Cytomorphologic Comparison of Type 1 and Type 2 Papillary Renal Cell Carcinoma: A Retrospective Analysis of 28 Cases

Martin J. Magers, MD; Carmen M. Perrino, MD; Harvey M. Cramer, MD; and Howard H. Wu, MD

BACKGROUND: Papillary renal cell carcinoma (pRCC) is classified as type 1 or type 2 on the basis of histomorphologic features. Type 1 pRCC typically carries a better prognosis, and renal cell carcinoma is often diagnosed by fine-needle aspiration (FNA). Thus, this study was designed to characterize cytomorphologic features present in FNA cases that could be used to discriminate between type 1 and type 2 pRCC. METHODS: Electronic records of Indiana University were searched for pRCC FNA cases (2007-2018). Corresponding surgical pathology reports were reviewed to classify patients as having type 1 or type 2 pRCC. FNA slides were reviewed to assess cytomorphologic features (ie, nuclear grade; cell size; cytoplasmic volume and quality; and the presence of single cells, papillary clusters, nuclear grooves, foamy histiocytes, hemosiderin pigment, psammoma bodies, and hyaline globules). A semiquantitative score was assigned to each feature. The nuclear grade was assigned with the World Health Organization/International Society of Urological Pathology grading system. The cytomorphologic features of type 1 and type 2 pRCC were compared. RESULTS: Sixteen patients with type 1 pRCC and 12 patients with type 2 pRCC were included in the study. Type 2 pRCC had a higher nuclear grade, a higher volume of cytoplasm, and more granular cytoplasm. Type 1 pRCC more frequently had nuclear grooves and clear cytoplasm. The remaining features (ie, cell size, papillary clusters, single cells, foamy histiocytes, hemosiderin pigment, psammoma bodies, and hyaline globules) were not statistically significant. CONCLUSIONS: Nuclear grade, cytoplasmic volume and granularity or clarity, and nuclear grooves are cytomorphologic features that may aid in the distinction between type 1 and type 2 pRCC. Cancer Cytopathol 2019;127:370-376. © 2019 American Cancer Society.

KEY WORDS: cytology; fine-needle aspiration; kidney; papillary renal cell carcinoma.

INTRODUCTION

Kidney cancer is relatively common: it has been estimated to be the 6th most common cancer diagnosis in men and the 10th most common cancer diagnosis in 2018, with approximately 65,000 new cases and 15,000 cancer deaths.1 The vast majority of kidney cancers are epithelial in origin, and this means that renal cell carcinoma (RCC) accounts for most of kidney cancer diagnoses. Papillary renal cell carcinoma (pRCC) is the second most common subtype of RCC and accounts for nearly 20% of all RCCs; it is further subtyped as type 1 pRCC or type 2 pRCC on the basis of histomorphologic features.2-4 Type 1 pRCC is characterized by low cuboidal epithelial cells with scant, pale or basophilic cytoplasm lining fibrovascular cores, most notably without pseudostatification, and usually with lower grade nuclei (Fig. 1). In contrast, type 2 pRCC is characterized by the presence of pseudostatification of neoplastic epithelial cells with more abundant eosinophilic cytoplasm and higher grade nuclei (Fig. 1).3-6 These histomorphologic differences are driven by different molecular abnormalities present in type 1 pRCC versus type 2 pRCC.7,8 Furthermore, it is important to accurately classify
type 1 and type 2 pRCC because type 1 pRCC confers a much better prognosis than type 2 pRCC.5,8

Although the cytologic features of pRCC are well described, the literature is lacking a detailed analysis of cytologic features that can be used to discriminate between type 1 pRCC and type 2 pRCC.9-16 However, this distinction is clinically important, and it may be important to specify type 1 pRCC or type 2 pRCC in the diagnosis of direct smears prepared from fine-needle aspiration (FNA) rather than simply diagnosing pRCC without a specific subtype. Here, we review our 12-year experience with FNA of pRCC in an attempt to identify

Figure 1. Caption (A,B) Type 1 pRCC is characterized by papillae with fibrovascular cores lined by a single layer of neoplastic epithelial cells (H & E, ×200 [panel A] or ×400 [panel B]), and (C) type 1 pRCC usually exhibits a relatively low nuclear/nucleolar grade (eg, grade 1 or 2; Papanicolaou-stained direct smear, ×400). (D,E) Type 2 pRCC is characterized by pseudostratified neoplastic epithelium lining fibrovascular cores (H & E, ×200 [panel D] or ×400 [panel E]), and (F) the nuclear grade is typically higher (eg, often grade 3; Papanicolaou-stained direct smear, ×400). pRCC indicates papillary renal cell carcinoma.
cytomorphologic features that can be used to discriminate between type 1 pRCC and type 2 pRCC.

MATERIALS AND METHODS

This study was approved by the Indiana University School of Medicine institutional review board (protocol 1901948415). The laboratory information system at Indiana University Health was searched over the 12-year period from August 2007 to July 2018 to identify all cases of pRCC that were sampled by FNA. Any corresponding surgical pathology reports, including the primary tumor nephrectomy specimens or core biopsies, were reviewed to classify all cases as being either type 1 pRCC or type 2 pRCC and to extract clinicopathologic information. FNA material included Papanicolaou- and Diff-Quik–stained direct smears in all cases and hematoxylin-eosin–stained slides cut from cell blocks and immunocytochemical slides in some cases. The cytology FNA slides were reviewed to assess for cytomorphologic features (ie, nuclear grade; cell size; cytoplasmic volume and quality; and the presence of single cells, papillary clusters, nuclear grooves, foamy histiocytes, hemosiderin pigment, psammoma bodies, and hyaline globules), and a semiquantitative score was assigned to each feature. The cell size was scored as 1 (equivalent to <3 red blood cells), 2 (3-5 red blood cells), or 3 (>5 red blood cells). The remaining cytomorphologic features were assigned scores of 0 (absent), 1 (scant), 2 (moderate), or 3 (abundant). The nuclear grade was assigned with the World Health Organization (WHO)/International Society of Urological Pathology (ISUP) grading system, according to which grade 1 tumors lack nucleoli or have only inconspicuous nucleoli at a magnification of ×400; grade 2 tumors possess conspicuous and eosinophilic nucleoli at ×400; grade 3 tumors possess conspicuous and eosinophilic nucleoli at 100×; and grade 4 tumors exhibit extreme nuclear pleomorphism, multinucleate giant cells, rhabdoid differentiation, or sarcomatoid differentiation.3,17,18 The cytomorphologic features of type 1 pRCC and type 2 pRCC were compared with a 2-tailed t test with a significance of \( P < .05 \).

RESULTS

The results are summarized in Tables 1 and 2. In total, 16 patients with type 1 pRCC and 12 patients with type 2 pRCC were included in the study. For the FNA cases, all specimens were acquired in house, whereas some of the corresponding surgical pathology specimens came from outside cases that were reviewed in consultation. Overall, patients’ ages varied from 44 to 87 years (mean, 65 years), and the overall male-to-female ratio was 3:1 (21 males and 7 females). There was no significant difference in age or sex between type 1 pRCC (age range, 44-78 years; mean, 64 years; 12 males and 4 females) and type 2 pRCC (age range, 53-87 years; mean, 67 years; 9 males and 3 females).

The FNA site for most cases of type 1 pRCC was the kidney (13 cases or 81%), with the lymph node (2 cases or 13%), lung (1 case or 6%), liver (0 cases), portal vein (0 cases), sacrum (0 cases), and retroperitoneum (0 cases).

| TABLE 1. Clinicopathologic Characteristics |
|----------------------------------------|
| Characteristic | Type 1 (n = 16) | Type 2 (n = 12) |
|----------------|----------------|----------------|
| Age, y | Median 65 | 65 |
| Mean 64 | 67 |
| Range 44-78 | 53-87 |
| Sex, No. (%) | Male 12 (75) | 9 (75) |
| Female 4 (25) | 3 (25) |
| Site of FNA, No. (%) | Kidney 13 (81) | 4 (33) |
| Lymph node 2 (13) | 3 (25) |
| Lung 1 (6) | 1 (8) |
| Liver 0 (0) | 1 (8) |
| Portal vein 0 (0) | 1 (8) |
| Sacrum 0 (0) | 1 (8) |
| Retropertioneum 0 (0) | 1 (8) |
| WHO/ISUP nuclear grade, No. (%) | 2 14 (88) | 0 (0) |
| 3 2 (13) | 11 (92) |
| 4 0 (0) | 1 (8) |
| Tumor size, cm | Median 5 | 10 |
| Mean 5 | 9 |
| Range 1-11 | 4-15 |

Abbreviations: FNA, fine-needle aspiration; ISUP, International Society of Urological Pathology; WHO, World Health Organization.

| TABLE 2. Cytomorphologic Features |
|-----------------------------------|
| Cytomorphologic Feature | Type 1, Mean (Range) | Type 2, Mean (Range) | P |
|-------------------------|----------------------|----------------------|---|
| Nuclear grade | 2.1 (2-3) | 3.1 (3-4) | <.001 |
| Nuclear grooves | 1.8 (1-3) | 1.2 (1-2) | .012 |
| Cytoplasmic volume | 2.1 (1-3) | 2.5 (2-3) | .048 |
| Granular cytoplasm | 0.6 (0-1) | 1.0 (1-1) | .007 |
| Clear cytoplasm | 0.8 (0-1) | 0.3 (0-1) | .007 |
| Cell size | 2.9 (2-3) | 3.0 (3-3) | .397 |
| Papillary clusters | 2.7 (1-3) | 2.5 (1-3) | 542 |
| Single cells | 1.4 (1-3) | 1.3 (1-2) | 1,000 |
| Foamy histiocytes | 1.5 (0-3) | 0.8 (0-3) | .071 |
| Hemosiderin pigment | 0.7 (0-3) | 0.7 (0-3) | .953 |
| Psammoma bodies | 0.3 (0-3) | 0.0 (0-0) | .276 |
| Hyaline globules | 1.0 (0-3) | 1.1 (0-2) | .776 |

The key for the semiquantitative scale is as follows: 0, absent; 1, scant; 2, moderate, and 3, abundant.

\( a \)The cell size was quantified as 1 (<3 red blood cells), 2 (3-5 red blood cells), or 3 (>5 red blood cells).

Cytomorphologic features which are statistically significant are bolded.
or 13%) and the lung (1 case or 6%) accounting for the few remaining cases. On the other hand, the FNA site in type 2 pRCC varied widely, with the kidney accounting for one-third of cases (4 cases or 33%); the next most common site was the lymph node (3 cases or 25%), and the lung, liver, portal vein, sacrum, and retroperitoneum all accounted for 1 case.

The original FNA diagnosis for patients with type 1 pRCC was usually pRCC without a designated subtype (n = 9), although 2 cases were diagnosed as type 1 pRCC on the basis of the FNA specimen. Nine patients with type 1 pRCC underwent core-needle biopsy concurrently with FNA, and 2 of these patients were diagnosed with type 1 pRCC on the basis of the core-needle biopsy; 1 of these 2 patients was also diagnosed as type 1 pRCC on the basis of the FNA specimen. No FNA specimens from patients with type 1 pRCC were called clear cell RCC, although 4 cases were diagnosed simply as RCC. Immunohistochemistry (IHC) was performed on 4 FNA specimens and 2 core biopsy specimens, and these typically included paired box 8 (PAX8), cytokeratin 7 (CK7), and α-methylacyl-coenzyme A racemase (AMACR); some cases also included carbonic anhydrase IX (CAIX), vimentin, CD10, human melanoma black 45 (HMB45), p63, or GATA3. IHC was performed on the resection specimen of a patient with type 1 pRCC in only 1 case, and this case was positive for CK7 and AMACR.

As for the original FNA diagnosis in patients with type 2 pRCC, there was an equal distribution of pRCC (n = 6) and RCC (n = 6). Three patients were diagnosed with type 2 pRCC by FNA. Only 3 patients with type 2 pRCC underwent concurrent core-needle biopsy, and all of these patients were diagnosed as having type 2 pRCC; 1 of these patients was among the 3 patients diagnosed with type 2 pRCC by FNA. IHC was performed on only 3 FNA specimens, and PAX8 was the most frequently used IHC stain. IHC was performed on surgical resection specimens of patients with type 2 pRCC in 3 cases, with AMACR and CK7 being the most frequently used IHC stains.

The nuclear grade of the primary kidney tumor tended to be lower in type 1 pRCC than type 2 pRCC: only 2 cases of type 1 pRCC (13%) were grade 3, and the rest (88%) were grade 2. In contrast, all type 2 pRCC cases were at least grade 3, and 1 case was grade 4 (8%). The size of the primary kidney tumor also tended to be smaller in type 1 pRCC (range, 1-11 cm; mean, 5 cm) versus type 2 pRCC (range, 4-15 cm; mean, 9 cm).

As for the cytomorphologic features of the FNA cases, type 2 pRCC cases had a higher nuclear grade (all type 2 pRCC cases were grade 3 or higher, whereas only 13% of type 1 pRCC cases were; P < .05; Fig. 1), a higher volume of cytoplasm (50% of type 2 pRCC cases contained abundant cytoplasm, whereas only 19% of type 1 pRCC cases did; P < .05; Fig. 2), and more granular cytoplasm (all type 2 pRCC cases had at least focal granular cytoplasm, whereas 56% of type 1 pRCC cases did; P < .05). In addition, type 1 pRCC cases more frequently had at least moderate nuclear grooves (63% of type 1 pRCC cases vs 17% of type 2 pRCC cases; P < .05; Fig. 2) and focal clear cytoplasm (75% of type 1 pRCC cases vs 25% of type 2 pRCC cases; P < .05; Fig. 2). The remaining cytomorphologic features (ie, cell size, papillary clusters, single cells, foamy histiocytes, hemosiderin pigment, psammoma bodies, and hyaline globules) were not found to be statistically significant.

**DISCUSSION**

The cytologic features of pRCC are relatively well described. FNA of pRCC typically yields cellular direct smears composed of true papillae, as evidenced by either the presence of neoplastic cells lining long fibrovascular cores or 3-dimensional spheres of neoplastic cells, intracytoplasmic hemosiderin, nuclear grooves, foamy histiocytes, and rare to occasional psammomatous calcifications. In contrast to most other types of RCC, the presence of long papillary fragments or tight spheres of neoplastic cells, intracytoplasmic hemosiderin, nuclear grooves, foamy histiocytes, and rare to occasional psammomatous calcifications were not found to be statistically significant. The remaining cytomorphologic features (ie, cell size, papillary clusters, single cells, foamy histiocytes, hemosiderin pigment, psammoma bodies, and hyaline globules) were not found to be statistically significant.
Lew et al\textsuperscript{11} opine that type 1 pRCC typically has cells that are small to medium in size and cuboidal in shape with scant cytoplasm, round nuclei, and inconspicuous nucleoli; this is in contrast to type 2 pRCC, which demonstrates larger cells with enlarged nuclei and prominent nucleoli.

However, they do not cite any studies that separate pRCC on the basis of subtyping, and they admit that these features are based on the presumption of type 1 pRCC being lower grade and type 2 pRCC being higher grade. The assumption that type 1 pRCC is lower grade and

\textbf{Figure 2.} Among the cytomorphologic features assessed in this study, (A) nuclear grooves, albeit scant to moderately abundant, were more commonly identified in type 1 pRCC (Papanicolaou-stained direct smear, \( \times 400 \)), (B) cytoplasmic volume was lower in type 1 pRCC (Papanicolaou-stained direct smear, \( \times 400 \)), and (C) type 1 pRCC more frequently possessed clear, vacuolated cytoplasm (Diff-Quik-stained direct smear, \( \times 400 \)). In contrast, (D) nuclear grooves were rarely identified in type 2 pRCC (Papanicolaou-stained direct smear, \( \times 400 \)), (E) type 2 pRCC contained more cytoplasm (Papanicolaou-stained direct smear, \( \times 400 \)), and (F) the cytoplasm of type 2 pRCC was more frequently finely granular (Diff-Quik-stained direct smear, \( \times 400 \)). pRCC indicates papillary renal cell carcinoma.
type 2 pRCC is higher grade is not always true, though. The original descriptions of type 1 pRCC and type 2 pRCC did not definitively subtype type 1 pRCC as lower grade or vice versa, and a subset of type 1 pRCC will be WHO/ISUP grade 3 or higher, whereas a subset of type 2 pRCC will be WHO/ISUP grade 2 or lower.\textsuperscript{5,4,19} Thus, although comparing the cytologic results of low-grade pRCC and high-grade pRCC may yield results similar to those from a comparison of type 1 pRCC and type 2 pRCC, these are not identical comparisons, and care must be taken to not assume that all type 2 pRCC cases will be high-grade or vice versa. As for the use of Fuhrman nuclear grading in these older studies, they were published before the adoption of the WHO/ISUP nucleolar grading system. Likewise, some of the nephrectomy specimens in our study were assigned a grade with the Fuhrman system. Nonetheless, high-grade pRCC has been described as having more abundant cytoplasm, pleomorphic nuclei, and prominent nucleoli, and this is in contrast to low-grade pRCC, which reportedly demonstrates smaller cells, less cytoplasm, inconspicuous nucleoli, and more frequent nuclear grooves.\textsuperscript{10,11,14} Papillary fragments are reportedly less frequent in high-grade pRCC, but intracytoplasmic hemosiderin deposition and foamy histiocytes may still be clues to the diagnosis of pRCC.\textsuperscript{10} Although these findings of low-grade pRCC versus high-grade pRCC are somewhat parallel to our findings from a comparison of type 1 pRCC and type 2 pRCC, in that we found type 1 pRCC to be lower grade with more frequent nuclear grooves and scant, clear cytoplasm in comparison with type 2 pRCC, they are not identical. For instance, we did find 2 cases of type 1 pRCC (13%) with WHO/ISUP grade 3 nucleoli. If WHO/ISUP grading had been considered definitive for subtyping pRCC, these cases would have incorrectly been diagnosed as type 2 pRCC. Furthermore, a small subset of type 2 pRCC cases had nuclear grooves and scant cytoplasm. In addition, in contrast to previous reports that described papillae as typically absent in FNA of high-grade pRCC, we found at least focal papillae in all type 2 pRCC cases.

Interestingly, a previous study included only 2 cases of pRCC, both of which were type 1 pRCC.\textsuperscript{12} Although the focus of this study was not the subtyping of pRCC, no cases of type 2 pRCC were included. Thus, this is perhaps the most pure cytologic description of type 1 pRCC currently in the literature. In that study, Lim and Wojcik\textsuperscript{12} described type 1 pRCC as cytologically characterized by 3-dimensional spheres of neoplastic cells that possess scant, foamy cytoplasm; variably present intracytoplasmic hemosiderin; occasional nuclear grooves; and small nucleoli. Their description is in agreement with our findings for type 1 pRCC.

Although pRCC is currently split into type 1 and type 2 on the basis of well-described morphologic features, there are some cases that demonstrate morphologic overlap.\textsuperscript{20} However, this was not noted in any cases included in this study. Because most cases of pRCC do relatively easily fit into one type,\textsuperscript{6} it is not surprising that our 28 cases fit into one type or another. The lack of significant morphologic overlap in our study likely aided in our ability to distinguish type 1 pRCC from type 2 pRCC.

In conclusion, we found that nuclear grade, cytoplasmic volume, cytoplasmic granularity versus clarity, and nuclear grooves are all cytomorphologic features that may aid in the distinction between type 1 pRCC and type 2 pRCC. Specifically, type 2 pRCC has a higher nuclear grade and more abundant, granular cytoplasm, whereas type 1 pRCC more frequently has nuclear grooves and clear cytoplasm. Although most type 1 pRCC cases are lower grade and most type 2 pRCC cases are higher grade, there is some overlap in nuclear grade, and the entire picture of cytologic features must be taken into account to potentially diagnose type 1 pRCC or type 2 pRCC on the basis of FNA material. Prospective validation of these findings may confirm the clinical utility of using these cytologic features to distinguish type 1 pRCC from type 2 pRCC.

FUNDING SUPPORT
No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES
The authors made no disclosures.

AUTHOR CONTRIBUTIONS
Martin J. Magers: Assistance with the curation of secondary data, writing of the original draft, discussion of the results, and contributions to the final manuscript. Carmen M. Perrino: Assistance with the curation of secondary data, discussion of the results, and contributions to the final manuscript. Harvey M. Cramer: Supervision of the findings of the work, discussion of the results, and contributions to the final manuscript. Howard H. Wu: Conception of the idea, performance of the primary investigation (ie, the slide review), curation of the data, performance of the formal statistical analysis, supervision of the findings of the work, discussion of the results, and contributions to the final manuscript.
REFERENCES
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68:7-30.
2. Amin MB, Amin MB, Tamboli P, et al. Prognostic impact of histologic subtyping of adult renal epithelial neoplasms: an experience of 405 cases. Am J Surg Pathol. 2002;26:281-291.
3. Moch H, Humphrey PA, Ulbright TM, Reuter VE, eds. WHO Classification of Tumours of the Urinary System and Male Genital Organs. Lyon, France: International Agency for Research on Cancer; 2016.
4. Alomari AK, Nettey OS, Singh D, Kluger H, Adeniran AJ. Clinicopathological and immunohistochemical characteristics of papillary renal cell carcinoma with emphasis on subtyping. Hum Pathol. 2015;46:1418-1426.
5. Delahunt B, Eble JN, McCredie MR, Berthwaite PB, Stewart JH, Bilous AM. Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patient survival in 66 cases. Hum Pathol. 2001;32:590-595.
6. Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol. 1997;10:537-544.
7. Linehan WM, Spellman PT, Ricketts CJ, et al; Cancer Genome Atlas Research Network. Comprehensive molecular characterization of papillary renal-cell carcinoma. N Engl J Med. 2016;374:135-145.
8. Antonelli A, Tardanico R, Balzarini P, et al. Cytogenetic features, clinical significance and prognostic impact of type 1 and type 2 papillary renal cell carcinoma. Cancer Genet Cytogenet. 2010;199:128-133.
9. Dekmezian R, Sneige N, Shabb N. Papillary renal-cell carcinoma: fine-needle aspiration of 15 cases. Diagn Cytopathol. 1991;7:198-203.
10. Granter SR, Perez-Atayde AR, Renshaw AA. Cytologic analysis of papillary renal cell carcinoma. Cancer. 1998;84:303-308.
11. Lew M, Foo WC, Roh MH. Diagnosis of metastatic renal cell carcinoma on fine-needle aspiration cytology. Arch Pathol Lab Med. 2014;138:1278-1285.
12. Lim JC, Wojcik EM. Fine-needle aspiration cytology of papillary renal cell carcinoma: the association with concomitant secondary malignancies. Diagn Cytopathol. 2006;34:797-800.
13. Masoom S, Venkataraman G, Jensen J, Flanagan RC, Wojcik EM. Renal FNA–based typing of renal masses remains a useful adjunctive modality: evaluation of 31 renal masses with correlative histology. Cytopathology. 2009;20:50-55.
14. Renshaw AA, Granter SR, Cibas ES. Fine-needle aspiration of the adult kidney. Cancer. 1997;81:71-88.
15. Truong LD, Todd TD, Dhurandhar B, Ramzy I. Fine-needle aspiration of renal masses in adults: analysis of results and diagnostic problems in 108 cases. Diagn Cytopathol. 1999;20:339-349.
16. Flint A, Coombe C. Cytologic diagnosis of the papillary variant of renal-cell carcinoma. Acta Cytol. 1987;31:325-329.
17. Delahunt B, Sika-Paotonu D, Berthwaite PB, et al. Grading of clear cell renal cell carcinoma should be based on nuclear prominence. Am J Surg Pathol. 2011;35:1134-1139.
18. Delahunt B, Cheville JC, Martignoni G, et al. The International Society of Urological Pathology (ISUP) grading system for renal cell carcinoma and other prognostic parameters. Am J Surg Pathol. 2013;37:1490-1504.
19. Eble JN, Sauter G, Epstein JL, Sesterhenn IA, eds. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon, France: International Agency for Research on Cancer; 2004.
20. Saleeb RM, Brimo F, Farag M, et al. Toward biological subtyping of papillary renal cell carcinoma with clinical implications through histologic, immunohistochemical, and molecular analysis. Am J Surg Pathol. 2017;41:1618-1629.