Original Article

Differentiation of renal cell tumors with morphological cocktails using a minimal panel of immunohistochemical markers

B. Vishal Rao, Tejomayi Regulavalasa, Daphne Fonseca, Sudha S. Murthy, Rakesh Sharma[1], K. V. V. N. Raju[1], T. Subramanyeshwar Rao[1], Challa Sundaram

Departments of Pathology and Laboratory Medicine and [1]Surgical Oncology, Basavatarakam Indo American Cancer Hospital and Research Institute, Hyderabad, Telangana, India

Abstract

Context: Morphological cocktails in renal cell carcinoma (RCC).

Aims: Minimal immunohistochemistry (IHC) panel to resolve the diagnosis of renal cell cacinoma (RCC) with morphological overlaps.

Settings and Design: RCC is the most common malignancy in kidney accounting for 90% of all kidney cancers. Clear cell RCC is the most common histological type followed by papillary RCC. However, many of the RCCs show morphological cocktails which may pose diagnostic difficulties in small biopsies and even in the resection specimens. Accurate diagnosis has both prognostic and therapeutic implications; hence, correct differentiation is necessary.

Subjects and Methods: This retrospective study includes all renal cell tumors diagnosed on core biopsies, radical and partial nephrectomies between January 2015 and September 2017 were studied. The demographic, clinical, and gross findings were noted. The cases that had morphological overlap among the subtypes were subjected to a panel of IHC markers, including CD10, CK7, alpha-methyl acyl-coenzymeA racemase (AMACR), and CD117.

Results: There were 128 RCC in the study period, and morphological overlap was seen in 36 (27.9%) specimens including 13 core biopsies, 16 radical, and 7 partial nephrectomies. IHC resolved 35/36 (97.2%) cases rendering a diagnosis of clear cell (11), papillary (15), chromophobe (4), and oncocytoma (5). However, in one case where the provisional diagnosis was oncocytic tumor, all IHC markers were negative rendering IHC noncontributory.

Conclusions: Difficulty in diagnosis was encountered in many core biopsies, resection specimens which when subjected to IHC panel of CD10, CK7, AMACR, and CD117 helped in resolving the diagnosis of subtypes of RCC.

Keywords: Immunohistochemistry, morphological cocktails, renal cell carcinoma

Address for correspondence: Dr. B. Vishal Rao, Basavatarakam Indo American Cancer Hospital and Research Institute, Road No. 14, Banjara Hills, Hyderabad - 500 034, Telangana, India.
E-mail: vishalrao1980@gmail.com

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INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignancy in kidney accounting for 90% of all kidney cancers.[1] The most common histological types include clear cell and papillary types. However, clear cell RCC may have papillary architecture, and the papillary RCC may contain clear cells. The two recently described, but less common RCCs are clear cell papillary RCC (CPRCC) and Xp11 translocation RCC, and characteristically both have papillary architecture and cells with clear cytoplasm.[2] The eosinophilic variant of clear cell RCC and chromophobe RCC may pose diagnostic difficulties, with each other and from oncocytic tumors. Oncocytoma shares a similar immunoprofile with chromophobe RCC, particularly the eosinophilic variant. Numerous studies have attempted to identify markers that can reliably differentiate oncocytoma from chromophobe RCC, with disappointing results.[3-6] In addition, ample evidence suggests that some tumors may have features of both oncocytoma and chromophobe RCC (the so-called hybrid tumor) as described in patients with Birt-Hogg-Dubé syndrome.[7] Precise histological categorization has both prognostic and therapeutic implications. The International Society of Urologic Pathology Consensus Conference also recommends the application of immunohistochemistry (IHC) in evaluating renal tumors with complex morphology.[8]

Alpha-methyl acyl-coenzymeA racemase (AMACR) is a useful IHC stain in the diagnosis of papillary RCC. CD10 is a proximal tubular marker which is highly sensitive and consistently positive in clear cell RCC but not specific to RCC alone. CD117 is positive in chromophobe RCCs and oncocytomas. CK7 is diffusely positive in chromophobe RCC; however, each marker is not specific by itself for the diagnosis of renal tumor subtype.[7,9] A concise and cost-effective IHC panel is necessary for a prompt and precise diagnosis in a resource-limited setting.

The aim of this study is to differentiate renal tumor subtypes with morphological overlap using a minimal panel of four IHC markers, including AMACR, CD10, CK7, and CD117.

SUBJECTS AND METHODS

A retrospective study was performed on all renal tumors diagnosed on core biopsies, radical and partial nephrectomies in our tertiary care cancer center between January 2015 and September 2017. The demographic, clinical, and gross findings were noted. The cases were diagnosed according to 2016 WHO Classification. RCCs that had mixed morphological patterns and difficult to render a definitive morphological diagnosis were subjected to a panel of IHC markers, including CD10, CK7, AMACR, and CD117. These included tumors with mixed patterns such as papillary, solid and tubulocystic, tumors showing clear cell features with papillary growth pattern, and tumors with features of oncocytic change.

The most common renal tumors were classified into subgroups by IHC as shown in Table 1.

Immunohistochemical study and evaluation

The IHC study was performed by Biocare's intelliPATH automated slide stainer using heat retrieval method. The following antibodies: CD10, AMACR, CK7, and CD117 were done. The source, type, dilution, and localization of antibody are given in Table 2. Immunostaining of >10% of tumor cells was scored as positive.[2] The initial morphologic diagnosis was correlated with the final diagnosis after IHC.

RESULTS

There were a total of 128 cases in the study period, which included 61 radical nephrectomies, 8 partial nephrectomies, and 59 core biopsies. The initial morphologic diagnosis was clear cell RCC in 80 (62.5%), papillary RCC in 25 (19.5%), chromophobe RCC in 5 (3.9%), oncocytic tumors in 10 (7.8%), sarcomatoid RCC in 3 (2.3%), urothelial carcinoma in 2 (1.6%), and one each of translocation RCC, sarcoma, and angiomyolipoma. Morphological overlap and diagnostic difficulty were encountered in 36/128 (28%) cases which were subjected to IHC. These included 13 core biopsies, 07 partial nephrectomies, and 16 radical nephrectomies.

Tumors with morphological overlap (n = 36)

These included tumors with papillary growth pattern and clear cell morphology (16) and tumors with oncocytic cells admixed with clear cell/chromophobe morphology and papillary growth pattern (20).

Contribution of immunohistochemistry to diagnosis

In the 16 cases with papillary pattern and clear cell

Table 1: Diagnosis of renal tumors by immunohistochemistry - The most common renal tumors were classified into subgroups by immunohistochemistry as follows

Subtype of renal tumor	CD10	AMACR	CK7	CD117
Clear cell RCC	+	+/−	−	−
Papillary RCC	+/−	+	+	−
Chromophobe RCC	−	−	+	+
Oncocytoma	−	−	Occasional cell +	+

RCC: Renal cell carcinoma; AMACR: Alpha-methyl acyl-coenzymeA racemase; +: Positive; −: Negative

morphology, IHC helped resolve them into papillary and clear cell RCC in 8 cases each. In the 20 cases with oncocytic cells admixed with clear cells, chromophobe like morphology and papillary patterns, IHC resolved them into papillary RCC in 7, eosinophilic variant of clear cell RCC in 3, chromophobe RCC in 4, and oncocytoma in 5. In one case of oncocytic tumor, all the four markers were negative rendering the IHC panel noncontributory. Further IHC studies and electron microscopy studies were not performed, and a report of the oncocytic tumor was given [Figure 1].

Hence, IHC helped in resolving the diagnosis in 35 out of 36 cases (97.2%) and was noncontributory in one case (2.8%). Immunohistochemical expression of various subtypes of renal cells tumors is depicted in Figure 2.

The demographic details, procedures performed, initial diagnosis on morphology, diagnosis with IHC, and final diagnosis are given in Table 3.

DISCUSSION

The World Health Organization classification of renal tumors incorporates morphological, immunohistochemical, and molecular data to define distinct entities that are biologically and clinically relevant.[2] Due to the availability of more effective molecular targeted therapy for certain specific renal neoplasms, IHC is playing an increasingly important role in the diagnosis, subclassification of primary tumors, prognosis, and prediction of renal neoplasms.[9-11] With an increase in the number of available markers, the challenge is to choose a concise and cost-effective panel for routine use, especially for core biopsies.[9] In the current study, a set of four immune markers were used to differentiate the major types of renal tumors with morphological overlap.

The application of IHC is specifically useful to differentiate various histological subtypes of RCC, to differentiate them from their benign mimics, and to establish a diagnosis of metastatic RCC. The utility of a marker depends on the differential diagnosis in question, grade of the RCC, sample size, and the specific clone/method used.[8] In the present study, primary renal tumors with complex morphology, including papillary, solid or tubular, and those with oncocytic features were included where there was a difficulty to classify into a subgroup.

The utility of IHC is increasing, especially in core biopsies. Core needle biopsy has recently become more frequently used for preoperative diagnosis, not only for traditional indications, such as inoperable tumors or tumors where surgical resection is considered to be contraindicated or ineffective, such as malignant lymphoma or metastatic tumors but also in response to new therapies where preoperative diagnosis will help make decisions about

Table 2: The antibodies used, their source, type, dilution, and localization

Antibody	Source, type, dilution	Localization
AMACR	Rabbit monoclonal antibody; clone 13H4; Dako, 1:200 dilution	Membranous Cytoplasmic
CD10	Monoclonal mouse anti-human antibody; clone 56c6; Dako, 1:100 dilution	Membranous
CK7	Monoclonal mouse anti-human antibody; clone 12,130; cell marque, 1:100 dilution	Membranous Cytoplasmic
CD117	Rabbit monoclonal antibody; clone YR145; cell marque, 1:100 dilution	Membranous

AMACR: Alpha-methylacyl-CoA racemase

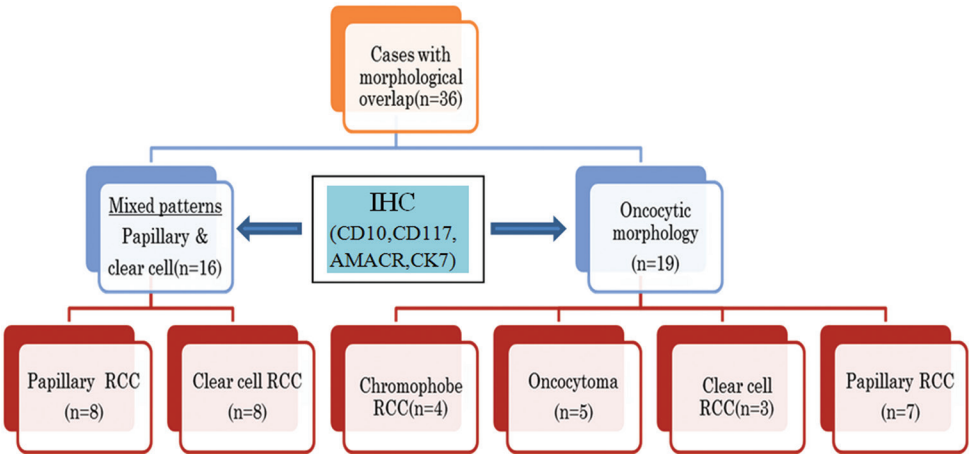

Figure 1: Immunohistochemistry of selected cases with mixed morphological patterns, immunohistochemistry was noncontributory in one case (1/36)

Table 3: Demographic details, type of procedure, initial diagnosis on morphology, and final diagnosis after immunohistochemistry in renal tumors (*n*=36)

Clinicopathological variables	Subtypes of renal tumors on morphology	Subtypes of renal tumors on IHC	Final Diagnosis
Gender: M: F: 5:4 (20:16)	Clear cell RCC with papillary pattern/ oncocytic cells :10	Clear cell RCC: (CD 10+; AMACR +/-; CK 7-; CD 117-)	Clear cell RCC: 11
Age: 39 to 73 (median 56) years	Papillary RCC with clear/oncocytic cells: 12	Papillary RCC: (AMACR +; CD 10 +/-; CK 7+; CD 117-)	Papillary RCC: 15
Laterality: R: L: 23:13	Chromophobe RCC: 4	Chromophobe RCC: (CK 7+; CD 117+; CD 10-; AMACR -)	Chromophobe RCC: 04
Procedure: Core biopsy: 13 Partial nephrectomy: 07 Radical Nephrectomy: 16	Oncocytic tumor/neoplasm: 8	Oncocytoma: (CD 117 +; CK 7 occasional cell+; CD 10 -; AMACR -)	Oncocytoma: 05
	Poorly differentiated carcinoma: 2	One case -Unresolved: All markers negative	Morphological diagnosis only (oncocytic tumor) : 01

RCC: Renal cell carcinoma; AMACR: Alpha-methyl acyl-coenzymeA racemose; ICC: Immunohistochemistry; +: Positive; −: Negative

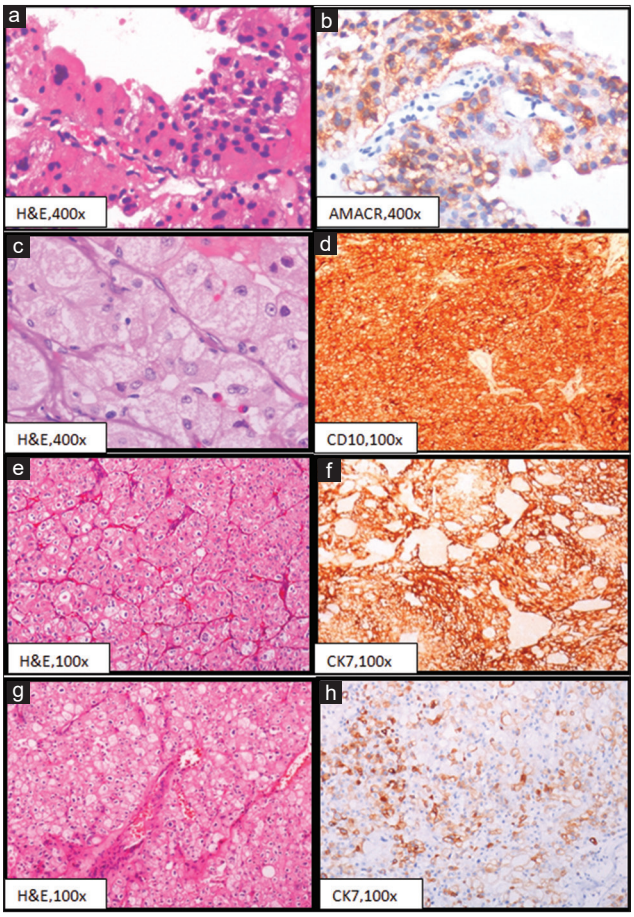

Figure 2: Immunohistochemical expressions of renal cell tumors. (a) Papillary renal cell carcinoma with oncocytic cells (H and E). (b) Alpha-methyl acyl-coenzyme racemase diffuse positivity; (c) clear cell renal cell carcinoma with oncocytic cells (H and E); (d) CD10 diffuse positivity; (e) chromophobe renal cell carcinoma (H and E); (f) CK7 diffuse positivity; (g) oncocytic tumor (H and E), (h) CK7 focal positive

the choice of treatment.[12,13] A preoperative diagnosis on core biopsy is important because 20%–45% of small renal masses are ultimately found to be benign, and active surveillance is an option for many patients.[14-17] In tumors with cells containing eosinophilic cytoplasm, the differential diagnosis includes oncocytoma, chromophobe RCC, succinate dehydrogenase-deficient RCC, papillary RCC eosinophilic variant, and tubulocystic RCC and oncocytic angiomyolipoma, indicating a need for the application of IHC.[17] Oncocytic lesions can be especially troublesome in renal mass biopsy, as the interpretation of a limited tissue may not be representative of the entire lesion. In one case of the oncocytic tumor where diagnosis could not be resolved on IHC was a core biopsy in the present study, highlighting the difficulties as well as sample adequacy.

In the differential diagnosis of clear cell RCC from chromophobe RCC, and clear CPRCC, inclusion of carbonic anhydrase was recommended.[8] However, with the IHC panel, including CD10, CD117, CK7 and AMACR in the present study, the issue was resolved in almost all the cases. Inclusion of CK7, CD117, Ksp-cadherin, and S100A1 were recommended. With the limited panel of IHC used in the present study, 19 of the 20 cases of tumors with oncocytic features could be resolved. However, Ksp-cadherin and S100A1 are expressed in both oncocytoma and chromophobe RCC, and their role in difficult to classify tumors is not yet validated.[8]

Al-Ahmadie *et al.* studied that standard morphologic evaluation in combination with the use of five markers including CAIX, CD117, AMACR, CK7, and CD10, to get an accurate diagnosis in >90% of cases.[18] They performed their study on *ex vivo* core biopsies on the nephrectomy specimens. Alshenawy studied the utility of CK7, AMACR, CAIX, and TFE3 in 66 cases of RCC with clear cell and papillary features.[2]

The current study is the first of its kind to use a minimal panel of four markers to differentiate the major subtypes of renal tumors when there is a histological overlap, and a definitive morphological diagnosis is difficult.

CONCLUSIONS

Difficulty in diagnosis was encountered in many core biopsies, resection specimens which when subjected to IHC panel of CD10, CK7, AMACR, and CD117 helped in resolving the diagnosis of subtypes of RCC.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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