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

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Abstract

Context: Morphological cocktails in renal cell carcinoma (RCC).
Aims: Minimal immunohistochemistry (IHC) panel to resolve the diagnosis of renal cell carcinoma (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

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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 characterized both have papillary architecture and cells with clear cytoplasm.[2,3] The eosinophilic variant of clear cell RCC and chromophobe RCC may pose diagnostic difficulties, with each other and from oncocyto 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.[4,5] 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 oncocytoic 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.[3] 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 angiomylipoma. 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

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

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

| Antibody | Source, type, dilution | Localization |
|----------|------------------------|--------------|
| AMACR    | Rabbit monoclonal antibody; clone 13H4; Dako, 1:200 dilution | Membranous   |
| 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   |
| 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)
the choice of treatment.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[8]}

Al-Ahmadie \textit{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.\textsuperscript{[18]} They performed their study on \textit{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.\textsuperscript{[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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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Rosai J, Ackerman L.V., editors. Rosai and Ackerman’s Surgical Pathology. 9th ed. New York: C.V. Mosby; 2004. p. 1251.
2. Alshenawy HA. Immunohistochemical panel for differentiating renal cell carcinoma with clear and papillary features. J Microsc Ultrastruct 2015;3:68-74.
3. McGregor DK, Khurana KK, Cao C, Tsao CC, Ayala G, Krishnan B, et al. Diagnosing primary and metastatic renal cell carcinoma: The use of the monoclonal antibody ‘renal cell carcinoma marker’. Am J Surg Pathol 2001;25:1485-92.
4. Martignoni G, Pea M, Chilosi M, Brunelli M, Scarpa A, Colato C, et al. Parvalbumin is constantly expressed in chromophobe renal carcinoma. Mod Pathol 2001;14:760-7.
5. Adley BP, Papavero V, Sugimura J, Teh BT, Yang XJ. Diagnostic value of cytokeratin 7 and parvalbumin in differentiating chromophobe renal cell carcinoma from renal oncocytoma. Anal Quant Cytol Histol 2006;28:228-36.
6. Li G, Barthelmy A, Feng G, Gentil-Perret A, Peoc'h M, Genin C, et al. S100A1: A powerful marker to differentiate chromophobe renal cell carcinoma from renal oncocytoma. Histopathology 2007;50:642-7.
7. Truong LD, Shen SS. Immunohistochemical diagnosis of renal neoplasms. Arch Pathol Lab Med 2011;135:92-109.
8. Reuter VE, Argani P, Zhou M, Delahunt B; Members of the ISUP Immunohistochemistry in Diagnostic Urologic Pathology Group. Best practices recommendations in the application of immunohistochemistry in the kidney tumors: Report from the International Society of Urologic Pathology Consensus Conference. Am J Surg Pathol 2014;38:e35-49.
9. Shen SS, Truong LD, Scarpelli M, Lopez-Beltran A. Role of immunohistochemistry in diagnosing renal neoplasms: When is it really useful? Arch Pathol Lab Med 2012;136:410-7.
10. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125-34.
11. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Oudard S, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol 2009;27:3584-90.
12. Gill IS, Aron M, Gervais DA, Jewett MA. Clinical practice. Small renal mass. N Engl J Med 2010;362:624-34.
13. Parks GE, Perkins LA, Zagoria RJ, Garvin AJ, Sirintrapun SJ, Geisinger KR. Benefits of a combined approach to sampling of renal neoplasms as demonstrated in a series of 351 cases. Am J Surg Pathol 2011;35:827-35.
14. Frank I, Blute MI, Cheville JC, Lohse CM, Weaver AL, Zincke H. Solid renal tumors: An analysis of pathological features related to tumor size. J Urol 2003;170:2217-20.
15. Lane BR, Babineau D, Kattan MW, Novick AC, Gill IS, Zhou M, et al. A preoperative prognostic nomogram for solid enhancing renal tumors 7 cm or less amenable to partial nephrectomy. J Urol 2007;178:429-34.
16. Patel HD, Druskin SC, Rowe SP, Pierorazio PM, Gorin MA, Allanf ME. Surgical histopathology for suspected oncocytoma on renal mass biopsy: A systematic review and meta-analysis. BJU Int 2017;119:661-6.
17. Wobker SE, Williamson SR. Modern pathologic diagnosis of renal oncocytoma. J Kidney Cancer VHL. 2017;4:1-2.
18. Al-Ahmadic HA, Alden D, Fine SW, Gopalan A, Touijer KA, Russo P, et al. Role of immunohistochemistry in the evaluation of needle core biopsies in adult renal cortical tumors: An ex vivo study. Am J Surg Pathol 2011;35:949-61.