoplasms were observed for chromosomal losses or gains of chromosome 3 (**Figures 1F** and **2D**).

Ultrastructural findings

In case 11, the tumor cells composed of numerous glycogens with scanty cell organelles, reminiscent of CCRCC (**Figure 1E**).

Discussion

CCPRCC is a recently recognized renal tumor, which have immunohistochemical and genetic profiles distinct from clear cell renal cell carcinoma and papillary renal cell carcinoma [4, 9]. In this study, we describe 11 patients with

CCPRCC, accounting for 1.2% of RCCs in our renal tumor series. The clinicopathological features are consistent with previous studies. The male-to-female ratio was 8:3, with a slight male predominance. The patients' ages ranged from 33 to 72 years (mean, 52.5 y; median, 45.4 y). Follow-up was available for 8 patients. All 8 patients are alive with no evidence of disease after initial resection, showing an indolent clinical behavior.

Histologically, all tumors exhibit morphologic features typical of CCPRCC as previously described including a mixture of cystic and papillary components, covered by small to medium-sized cuboidal cells with abundant clear cytoplasm [1-3]. In accord with previous studies [1-3, 10], all 11 cases exhibited moderate to strong positivity for CK7, CA9, Vim, and HIF-1α, coupled with negative reactions for CD10, P504S, and RCC. The immunohistochemical panel we used in this study allows one to distinguish between CCRCC, PRCC and CCPRCC. CCRCC typically show immunoreactivity to antibodies against CA9 and CD10 and lack immunoreactivity for P504S and CK7. PRCC characteristically show positive immunostaining for CD10, P504S and CK7, but less frequently express CA9. Ultrastructurally, the tumor cells composed of numerous glycogens with scanty cell organelles, reminiscent of CCRCC suggesting the close pathogenesis relationship of CCPRCC with CCRCC.

The *VHL* gene product protein is important in the regulation of HIF-1α and vascular endothelial growth factor (VEGF), whereas Loss of function of the *VHL* gene ultimately leads to overexpression of a variety of proteins that are targets of the HIF pathway including HIF-1α, glucose transporter-1 (GLUT-1), and CA9 [11]. CA9 is one of the better-known HIF targets and has been shown to be useful as an immunohistochemical marker of CCRCC [11]. Previous studies have documented CCPRCC lack trisomies of chromosomes 7 and 17, deletions of 3p25, *VHL* gene mutations, and *VHL* promoter hypermethylation [1-3, 11]. The comparative genomic hybridization study of Adam et al. demonstrated no genomic imbalance in seven tumors tested [10], whereas other scattered reports have shown abnormalities at various chromosomal loci, including trisomies 10 and 12, monosomies 16, 17, 20, and gains at 5p, 5q, 7pq, 12pq, and 16pq, by various methods [12-14]. In our

study, we did not find any *VHL* gene mutations in all 11 cases, in keeping with the findings of previous studies [1-3, 11], however, losses of chromosomes 3 (monosomies chromosome 3) was detected in 3 cases suggesting that at least some of these lesions have demonstrated abnormalities of chromosomes 3. The frequency of the cytogenetic heterogeneity for these tumors remains uncertain and need further investigation. In addition, the strong expression of HIF-1α and CA9 in all tumors provide supporting evidence that upregulation of the HIF pathway is independent of *VHL* gene mutation. Other events if any, such as post transcriptional deregulation, translational control or microRNA deregulation may be a possible explanation for the mechanism of loss of function of the *VHL* gene.

In summary, we reported 11 CCPRCC, arising in kidneys unaffected by ESRD. All tumors exhibit morphologic and immunochemical features typical of CCPRCC. The coexpression of CA9 and HIF-1α in the absence of *VHL* gene abnormalities in CCPRCC suggests activation of the HIF pathway by mechanisms independent of *VHL* gene mutation. Losses of chromosomes 3 (monosomies chromosome 3) was detected in 3 cases suggesting that at least some of these lesions have demonstrated abnormalities of chromosomes 3. Ultrastructurally, CCPRCC composed of numerous glycogens with scanty cell organelles, reminiscent of CCRCC suggesting the close pathogenesis relationship of CCPRCC with CCRCC.

Disclosure of conflict of interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

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