

Morphologic, Immunohistochemical and Molecular Analyses of a Huge Clear Cell Papillary Renal Cell Carcinoma

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Abstract

Among the tumors of the kidney, the clear cell papillary renal cell carcinoma (CCPRCC) is a recently described entity. This tumor is well circumscribed, with a fibrous capsule, showing mostly a prominent cystic component and a diameter less than 4 cm. Necrosis is not a feature. Tumor cells are clear with low grade nuclei with a predominantly tubule-papillary architecture and immunohistochemical staining strongly positive for CK7, and for CA IX and negative for RACEMASE, features which differentiate this tumor from clear cell renal cell carcinoma (CCRCC) and papillary renal cell carcinoma (papillary RCC). In these latter tumors, it has been reported, unlike the other two, no loss or gain of chromosomes 7 and Y, no chromosome 3p deletion, and no KRAS mutation. In this report, a huge clear cell papillary renal cell carcinoma case, 9 cm diameter, in a 42-year-old male is described. Pathologic diagnosis of the tumor was confirmed by immunohistochemical analysis including CD10, CA IX, CK7, RACEMASE, and 34 beta E12 stainings. Molecular detection of *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *ALK*, *ERBB2*, *DDR2*, *MAP2K1*, *RET*, and *EGFR* gene mutational analysis has also been performed. Molecular findings are in accordance with the speculation that the CCRCC is an indolent tumor which may be defined as low malignant potential. In fact, in the molecular analysis, none out of all genes evaluated showed mutation.

Keywords: Clear cell papillary renal cell carcinoma; *KRAS*; *NRAS*; *BRAF*; *PIK3CA*; *ALK*; *ERBB2*; *DDR2*; *MAP2K1*; *RET*; *EGFR*; Kidney; Molecular pathology

Abbreviations: RCC: Renal Cell Carcinoma; CCRCC: Clear Cell Renal Cell Carcinoma; PRCC: Papillary Renal Cell Carcinoma; ESDR: End-Stage Renal Disease; VHL: Von Hippel-Lindau Syndrome

Introduction

A recent update of renal tumors is reported by the 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs [1]. WHO classification underlines that genetic profiling is the clue for differentiating subtypes of renal cell carcinomas (RCCs), although little is still known about the genetic events involved in tumor progression. Among the tumors of the kidney, the clear cell papillary renal cell carcinoma (CCPRCC) is an important entity. This tumor is well circumscribed, with a fibrous capsule, showing, in the majority of the cases, a prominent cystic component and a diameter less than 4 cm. Necrosis is not a feature. Tumor cells are clear with low grade nuclei with a predominantly tubule-papillary architecture and immunohistochemical staining is strongly positive to CK7, basolateral (cup-shape) CA IX expression and negative to RACEMASE, a feature which differentiates this tumor from clear cell renal cell carcinoma (CCRCC) and papillary renal cell carcinoma (papillary RCC). In these latter tumors, it has been reported, unlike CCRCCs and papillary RCCs, no loss or gain of chromosomes 7 and Y, no chromosome 3p deletion, and no KRAS mutation [2]. Therefore, histologic features alone are not enough to perform a differential diagnosis, while immunohistochemical and molecular analyses are also critical to assess appropriate surveillance schedules. An analysis of 327 reported cases with follow-up supports the concept that CCPRCC is an indolent tumor worth to be named clear cell papillary neoplasm of low malignant potential [3]. The differential diagnosis remains an issue since clear cell renal cell carcinoma (CCRCC) and papillary RCCs might potentially metastasize [4-6] while CCPRCC has no metastatic potential [7,8]. We report a case of a huge renal tumor in a young male patient characterized by solid areas and cysts lined with

clear cells. We evaluated the immunohistochemical characteristics of the tumor cells and we analysed any mutations of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *ALK*, *ERBB2*, *DDR2*, *MAP2K1*, *RET*, and *EGFR* genes.

Case Report

A 42-year-old man underwent radical nephrectomy for a large mass. There was no personal or family history suggestive for end-stage renal disease (ESDR), chronic kidney disease or von Hippel-Lindau syndrome (VHL). Macroscopic examination showed a solitary solid-cystic mass of 9 cm × 8 cm × 8 cm in diameters. Microscopic examination showed cystic areas lined by a single layer of cell intermixed with solid and tubular-papillary areas. The cells had vacuum clear appearance cytoplasm and a low nucleolar grade. No necrosis was observed. No sarcomatoid or rhabdoid areas were identified. Immunohistochemical assessment showed a strong positivity for CK7, basolateral distribution “cup -shape” CA IX expression and negative RACEMASE and CD10 (Figure 1). The histological diagnosis was of CCPRCC in accordance with 2016 World Health Organization (WHO) classification [1] and the Vancouver ISUP reported criteria [9].

The neoplasm was well circumscribed and there was no evidence of capsular or perirenal fat tissue invasion. So pT2a category was assessed according to 2017 revision of TNM [10].

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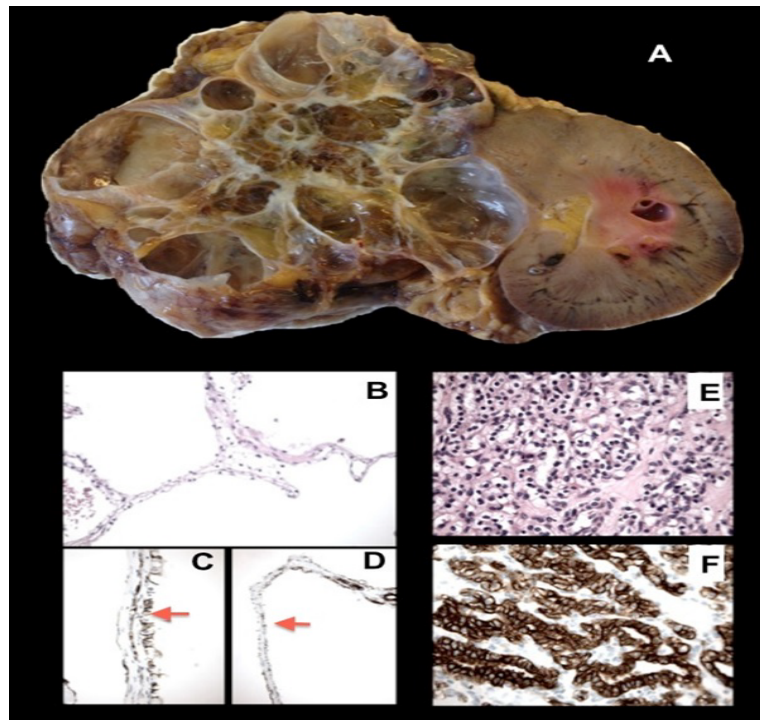


Figure 1: A: Gross feature of a huge CCPRCC case. Nephrectomy has been performed in a 42-year-old male patient for a solid and cystic mass measuring 9 × 8 × 8 cm (A). Histological and immune-histochemical features of a cystic area: hematoxylin and eosin staining (B), CA IX (C) and CK7 (D) positive staining. Arrowheads show the positive staining of the tumor cells which are lining the cysts. Histological and immune-histochemical features of a solid area: hematoxylin and eosin staining (E), and CK7 positive staining (F).

In the follow up period, no recurrence, distant metastasis or death caused by disease have been reported. No mutations in the investigated genes (*NRAS*, *BRAF*, *PIK3CA*, *ALK*, *ERBB2*, *DDR2*, *MAP2K1*, *RET*, and *EGFR*) have been detected.

Molecular Analysis

Two microliters were tested with the kit Myriapod Lung Status (Diatech Pharmacogenetics, Ancona, Italy). The Mass-ARRAY iPLEX system involves 4 main steps: PCR (Polymerase Chain Reaction), SAP (Shrimp Alkaline Phosphatase) reaction, single-base primer extension (iPLEX reaction), and separation of the products on a matrix-loaded silicon chip by MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry. The PCR, SAP and iPLEX reactions were performed in a thermal cycler (Labcyler, SensoQuest, Göttingen, Germany), whereas the extension products were analyzed using the matrix-assisted laser desorption ionization time-of-flight Mass-ARRAY Analyzer 4 (Agena Bioscience), and using all reagents and consumables contained in Myriapod Lung Status Kit (Diatech).

Assay amplification primers and extension oligos in the Myriapod Lung Status were designed with a maximum of 12 assays per well. Every single test is split in 8 wells to detect single nucleotide polymorphism (SNPs) involving 184 mutations of the genes *KRAS* (codons 12, 13, 18, 59, 61, 117, 146), *NRAS* (codons 12, 13, 18, 59, 61, 117, 146), *BRAF* (codons 11 and 15), *PIK3CA* (codons 9, and 20), *ALK* (codons 22, 23, and 25), *ERBB2* (codon 20), *DDR2* (codons 9, 16, and 18), *MAP2K1* (codon 2), *RET* (codon 16), and *EGFR*.

Discussion and Conclusion

We reported an uncommon huge CCPRCC in a 42-year old male. The present case was unusual for the size, but the extensive samplings

and the careful observation of histologic and immunohistochemical features were perfectly compliant to the diagnosis of clear cell papillary renal cell carcinoma. Molecular findings reinforced the morphologic data. In the molecular analysis, none out of a panel of all genes evaluated showed mutation.

CCPRCC was first described in 2006 in end-stage kidney [11]. After 10 years this entity has been recognized by the 2016 World Health Organization (WHO) classification of urologic tumors as both sporadic and end-stage renal disease or von Hippel-Lindau syndrome associated neoplasm [1]. An African-American patient's prevalence and an equal female-to-male distribution have been described [12]. Clear cell papillary renal cell carcinoma is now recognized as a distinct tumor with unique morphology, immunohistochemistry, and cytogenetics [13-15]. Zhou et al. in a series of 290 consecutive nephrectomies documented that CCPRCC is the fourth renal cell carcinoma by incidence accounting 4.1% of renal tumors [16]. Rare cases of multifocal or bilateral neoplasms have been described [17,18]. Metastases have not been reported, emphasizing the non-aggressive behaviour of this tumor [19]. Mostly these neoplasms are detected at early stage, generally pT1a [20].

Current experience is limited to support these neoplasms as benign entities although, recent literature data is supporting the concept to rename this entity as "low malignant potential tumor" [3].

The most challenging differential diagnosis is with CCRCC, but a careful evaluation of microscopic appearance and a proper immunohistochemistry use can drive to the correct diagnosis. A recent paper highlights the pitfalls of overlapping features of CCPRCC and CCRCC, suggesting the strict use of both morphologic and immunohistochemical criteria for the correct diagnosis [21]. CCRCC is associated with a capability of recurrence in up to 30% of cases [22],

while the CCPRCC has an indolent behaviour. Unlike CCRC [2,23], the present case did not show RAS mutations, and this is in accordance with our previous data [24], and, in addition, it did not show mutations in *PIK3CA*, *ALK*, *ERBB2*, *DDR2*, *MAP2K1*, *RET*, and *EGFR* genes in agreement with the results of a series of small CCPRCC recently described [25].

The molecular analysis of the huge CCPRC was of particular help in the differential diagnosis of this challenging case.

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