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Malignant tumors with clear cell morphology: a comparative immunohistochemical study with renal cell carcinoma antibody, Pax8, steroidogenic factor 1, and brachyury

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ABSTRACT

This study aimed to identify an immunohistochemical panel to aid in the differential diagnosis for tumors with clear cell morphology. Twenty-five clear cell renal cell carcinomas (CCRCCs), 19 clear cell ovarian carcinoma (CCOCs), 20 cases of adrenal cortical carcinomas(ACCs), and 10 chordomas were stained for renal cell carcinoma marker (RCC Ma), Pax8, brachyury, and steroidogenic factor 1 (SF-1). The extent of stains was scored as focal (<25%), nonfocal (25%-50%), and diffuse (>50%). The intensity was scored as weak, moderate, and strong. Twenty-two CCRCCs were positive for RCC Ma (88%) and Pax8 (88%), respectively. The RCC Ma cytoplasmic staining was largely diffuse (76%) and strong (76%). The nuclear Pax8 staining was usually diffuse (76%) and moderate (64%) to strong (8%). All of CCRCCs were negative for brachyury and SF-1. All of 19 CCOCs were positive for Pax8 nuclear staining. The staining was diffuse, moderate (21%) to strong (79%). All of CCOCs were negative for RCC Ma, brachyury, and SF-1. All of 20 ACCs were positive for SF-1 nuclear staining. The staining was largely diffuse (95%), moderate (55%) to strong (15%). All of ACC were negative for RCC Ma, Pax8, and brachyury. All of 10 chordomas were positive for brachyury nuclear staining. The staining was diffuse and strong. All of chordomas were negative for RCC Ma, Pax8, and SF-1. In summary, the panel of RCC Ma, Pax8, brachyury, and SF-1 is useful in the differential diagnosis of tumors with clear morphology.

1. Introduction

Tumors with clear cell morphology may pose a diagnostic challenge, especially on biopsies with limited material. As treatment modalities for various tumors with clear cell morphology differ, misdiagnosing a clear cell lesion's tissue of origin—as well as its primary vs metastatic nature—carries significant clinical consequences. The differential diagnosis of a primary tumor with clear cell morphology includes clear cell renal cell carcinoma (CCRCC), clear cell ovarian carcinoma (CCOC), adrenal cortical carcinoma (ACC), and chordoma, among others. Because of the overlapping morphology of these tumors, a focused immunohistochemical panel is useful for rendering an accurate diagnosis.

Renal cell carcinoma marker (RCC Ma) is a well-recognized immunohistochemical stain useful for identifying renal cell carcinomas [1-4]. Following the recognition that RCC Ma does not stain chordomas [5], brachyury was identified as a sensitive and specific marker for chordomas [6-8]. In recent years, Pax8 emerged as a more sensitive marker for renal neoplasms [9-11] as well as tumors derived from thyroid [12] and müllerian tissue [9]. Similarly, steroidogenic factor 1 (SF-1), also known as Ad4-binding protein, was identified as sensitive and specific for nonneoplastic and neoplastic adrenal tissues [10,13-15]. The objective of our study was to assess the utility of RCC Ma, Pax8, SF-1, and brachyury as a diagnostic panel for tumors with clear cell morphology.

2. Materials and methods

In this retrospective study, 25 CCRCCs, 19 CCOCs, 20 ACCs, and 10 chordomas were identified from our institution's pathology archives. All CCRCCs, CCOCs, and ACCs were primary tumors. The ACCs were assembled onto tissue microarrays as previously described by Enriquez et al [13].

Immunohistochemistry of formalin-fixed, paraffin-embedded tissue was performed using antibodies against RCC Ma (NCL-RCC; 1:10; Leica, Richmond, IL), Pax8 (363A-15; 1:100; Cell Marque, Rocklin, CA), SF-1 (PP-N1665-00; 1:200; R&D Systems, Minneapolis, MN), and brachyury (sc-20109; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA). Renal cell carcinoma, Pax8, and brachyury immunohistochemistry were performed on a Leica Bond instrument using the Novocastra Bond Polymer Refine Detection System. Steroidogenic factor 1 immunohistochemistry was performed manually using the Dako Envision + Detection System.

Staining was reviewed by 2 pathologists (EC and ZB). Membranous staining was considered positive for RCC Ma, and nuclear staining was considered positive for Pax8, brachyury, and SF-1. The extent of staining was semiquantitatively scored as focal (<25%), nonfocal (25%-50%), or diffuse (>50%). The intensity was scored as weak (1+), moderate (2+), or strong (3+).

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This study was approved by the Institutional Review Board of the University of Pennsylvania.

3. Results

All antibodies were detected in stained paraffin-embedded tissue sections with adequate positive and negative controls. The immunohistochemical staining results were summarized in the Table.

Of the 25 CCRCCs, 12 were Fuhrman grade II, 11 were Fuhrman grade III, and 2 were Fuhrman grade IV. Twenty-two CCRCCs were positive for RCC Ma (88%). The RCC Ma showed membranous/cytoplasmic staining. The staining was largely diffuse (88%) and moderate (2+) to strong (3+) with an average score of 2.2 ± 0.9 (Fig. 1B; Table), particularly in Fuhrman grade II and III tumors. Twenty-two CCRCCs were positive for Pax8 (88%). Pax8 showed specific nuclear staining. The staining was predominantly diffuse (88%), with variable intensity ranging from weak (1+) to strong (3+) with an average of 2.2 ± 0.8 (Fig. 1C and F; Table). Pax8 positivity remained largely diffuse and moderate to strong across all Fuhrman grades. All of the CCRCCs were negative for brachyury (Fig. 1G) and SF-1 (Fig. 1H).

All 19 CCOCs showed diffuse nuclear positivity for Pax8 (Fig. 2D). The intensity ranged from moderate (2+) to strong (3+) with an average of 2.8 ± 0.3 (Table). All CCOCs were negative for RCC Ma (Fig. 2C), brachyury (Fig. 2E), and SF-1 (Fig. 2F).

The tissues from all 20 ACCs on the adrenal tissue microarrays were intact and adequate for review. The SF-1 showed specific nuclear staining. All 20 ACCs were positive for SF-1 (Fig. 3F). The nuclear staining was largely diffuse with variable but predominantly moderate (2+) intensity (Table). All ACCs were negative for RCC Ma (Fig. 3C), Pax8 (Fig. 3D), and brachyury (Fig. 3E).

Nine chordomas were axial (4 in clivus, 3 in cervical spine, 1 in thoracic spine, 1 in lumbar spine). One chordoma was extra-axial and located in the paraspinal soft tissue at L3-L4. All 10 chordomas showed diffuse and strong (3+) nuclear positivity for brachyury (Fig. 4 E). All chordomas were negative for RCC Ma (Fig. 4C), Pax8 (Fig. 4D), and SF-1 (Fig. 4F).

4. Discussion

Clear cell renal cell carcinoma, CCOC, ACC, and chordoma are generally distinguishable from one another when received as sizeable resection specimens accompanied by adequate clinical and radiographic information. However, in instances where clinical information and diagnostic tissue are limited, the overlapping clear cell morphology with these tumors can pose a diagnostic challenge. As previous studies have shown, immunohistochemistry is a practical and reliable method for differentiating among tumors with clear cell morphology [5,6,13,14,16-18]. To our knowledge, this is the first study to incorporate RCC Ma, Pax8, brachyury, and SF-1 into a single diagnostic panel.

Renal cell carcinoma marker is a commercially available monoclonal antibody known for its high detection rate for renal epithelial neoplasms. Directed against a 200-kd glycoprotein in the proximal renal tubular brush border, its reported sensitivity in primary CCRCCs ranges from 72% to 92% [1-4]. However, its decreased expression in higher grade tumors [1,2,4], cystic renal cell carcinomas [3], and metastases [2-4] limits its sensitivity. In our study, the RCC Ma detection rate in primary CCRCCs was 88% and was predominantly diffuse and strong cytoplasmic staining. The 3 cases that were negative for RCC Ma included both lower grade (Fuhrman II) and higher grade (Fuhrman IV) tumors. Renal cell carcinoma marker has limited specificity, as it stains renal tumors other than CCRCCs [1-4]. Although the other tumors with clear cell morphology in our study did not stain for RCC Ma, others have reported RCC Ma positivity in nonrenal tumors including parathyroid [2,3], breast [2,3], testicular embryonal carcinoma [3], adrenal cortical, ovary, colon, prostate, melanoma, and lung [2]. It is interesting to note that the 3 CCRCCs that were negative for RCC Ma were positive for Pax8.

Pax8 is a member of Pax family whose members contain a conserved paired box, a DNA binding domains of 128 amino acids located at the N-terminal [19], and play key roles in regulating cell proliferation, differentiation, apoptosis, migration, and stem cell maintenance [20]. Pax8 has been shown to be involved in the normal development of renal, thyroid, and müllerian tissues [9,11]. It has been shown to be specifically expressed in tumors of thyroid, müllerian, and renal origins and is useful markers for the differential diagnosis of tumors from these organs [21-26]. Similar to previous studies [3,23], we demonstrated high sensitivity with nuclear Pax8 staining in 88% of our CCRCCs. The three Pax8-negative CCRCCs showed RCC Ma positivity, which demonstrated the complementary nature of these 2 stains and the benefit of using both when evaluating suspected renal neoplasms. In our study, diffuse nuclear Pax8 positivity was also demonstrated in all 19 CCOCs (100%). Although rare Pax8 positivity in chordomas has been reported [6], none of the chordomas in our study were positive for Pax8. Likewise, none of our ACCs were positive for Pax8, a finding similar to that described by Sangoi et al [10]. Although both CCRCCs and CCOCs exhibit diffuse Pax8 positivity, the staining intensity may differentiate between the 2 entities. Most CCOCs showed strong intensity, whereas CCRCCs typically displayed weak-to-moderate intensity (Table). Although this distinction has essentially no utility in male patients, it may assist in the workup of females in whom ovarian and renal cell carcinomas are considered.

Brachyury is a founding member of a gene family of transcription factors with a novel DNA-binding domain called T-domain, which consists of approximately 180 ± 200 amino acids at the N-terminal portion of the protein [27,28]. It is a transcription factor involved in mesoderm differentiation and notochord development [29]. Emerging evidence shows that a network of T-box genes, by interacting with Wnt/β-catenin and Notch/Delta signaling pathways, control mesoderm specification, somite segmentation, and left/right body axis determination [30]. The brachyury antibody has been shown to be specific for notochord and notochord-derived tumors, including axial [6,8] as well as extra-axial chordomas [7]. Our study, which included 9 axial and 1 extra-axial tumors, showed 100% sensitivity for chordomas with diffuse and strong nuclear positivity. Although brachyury positivity is essentially pathognomonic for chordomas, reports exist of rare cases with brachyury reactivity in testicular germ cell tumors [6,7]. In our study, all of CCRCCs, CCOCs, or ACCs were negative for brachyury.

Steroidogenic factor 1 is an orphan hormone receptor and has a modular domain structure consisting of an N-terminal zinc finger

Table
Immunohistochemical stains of tumors with clear cell morphology

Tumor	No.	Pax8		RCC Ma		SF-1		Brachyury	
		Positivity (%)	Score	Positivity (%)	Score	Positivity (%)	Score	Positivity (%)	Score
CCRCC	25	88	2.2 ± 0.8	88	2.2 ± 0.9	0	0	0	0
CCOC	19	100	2.8 ± 0.3	0	0	0	0	0	0
ACC	20	0	0	0	0	100	2.5 ± 0.5	0	0
Chordoma	10	0	0	0	0	0	0	100	3