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Expression of vitamin D receptor in clear cell papillary renal cell carcinoma

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ABSTRACT

Clear cell papillary renal cell carcinoma (ccpRCC) is a recently recognized subtype of renal cell carcinoma. In this study, we investigated the clinicopathological and immunohistochemical features in a group of 26 cases of ccpRCC, with a special emphasis on the expression of vitamin D receptor (VDR). The mean age of patients was 53.3 years (range 36–74 years), and the mean tumor size was 2.5 cm (range 0.5 to 6.5 cm). During follow-up (range 12–121 months, median 50 months), no recurrence or metastasis was observed. Histopathologically, all cases of ccpRCC exhibited a tubular and papillary architecture, covered by tumor cells with clear cytoplasm. Immunohistochemistry showed intermediate (5/26, 19%) to diffuse (21/26, 81%) and moderate (2/26, 8%) to strong (24/26, 92%) membranous staining for VDR in each case. All cases (26/26, 100%) were diffuse and strong cytoplasmic and fibrillar staining for cytokeratin 7 (CK7), but negative forα-methylacyl-CoA-racemase (AMACR). Each case showed diffuse (26/26, 100%) and moderate (4/26, 15%) to strong (22/26, 85%) membranous staining for carbonic anhydrase IX (CA IX). In addition, the majority of cases showed negative for cluster of differentiation 10 (CD10) (20/26, 77%) and renal cell carcinoma maker (RCC-Ma) (24/26, 92%). This unique staining pattern is helpful for distinguishing ccpRCC from its mimics. Furthermore, VDR positive expression suggests that ccpRCC originates from the precursor epithelium of distal nephron.

1. Introduction

The fourth version of the World Health Organization (WHO) Classification of Tumors of the Urinary System and Male Genital Organs was formally published in the spring of 2016, in which identified clear cell papillary renal cell carcinoma (ccpRCC) as a new type of renal cell carcinoma [1]. This unique neoplasm was initially found in patients with end-stage renal disease (ESRD) and subsequently in patients with normal kidneys as well [2, 3]. Histologically, ccpRCC shows a mixture of cystic, tubular and papillary components, covered by low Fuhrman nuclear grade cells with abundant clear cytoplasm [4, 5]. Clinically, it is widely believed that ccpRCC is an indolent tumor and no tumors of the reported cases have metastasized [6]. However, the origin of ccpRCC is rarely reported in the literature.

The vitamin D receptor (VDR) is a member of the nuclear receptor superfamily, which is activated after binding to the 1α,25-dihydroxyvitamin D3 and then induces target gene expression at the transcriptional level [7, 8]. It plays an important role in calcium homeostasis and bone metabolism, immunoregulation, angiogenesis, and cellular proliferation and differentiation in multiple tissues [8]. The kidney is not only a primary vitamin D target organ but also is a key site of vitamin D metabolism [9]. Previous studies have shown that the major site of action of 1α,25-dihydroxyvitamin D3 and the expression of VDR were in the distal convoluted tubule of the kidney, whereas the proximal tubules only synthesized 1α,25-dihydroxyvitamin D3 and did not express VDR [9, 10]. Therefore, as a reliable marker specific for distal nephron, VDR can be used to differentiate subtypes of renal cell carcinoma and determine the origin of a renal neoplasm [11]. In this study, we evaluated the expression of VDR as well as cytokeratin 7 (CK7), α-methylacyl-CoA-racemase (AMACR), carbonic anhydrase IX (CA IX), renal cell carcinoma maker (RCC-Ma), and cluster of differentiation 10 (CD10) in a group of ccpRCC by immunohistochemistry. In addition, we reviewed the clinicopathological features of ccpRCC in order to better understand this newly recognized entity.

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2. Materials and methods

2.1. Case selection

Twenty-six cases of ccpRCC were retrieved from the pathology archives of two hospitals. All cases were reviewed by three experienced pathologists and diagnosed according to the criteria described by Tickoo [12]. The institutional review boards from both hospitals approved this study.

2.2. Immunohistochemistry

Samples of all tumors were fixed in formalin and embedded in paraffin. The 4-um thick sections were mounted on glass slides coated with poly-L-lysine for tissue section adhesion (Sigma) and dried overnight at 60 °C. After deparaffinization and rehydration of sections, heat-induced epitope retrieval was performed in sodium citrate monohydrate (0.01 mol/L, pH 6.0) using a microwave oven, heated for three cycles of 5 min (approximately 700 W) and cooled down to room temperature over a period of 10 min. Subsequently, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Primary monoclonal antibodies against VDR (D-6, 1/1000 dilution, Santa Cruz Biotechnology, Santa Cruz, US), CK7 (OV-TL 12/30, 1/200 dilution, Dako, Carpinteria, US), AMACR (13H4, ready-to-use, Dako, Carpinteria, US), CA IX (TH22, 1/100 dilution, Novocastra, Newcastle upon Tyne, UK), RCC Ma (SPM314, 1/100 dilution, Dako, Carpinteria, US) and CD10 (56C6, 1/100 dilution, Novocastra, Newcastle upon Tyne, UK) were used for 0.5–1 h at 37 °C. Detection was performed with a biotin-free horseradish peroxidase labeled dextran polymer (Dako Real Envision detection system, Dako, Carpinteria, US) and hematoxylin was used as counterstain. The immunohistochemistry results were interpreted as negative, focal (< 30% staining), moderate (30%–70% staining) or diffuse (> 70% staining) according to the distribution of positive cells, and negative, weak, moderate, or strong according to the staining intensity.

3. Results

3.1. Clinical features

Twenty-six patients were included in this study: 19 were men and 7 were women. The mean age was 53.3 years (range 36–74 years). Eighteen tumors were from the left side and 8 from the right side. Eleven patients underwent radical nephrectomy, the rest had partial nephrectomy. There were 4 patients with multilocular renal cyst (MRC), 1 with ESRD and 1 with bilateral renal tumors (the right side was ccpRCC and the left side was clear cell renal cell carcinoma). None of these patients was treated with chemotherapy or radiation therapy after surgery. During follow-up (range 12–121 months, median 50 months), no patient developed recurrence or metastasis.

3.2. Pathologic features

The mean size of the tumors was 2.5 cm and ranged from 0.5 to 6.5 cm. Macroscopically, all tumors were either encapsulated by a fibrous capsule or well demarcated. Microscopically, most tumors exhibited a combination of architectural patterns, including small blunt papillae, tubular acinar and variably sized cysts (Fig. 1). The tumor cells were oval or cuboidal with abundant clear cytoplasm and had low Fuhrman nuclear grade (8 were grade 1, 18 were grade 2). In most of these tumors, a characteristic nuclear horizontally linear arrangement away from the basement membrane was identified. Neither necrosis nor hemosiderin was present in these cases.

The immunohistochemical staining results were summarized in Table 1. All cases (26/26, 100%) were diffuse and strong cytoplasmic and fibrillar staining for CK7 (Fig. 2A), but negative for AMACR

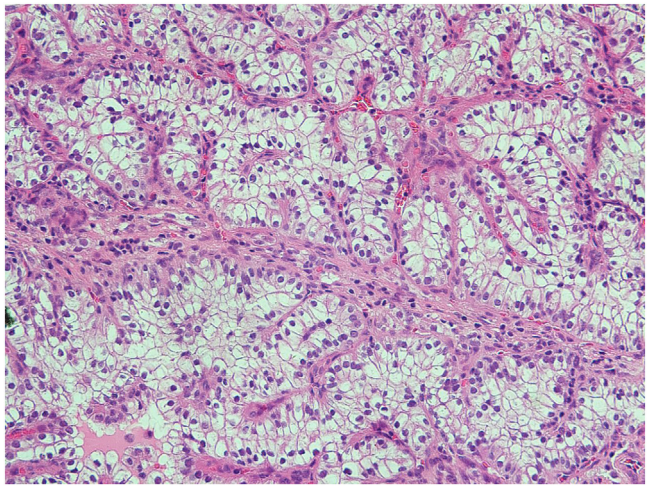

Fig. 1. Histomorphological features of ccpRCC. Hematoxylin and eosin, ×200.

(Fig. 2B). Moreover, each case showed diffuse (26/26, 100%) and moderate (4/26, 15%) to strong (22/26, 85%) membranous staining for CA IX (cup-shaped distribution) (Fig. 2C). Among 26 cases stained for RCC-Ma, 24 cases (92%) were completely negative (Fig. 2D). The remaining 2 cases (8%) were focal positive with weak or moderate cytoplasmic staining respectively. The majority of cases also showed negative for CD10 (Fig. 2E). Only 6 cases (23%) were focally membranous positive, including 2 cases (8%) of strong staining, 3 cases (11%) of moderate staining and 1 case (4%) of weak staining. For VDR, each case showed intermediate (5/26, 19%) to diffuse (21/26, 81%) and moderate (2/26, 8%) to strong (24/26, 92%) membranous staining (Fig. 2F).

4. Discussion

CcpRCC is a recently described subtype of renal cell carcinoma, which is estimated to account for 1 to 4.3% of all renal epithelial neoplasms [6]. It has unique histomorphological and immunohistochemical characteristics. In addition, molecular genetic findings of this tumor indicate neither loss of chromosome 3p as seen in clear cell renal cell carcinoma (ccRCC), nor gain of chromosome 7 or 17 as seen in papillary renal cell carcinoma (pRCC) [3, 13]. However, little is known about the pathogenesis and origin of ccpRCC.

VDR has been shown to be widely distributed in normal human tissues. It is mainly expressed in intestinal epithelial cells, skeletal osteoblasts and chondrocytes, activated T lymphocytes, and epithelial cells of distal renal tubules and collecting ducts [14]. In renal tumors, however, the expression of VDR is different in the various subtypes. It has been demonstrated that VDR expression was absent in ccRCC, which originated from the proximal convoluted tubules [9, 11]. In contrast, VDR expression was positive in chromophobe RCC, renal collecting duct carcinoma and oncocytomas, which originated from the distal nephron [9]. No previous study has been performed to investigate the expression of this marker in ccpRCC. In this study, we showed the presence of diffuse or intermediate cytoplasmic staining for VDR in all of 26 cases of ccpRCC. Our results suggest that ccpRCC may arise from the precursor epithelium of distal nephron. In addition, CD10 and RCC-Ma, specific for proximal nephron in normal human kidney [15, 16], have also been tested in this study. The immunohistochemical results showed they were focal or negative staining, which provides additional evidence to support a possible distal tubular cell origin of ccpRCC.

The main differential diagnosis for ccpRCC is ccRCC and pRCC with low Fuhrman nuclear grade [17]. Although ccpRCC exhibits distinct morphological features, immunohistochemistry is still required to confirm the diagnosis, especially for those untypical cases. In this study,

Table 1
Immunohistochemical staining results of ccpRCC.

Stains	Distribution of positive cells				Staining intensity				Total cases
	Negative	Focal	Intermediate	Diffuse	Negative	Weak	Moderate	Strong	
CK7	–	–	–	26 (100%)	–	–	–	26 (100%)	26
AMACR	26 (100%)	–	–	–	26 (100%)	–	–	–	26
CA IX	–	–	–	26(100%)			4 (15%)	22 (85%)	26
RCC-Ma	24 (92%)	–	2 (8%)	–	24 (92%)	1 (4%)	1 (4%)	–	26
CD10	20 (77%)	6 (23%)	–	–	20 (77%)	1 (4%)	3 (11%)	2 (8%)	26
VDR	–	–	5 (19%)	21 (81%)	–	–	2 (8%)	24 (92%)	26

we showed VDR in combination with CK7, CA IX and AMACR was a useful panel to differentiate ccpRCC from its mimics. The classic immunochemical profiles of ccpRCC are positive for CK7, CA IX, VDR and negative for AMACR [3, 6]. On the contrary, ccRCC usually exhibits negative for CK7, AMACR, VDR and positive for CA IX. For pRCC, it is divided into type I and type II according to the histopathological morphology. Type I pRCC usually shows strong and diffuse staining for CK7 and AMACR, while type II pRCC sometimes shows absent or weak staining for these two markers [18]. Nonetheless, regardless of what type of pRCC, the tumor cells show negative for CA IX and positive for VDR [19]. Therefore, it is easy to identify ccpRCC with ccRCC and pRCC. In addition, one thing to note is that type I pRCC demonstrated cytoplasmic VDR staining pattern [9], which is different from diffusely membranous VDR staining of ccpRCC and type II pRCC.

CcpRCC is considered to be a low grade malignant tumor with good prognosis [1, 5, 6]. Most patients with ccpRCC present at an early stage with small renal tumors [17]. Up to now, ccpRCC with perirenal tissue invasion, distant metastasis and postoperative recurrence have not been reported [5]. Interestingly, several previous studies have shown that high expression of VDR may correlate with good prognosis in multiple types of cancer, such as breast cancer, colorectal cancer, cholangiocarcinoma and renal cancer [20-23]. Therefore, it is reasonable to presume that VDR plays a protective role in ccpRCC patients. Furthermore, previous studies have also shown that targeting VDR with 1α,25-

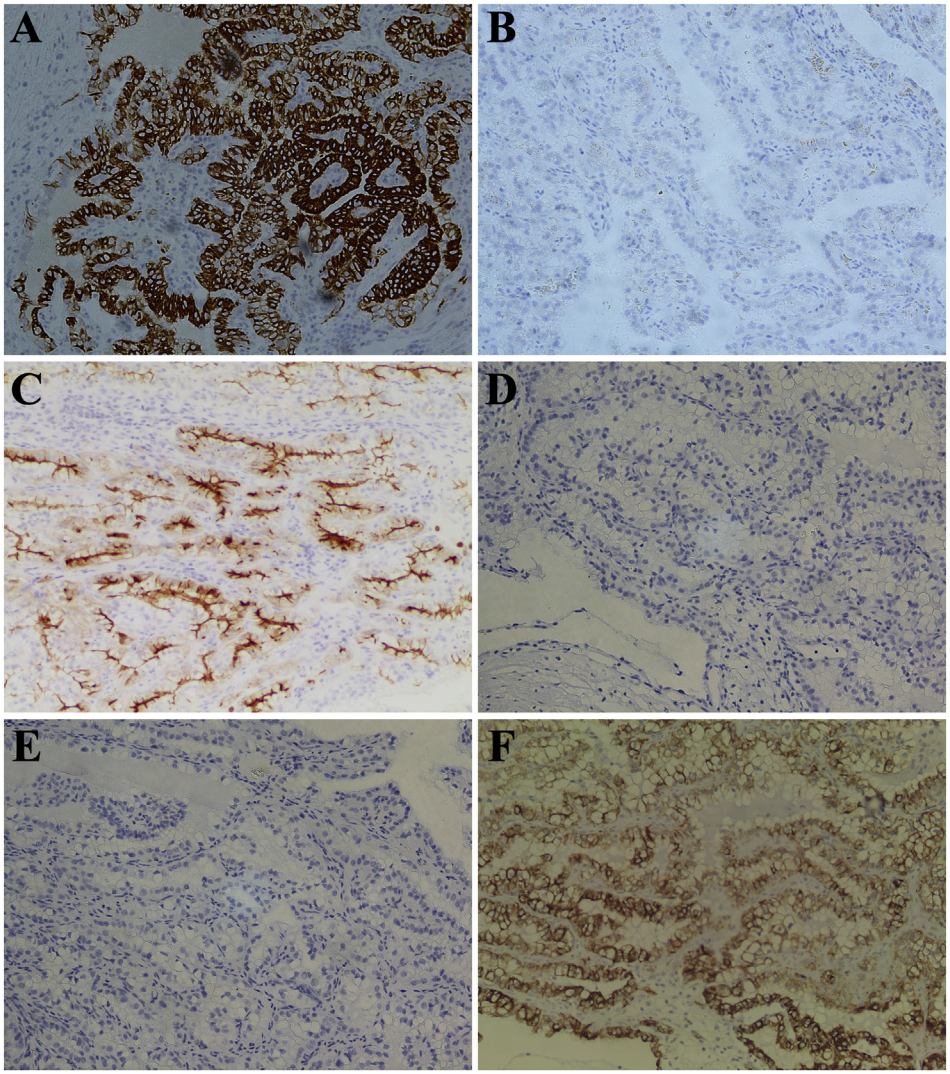

Fig. 2. Immunohistochemical features of ccpRCC. A, CK7, ×200; B, AMACR, ×200; C, CA IX, ×200; D, RCC-Ma, ×200; E, CD10, ×200; F, VDR, ×200

dihydroxyvitamin D3 or its synthetic analogs may have antitumor activity in many cancer models, including breast cancer, colorectal cancer, ovarian cancer, pancreatic cancer, testis cancer, prostatic cancer and primary liver cancer [24-29]. The main mechanism is currently considered to block cell cycle by up-regulation of several cell cycle inhibitors, induce tumor cell apoptosis through interaction with Bax family of proteins and suppress tumor metastasis by the inhibition of angiogenesis [30, 31]. In view of the high expression of VDR in ccpRCC, whether 1α,25-dihydroxyvitamin D3 or its synthetic analogs can be used to treat this kind of tumor needs further study.

In summary, this is the first report on the diffuse or intermediate positivity of VDR in ccpRCC and such staining property has the following significance: (1) The immunohistochemical spectrum of ccpRCC is further expanded. (2) CcpRCC may arise from the precursor epithelium of distal nephron. (3) The panel composed of VDR, CK7, CA IX and AMACR can be used to differentiate ccpRCC from its mimics including ccRCC and pRCC. (4) The VDR expression may correlate with the relative indolent behavior of ccpRCC. (5) VDR may be a target for ccpRCC treatment.

Conflict of interests

The authors declare no conflict of interests.

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