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Original Article

DOG1, cyclin D1, CK7, CD117 and vimentin are useful immunohistochemical markers in distinguishing chromophobe renal cell carcinoma from clear cell renal cell carcinoma and renal oncocytoma

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ABSTRACT

The distinction between chromophobe renal cell carcinoma (ChRCC), clear cell renal cell carcinoma (CRCC) and renal oncocytoma may cause a diagnostic dilemma. The usefulness of DOG1, cyclin D1, CK7, CD117 and vimentin in the differential diagnosis of these renal epithelial tumors was investigated. DOG1 was positive in ChRCC (32 of 32, 100%) and in renal oncocytoma (21 of 21, 100%). In contrast, DOG1 was absent in all CRCC (0 of 30). Cyclin D1 was positive in renal oncocytomas (17 of 21, 81%) but negative in the ChRCC (0/23) and CRCC (0 of 30). CK7 was positive in ChRCC (30 of 32, 94%), but was negative in oncocytoma (only scattered single positive cells), and was only focal positive in two cases of CRCC. CD117 was expressed in 88% of ChRCC (28 of 32), 86% of renal oncocytoma (18 of 21), and was negative in all CRCC (0 of 30). Twenty-six of the 30 cases of CRCC were positive (87%) for vimentin with prominent membrane staining patterns. All 23 chromophobe carcinomas were negative for vimentin and 15 of 21 oncocytomas demonstrated focal vimentin positivity, but less than 10%. The above results demonstrate that: (1) DOG1 was very sensitive and specific marker for distinguish ChRCC from CRCC; (2) Cyclin D1 was a useful marker to discriminate between ChRCC and renal oncocytoma; (3) CK7 and CD117 were useful markers to distinguish ChRCC from renal oncocytoma and CRCC. (4) Vimentin was helpful for distinguishing clear cell RCC from chromophobe and oncocytoma (87% of clear cell RCC positive, negative in chromophobe, only focally positive in oncocytoma). (5) CK8/18, CK19, CD10, β-catenin and E-cadherin could not be used to distinguish ChRCC from renal oncocytoma and CRCC.

Introduction

Chromophobe renal cell carcinoma (ChRCC) was first described by Thoenes et al. [1] in 1985, and now it is accepted universally as a distinct subtype of malignant epithelial tumor of renal parenchyma that accounts for 5% of malignant renal neoplasms [2,3]. ChRCC is considered to have low malignant potential [4–6] and it has been suggested that the cells of ChRCC are related to the normal intercalated cells of the collecting ducts [5–7]. Two subtypes of chromophobe carcinoma have been described, a typical variant and an eosinophilic variant [5]. The classical variant is characterized by large polygonal cells with transparent, slightly reticulated cytoplasm and can be confused with conventional clear cell renal cell carcinoma (CRCC), whereas the eosinophilic variant, composed of intensely eosinophilic cells, resembles oncocytoma [7].

Immunohistochemistry is currently the mainstay for attempts to differentiate ChRCC from other subtypes of renal tumors, such as CRCC and oncocytoma. In this study, we investigated the immunohistochemical expression of a series of antibodies in the three renal epithelial tumors most likely to be confused with each other: ChRCC, CRCC, and renal oncocytoma.

Materials and methods

Tissues

Our study group consisted of 32 cases of ChRCC, 21 cases of renal oncocytoma and 30 cases of CRCC. All cases were retrieved from the

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Table 1
The immunochemical stain of 10 antibodies on ChRCC, CRCC and oncocytoma.

Protein	ChRCC	CRCC	Oncocytoma
CK7	30/32	2/30[a]	0/21[b]
DOG1	32/32	0/30	21/21
CD117	28/32	0/30	18/21
Cyclin D1	0/32	0/30	17/21
Vimentin	0/32	26/30	15/21[a]
CK8/18	29/32	25/30	14/21
CK19	26/32	22/30	13/21
CD10	25/32	29/30	14/21
β-catenin	3/32	1/30	2/21
E-cadherin	20/32	11/30	15/21

[a] Focal positive and less than 10% overall staining.
[b] There were scattered single positive cells in oncocytomas, and less than 10% overall staining.

electronic database of our Department of Pathology between 2001 and 2012. All tumors were classified on the basis of H&E morphology according to the criteria of the WHO 2013 classifications by two renal tumor pathologists blinded to the immunohistochemical findings.

Immunohistochemistry

Immunohistochemical analysis was performed on 5-μm thick, formalin-fixed, paraffin-embedded tissue sections of each case and all slides were stained on a BENCHMARK Autostainer with the EnVision detection system with appropriate positive and negative controls. The antibodies used were DOG-1, cyclin D1, CK7, CD117, vimentin, E-cadherin, β-catenin, CK8/18, CK19 and CD10 (DAKO, Carpinteria, CA). For each stain, the percentage of positive cells was recorded. The presence of more than 10% expression of the marker within tumor cells was considered positive. Cases with focal positive and less than 10% overall staining were considered negative.

Results

All 32 (100%) cases of ChRCC were diffusely and strongly positive for DOG1 with staining of the cell membrane and cytoplasm positive (Table 1 and Fig. 1). Thirty of the 32 cases of ChRCC were positive for CK7, all of them exhibiting a membranous staining profile (Table 1 and Fig. 1). CD117 was diffusely and strongly positive for 28 of 32 ChRCC (91%), with both cell membrane and fine granular cytoplasmic staining (Table 1 and Fig. 1). Prominent cell membrane staining was observed whenever the tumor cells showed distinct cell borders. All 23 chromophobe carcinomas were negative for vimentin and cyclin D1 (Table 1 and Fig. 1).

Twenty-six of the 30 cases of CRCC were positive (87%) for vimentin with prominent membrane staining patterns (Table 1 and Fig. 2). Twenty-eight of the 30 cases of CRCC were negative (93%) for CK7. Only two cases of CRCC were focal positive for CK7 with granular cytoplasmic staining (Table 1 and Fig. 2). None of the 30 cases of CRCC exhibited immunopositivity for CD117, DOG1 and cyclin D1 (Table 1 and Fig. 2).

Seventeen of 21 cases of renal oncocytomas displayed cyclin D1 immunoreactivity with nuclear staining (Table 1 and Fig. 3). All 21 cases (100%) of renal oncocytomas were positive for DOG1 with staining of cell membranes, cytoplasm, or both (Table 1 and Fig. 3). Eighteen of 21 cases of renal oncocytomas showed diffuse and strong CD117 expression. The tumor cells showed both strong cytoplasmic and membrane, as well as diffuse, finely granular cytoplasmic staining (Table 1. and Fig. 3). Fifteen of 21 oncocytomas demonstrated focal vimentin positivity, but less than 10% (Table 1 and Fig. 3). There were scattered single positive cells for CK7 in oncocytomas, but less than 10% (Table 1 and Fig. 3).

The results of immunohistochemical staining on resected specimens for ChRCC, CRCC and renal oncocytoma are summarized in Table 1. The sensitivity, specificity, positive predictive value, and negative predictive value of the immunohistochemical stains for distinguishing ChRCC from CRCC are summarized in Table 2. The sensitivity, specificity, positive predictive value, and negative predictive value of the immunohistochemical markers for distinguishing oncocytoma from ChRCC are summarized in Table 3.

Discussion

It is important to be able to recognize ChRCC, since this neoplasm has a more favorable prognosis than CRCC [8]. Distinction of ChRCC, classic CRCC, and renal oncocytoma generally causes no diagnostic difficulty. However, the eosinophilic variant of CRCC and the ChRCC can be confused with each other and with renal oncocytoma. In such cases, hematoxylin and eosin-based morphology may not be sufficient to render a definitive diagnosis. We investigated the utility of a series of markers as aids in making this distinction.

Our result of immunohistochemical staining with CK7 is similar to those of most previously reported studies [9,10]. CK7 seems to be valuable in the differentiation of ChRCC from other tumors, especially the eosinophilic variant of CRCC which is generally CK7−, with high sensitivity (94%) and specificity (100%) (Table 2). However, immunostaining with a single antibody in individual cases can be notoriously unreliable. When addressing a differential diagnostic question in a difficult case, it is useful to have a panel of markers, some relatively specific for one condition and others for the alternative conditions. The demonstration of DOG1 and CD117 staining in ChRCC and oncocytomas and not in CRCC provides such markers.

DOG1 is a novel gene encoding for a hypothetical protein that has been ubiquitously expressed on GISTs. In a study conducted by West et al. [11], immunoreactivity for DOG1 on GIST samples was 97.8% reactive. Another study, conducted by Espinosa et al. [12] on DOG1 antibody, showed a high sensitivity and specificity, with 87% immunoreaction to GISTs. DOG1 expression in renal tumors has not been reported by other investigators since it has only recently emerged. In our hands, DOG-1 showed diffuse, strong staining in all 23 ChRCCs and 21 oncocytomas but negative in all 30 cases of CRCCs. We recommend inclusion of DOG1 in our immunohistochemical panel because its high sensitivity (100%) and specificity (100%) to distinguish ChRCC/oncocytoma group from CRCC (Table 2). Our results demonstrated that DOG1 was even superior to CK7 and CD117 because it was more sensitive than CK7 and CD117 for distinguishing the oncocytoma/chromophobe group from clear cell RCC. Potentially it may be the first choice to distinguish ChRCC/oncocytoma group from CRCC.

CD117 is a proto-oncogene located in the long arm of chromosome 4 (4q11-12) that encodes a transmembrane receptor with intrinsic tyrosine-specific protein kinase activity in its intracellular domain [13]. The ligand for c-kit is stem cell factor [14]. The c-kit receptor is expressed in several normal tissues including mast cells, skin basal cells and melanocytes, breast epithelium, germ cells [15], and interstitial cells of Cajal [16]. Overexpression of CD117 also is observed in a spectrum of human neoplasms, chiefly in mast cell disease, germ cell tumors, chronic myeloid leukemia, and gastrointestinal stromal tumor [17,18]. The constitutive expression of CD117 due to gain of function mutations in its proto-oncogene has been related to the growth and progression of the disease. In our study, CD117 expression was observed in 21/23 cases of ChRCCs and 18/21 cases of oncocytomas, whereas it was negative in all 30 cases of CRCCs. Similar findings have been reported by other investigators [19,20],