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Morphologic Spectrum of Renal Cell Carcinoma, Unclassified: An Analysis of 136 Cases

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Abstract

Aims: Renal cell carcinoma, unclassified (RCCU) is a category that includes a morphologically and biologically heterogeneous group of tumors that are unable to be diagnosed as other well-defined entities. We aim to describe the morphologic findings of tumors within this category and to determine the most frequent morphologic features leading to classification difficulty.

Methods and results: One hundred and thirty-six cases of RCCU were examined. Patients ranged in age from 23 to 87 years. Seventy-seven patients were men and 59 were women. International Society of Urological Pathology (ISUP) grade was most commonly 3 (n=66), followed by 2 (n=42) and 4 (n=28). Tumor size ranged from 0.6 cm to 24.9 cm. The AJCC pathologic T categories included pT1a (n=50), pT1b (n=14), pT2a (n=7), pT2b (n=4), pT3a (n=50), and pT4 (n=9). Forty-four cases included lymph node(s), of which 41% (n=18) had metastases. Tumors were assessed for a variety of histologic features and assigned to the following morphologic groups: predominantly oncocytoma/chromophobe RCC-like; clear cell RCC-like; papillary RCC-like; collecting duct-like; and pure sarcomatoid differentiation. The majority of the oncocytoma/chromophobe and clear cell RCC-like phenotypes were low stage (pT1 or pT2). The papillary RCC-like, collecting duct-like, and pure sarcomatoid phenotypes were mostly high stage (pT3 or pT4).

Conclusions: RCCU is a term that encompasses tumors with a variety of morphologic features and a wide biologic spectrum. The most common source of diagnostic difficulty was tumors composed of predominantly eosinophilic cells.

Introduction

Renal epithelial tumors that do not fit into any of the defined entities in the 2016 World Health Organization Classification are generally diagnosed as renal cell carcinoma, unclassified (RCCU). [1] This category may include tumors with unique morphologic patterns that fall outside of the known categories, have overlapping features of known subtypes, or are pure sarcomatoid tumors demonstrated to be of epithelial derivation. [1, 2] In practice, it can be difficult for the pathologist to decide exactly which or how many features do not allow a case to be assigned a more specific diagnosis. Adding to this dilemma, these tumors comprise a small portion of renal cell carcinomas (<5%) and occur in patients of all ages. Therefore, pathologists in any setting can encounter them, but individual experience with RCCU may be limited. [1-4]

Overall, in contemporary series, most renal cell carcinomas (RCC) are low stage (pT1 and pT2, 73.5% versus pT3 and pT4, 26.5%) and low nuclear grade (Fuhrman grade 1 or 2, 61% versus 3 or 4, 32%). [5] A number of studies addressing the prognosis of RCCU have reported that this diagnosis portends a worse outcome, giving the impression that these lesions are uniformly high grade and high stage. [6-10] The many unknowns surrounding tumors diagnosed as RCCU will likely be elucidated over time as distinct diagnostic entities emerge from this group. Only one comprehensive molecular study has been published to date in an attempt to further understand these lesions, and it underlined the heterogeneity of this category. The authors applied multiple molecular assays to 62 cases, detected recurrent somatic mutations in 29 genes, and outlined multiple different potential molecular subtypes (NF2 loss, mTORC1 hyperactive, *FH*-deficient, *ALK* translocation) with differing clinical outcomes. [11]

The purpose of this study is to describe the morphologic spectrum of RCCU in 136 cases diagnosed at our institution. We assigned each tumor to one of several morphologic groups in order to describe the main histopathologic features that present problems when attempting to classify primary renal epithelial tumors. We also report the basic epidemiologic data, AJCC pathologic TNM categories, and pertinent findings in the adjacent uninvolved kidney. We hope that this manuscript

further emphasizes the heterogeneity of this category.

Materials and Methods

The pathology database at our institution was queried for cases with a final diagnosis of "renal cell carcinoma, unclassified" or "renal cell carcinoma, not otherwise specified" between July 2007 and September 2016. Cases were included in this study from the queried time frame only if slides were available for re-review. Needle biopsy cases were excluded. A total of 136 cases were found that met these criteria. Most cases had been reported by (97%, 132/136), and all cases documented review by pathologists with subspecialty interest in urologic pathology. This study was approved by the Institutional Review Board.

Hematoxylin and eosin-stained slides were reviewed and the tumors were classified according to the 2016 World Health Organization classification system. [1] The 2010 American Joint Committee on Cancer (AJCC) tumor, lymph node, and metastasis (TNM) classification system was used for pathologic staging. [12] An International Society of Urological Pathology (ISUP) grade was assigned to each tumor. [13]

Pathology reports were reviewed and data was collected including patient age, sex, specimen type, and pathologic TNM stage. All available slides were reviewed and the presence-or absence of 36 different morphologic features was recorded for each case. In addition, the predominant morphologic pattern was noted for each case. These patterns included: predominantly oncocytoma/chromophobe RCC-like phenotype, predominantly papillary RCC-like phenotype, predominantly clear cell RCC-like phenotype, predominantly collecting duct-like phenotype, and pure sarcomatoid phenotype. The oncocytoma/chromophobe RCC-like category consisted of tumors that were mainly composed of sheets and/or nests of cells with eosinophilic cytoplasm, however focal papillary architecture was occasionally seen. The top differential diagnoses in this category were mainly eosinophilic chromophobe RCC versus oncocytoma. In the papillary RCC-like category the majority of neoplastic cells lined fibrovascular cores. The main differential diagnoses were papillary RCC versus

translocation-associated RCC. Tumors in the clear cell RCC-like category consisted mainly of cells with abundant, clear cytoplasm with a delicate, "chicken-wire" vasculature; focal granular, eosinophilic cytoplasm was permitted. The main differential diagnoses were clear cell RCC, translocation-associated RCC, and clear cell papillary RCC. Collecting duct-like phenotype included cases with high grade cytology, tubulopapillary architecture, and desmoplastic stroma. The main differential diagnoses were collecting duct carcinoma, medullary carcinoma, and urothelial carcinoma. For each of these 4 categories individual cases had one or more morphologic features that were considered sufficiently unusual as to exclude definitive classification. The pure sarcomatoid category consists of cells entirely composed of spindle-shaped cells without any epithelioid elements. Of note, an AJCC pathologic TNM stage could not be assigned to two tumors that were consults with only select slides available for re-review.

Results

Among the 136 cases included in this study, 66% (90/136) were from radical nephrectomy, 33% (45/136) were from partial nephrectomy, and 1% (1/136) was from a nephroureterectomy specimen. The majority of cases were resected at our institution (71%, 97/136), versus 27% (36/136) that were personal consultation cases.

Clinicopathologic Features

The 136 cases were from 136 different patients, 77 male and 59 female, who ranged in age from 23 to 87 years (median age: 61 years). The uninvolved kidney was unremarkable (n=103) or only had simple cortical cysts (n=21) in 91% (124/136) of cases. The uninvolved kidney had end-stage renal disease and/or acquired cystic kidney disease in 6% (8/136) of cases. A horseshoe kidney was present in one case. Other minor findings were noted, including infarct (n=3), ectopic adrenal gland (n=1), and a calcified fibrotic nodule (n=1).

The ISUP grade was 2 for 31% (42/136), 3 for 49% (66/136), and 4 for 21% (28/136) of tumors. The tumors ranged in size from 0.6 cm to 24.9 cm (median size: 5 cm). Most tumors were unifocal (90%, 123/136). A minority of tumors were multifocal (9%, 12/136), all of which had two or three separate foci of tumor. Focality wasn't specified in one consultation case with limited slides available for re-review. Sarcomatoid differentiation was present in 9% (12/136) of cases.

In addition to RCCU, between 1 and 5 other neoplastic lesions were seen in 15% (21/136) of cases. These were most frequently a papillary adenoma (n=9), followed by papillary RCC (n=5), clear cell RCC (n=3), angiomyolipoma (n=3), renomedullary interstitial cell tumor (n=3), chromophobe RCC (n=1), acquired cystic disease-associated RCC (n=1), multilocular cystic renal neoplasm of low malignant potential (n=1), and metanephric adenoma (n=1).

Among all specimens with a pathologic TNM stage assigned (n=134), the most common pT categories were pT3a (37%, 50/134) and pT1a (37%, 50/134) (**Table 1**). Overall, approximately half of the cases were organ confined (pT1a, pT1b, pT2a, or pT2b; 56%, 75/134), whereas the remaining 44% (50/134) were pT3a (n=41) or pT4 (n=9). Lymph node metastases were present in 41% (18/44) of cases with lymph nodes present. Among these cases, most were pT3a (61%, 11/18) or pT4 (33%, 6/18), and only 1 case was assigned a low pT category (pT1b). Distant metastases were documented in 4 cases, 3 of which were to the adrenal gland and one was to the liver.

Morphologic Features

For the entire group of tumors included in the study, the presence of eosinophilic cytoplasm in at least some tumor cells was the most commonly observed morphologic feature and was present in 83% (113/136) of cases (**Table 2, Figures 1 and 2**). Other common cytologic features included the presence of oncocytic cells (defined as polygonal cells with granular eosinophilic cytoplasm similar to those of renal oncocytoma; 24%, 32/136), large volume clear cytoplasm (21%, 28/136), prominent cell borders (17%, 23/136), and cytoplasmic vacuoles (15%, 20/136). Common architectural patterns observed included tubules (60%, 81/136), solid sheets (57%, 77/136), nests (39%, 53/136), papillary

structures (39%, 53/136), and cystic spaces (26%, 35/136). More cases had a circumscribed border (63%, 85/136) than an infiltrative one (29%, 40/136). There were 8 cases with two or more clearly defined morphologic areas that were abruptly juxtaposed, rather than intimately intermingled.

Each of the tumors was assigned to one morphologic category depending on the predominant pattern observed on re-review (**Table 3**). The most commonly assigned patterns were predominantly oncocytoma/chromophobe RCC-like phenotype (73%, 99/136), predominantly clear cell RCC-like phenotype (14%, 19/136), and predominantly papillary RCC-like phenotype (9%, 12/136) (**Figures 3, 4, and 5**). In reality there was a great deal of overlapping morphology in these tumors, therefore the purpose of assigning a predominant pattern was to determine if there was an overall trend showing which patterns were most challenging diagnostically. For example, tumors with features of translocation-associated RCC have both eosinophilic cytoplasm and papillary architecture and were mainly placed in the papillary RCC-like group.

Among cases with an oncocytoma/chromophobe RCC-like phenotype as their primary pattern, 31% (31/99) were consults, 68% (69/99) institutional cases, 36% (36/99) partial nephrectomies, and 64% (63/99) radical nephrectomies. ISUP grade was 2 in 34% (34/99) and 3 or 4 in 66% (65/99) of cases. AJCC pathologic TNM stage was not assigned to two cases, given that they were consults with only select slides available for re-review. AJCC pT category was pT1a in 39% (38/97), pT1b in 10% (10/97), pT2a in 6% (6/97), pT2b in 4% (4/97), pT3a in 36% (35/97), and pT4 in 4% (4/97) of cases. AJCC pN category was pN1 in 9% (9/97) of cases. Three cases were AJCC pM category pM1.

AJCC pT and pN Categories by Morphologic Subtype

Although the number of cases was too small to draw statistically significant conclusions, the

AJCC pT category did vary depending on the morphologic subtype assigned (**Figure 6**). However, given that outcome data was not available for many cases we are unable to further corroborate these findings. Pathologic category pT1 and pT2 tumors were considered low stage, whereas pT3 and pT4 tumors were considered high stage. When dividing cases into high and low stage, three main "types" of tumors emerged. The oncocytoma/chromophobe RCC-like phenotype was considered group I, the clear cell RCC-like phenotype group II, and papillary RCC-like/collecting duct/pure sarcomatoid tumors were group III. Groups I and II tumors both contained more low stage (58/99, 59% and 12/19, 63%, respectively) than high stage tumors. Overall, group III tumors were more commonly high stage (72%, 13/18). Specifically, the papillary RCC-like group contained more high stage (7/12, 58%) than low stage tumors, but this difference was minimal. The collecting duct-like and pure sarcomatoid phenotypes contained only high stage tumors (6/6, 100%).

Among tumors in the oncocytoma/chromophobe RCC-like phenotype that were pT3 (n=35), perinephric fat invasion was present in 69% (24/35), renal sinus fat invasion in 66% (23/35), and renal vein invasion in 54% (19/35).

Among tumors in the papillary RCC-like phenotype that were pT3 (n=4), perinephric fat invasion was present in 0% (0/4), renal sinus fat invasion in 100% (4/4), and renal vein invasion in 25% (1/4). Among tumors in the collecting duct-like phenotype that were pT3 (n=3), perinephric fat invasion was present in 100% (3/3), renal sinus fat invasion in 67% (2/3), and renal vein invasion in 33% (1/3). Among tumors in the clear cell-like phenotype that were pT3 (n=7), perinephric fat invasion was present in 43% (3/7), renal sinus fat invasion in 57% (4/7), and renal vein invasion in 71% (5/7).

The incidences of lymph node metastasis were 12%, 7% and 44%, respectively, in group 1, 2, and 3 RCCU (**Figure 7; Table 3**). Interestingly, none of these tumors with lymph node metastasis contained "oncocytic" cells.

Discussion

Renal cell carcinoma, unclassified is a diagnosis that is recommended for "tumours that do

not readily fit into any of the recognized subtypes of RCC" according to the 2016 World Health Organization Classification. [1] This category also includes "low-grade … unclassified oncocytic neoplasms" where the differential diagnosis would include oncocytoma, a benign entity. The growing awareness of emerging RCC subtypes and increasing utilization of immunohistochemical stains and ancillary studies may also contribute to an increasing use of this category in practice. Likewise, variability between pathologists and institutions regarding stringency of diagnostic criteria for each category of RCC may also play a role in the frequency that this category is assigned.

Very few studies have attempted to describe the morphologic features present in tumors diagnosed as RCCU. One study found that microvascular invasion, necrosis, tumor size, and histotype were independent predictors of disease-free survival on multivariate analysis. [14] Although this study defined "histotype" as either unrecognized cell type, a composite of recognized cell types, or pure sarcomatoid differentiation, the authors did not include further explanation of these categories or photomicrographs of the tumors. [14] Talento et al. described the ultrastructural findings in 10 cases of RCCU as supporting an epithelial and possible lower nephron origin; however a description of the findings of the hematoxylin and eosin-stained slides is limited to "unrecognizable cell types." [15]

Most studies have reported that the histologic subtype of RCC is an independent predictor of outcome, and the literature is dominated by studies which state RCCU has a poor prognosis compared to other RCC subtypes. [6-10, 16-18] Many of these studies did not have slides re-reviewed by a pathologist, had an absence of RCCU tumors that were low grade and low stage, and didn't exclude tumors with sarcomatoid differentiation (shown to portend a worse prognosis regardless of concomitant subtype of RCC). [19] In one such study, 71% (60/85) of the RCCU cases were high stage (pT3 or pT4) versus 38% (1624/4322) of clear cell RCC cases, and 80% (68/85) of RCCU cases were high Fuhrman nuclear grade (3 or 4) compared to 38% (1632/4322) of the clear cell RCC cases. [6] Another study matched RCCU cases with clear cell RCC cases stage for stage, but 100% (19/19) of RCCU cases versus 52% (148/256) of clear cell RCC cases were Fuhrman nuclear grade 3 or 4. [8] A third study did not have RCCU cases re-reviewed and therefore did not include Fuhrman nuclear grade, since this is only reported for clear cell RCC at their institution. [7] Recently, Kuthi et al. applied

the 2016 WHO renal tumor classification to 928 nephrectomies and reported that RCCU (n=28; 3%) had a lower 5 year cancer-specific survival rate compared to clear cell RCC (46% versus 83%). Most RCCU tumors were ISUP grade 3 or 4 (77%), had sarcomatoid/rhabdoid differentiation (41%), and were AJCC pT category pT3a or pT3b (68%). [20]

A study of 5,339 cases of RCC (clear cell, papillary, chromophobe, collecting duct, unclassified) from all patients undergoing radical or partial nephrectomy (1995-2007) at 16 different academic centers in Italy showed that most tumors were low stage and low grade (pT1 and pT2, 73.5% versus pT3 and pT4, 26.5%). [5] While these authors found that 73.5% of tumors were low stage, in the current series 56% (75/134) of RCCU tumors were low stage. Conversely, 26.5% of all tumors in the series were high stage, compared to 43% (59/136) in the current cohort of RCCU. Most tumors from this large overall RCC cohort (51%) were low nuclear grade, compared to only 31% in the current series (69% high nuclear grade). [5]

In our cohort, renal neoplasms predominantly composed of cells with eosinophilic cytoplasm posed the greatest diagnostic challenge. On re-review, cells with eosinophilic cytoplasm were present in 83% (113/136) of cases, and the oncocytoma/chromophobe RCC-like phenotype was the most commonly assigned primary pattern (73%, 99/136). Features of oncocytoma (oncocytic cells, 32%; loose hypocellular stroma, 28%; entrapped nonneoplastic renal tubules, 17%) and chromophobe RCC (prominent cell borders, 20%; perinuclear halos, 14%; thick-walled vessels, 7%) were commonly observed, evidence that this was a common diagnostic dilemma. However, some tumors with a minor papillary component were included in this group, which would almost by definition exclude the possibilities of oncocytoma or eosinophilic chromophobe. The distinction between low grade oncocytic tumors is challenging, as evidenced in a comprehensive survey detailing the differing opinions of expert urologic pathologists regarding the interpretation of a variety of morphologic and immunohistochemical features. [21] As oncocytoma is recognized as a benign entity, criteria for this diagnosis have generally been quite stringent and so it is likely that cases where the diagnosis is strongly favored nonetheless are not so designated. This has become a particularly challenging area in needle biopsy diagnosis. This category of tumors has prompted some pathologists to introduce

terminology in the literature such as “low-grade unclassified RCC” or “oncocytic neoplasm” preceded by a modifier such as “low-grade,” “borderline,” “oncocytic neoplasm, favor oncocytoma,” among others. [1, 21, 22] A significant proportion of tumors in the oncocytoma/chromophobe RCC-like category were high stage (40% pT3 or pT4), and a lower, but not negligible, number of these had lymph node metastases (**Figure 7**). In renal oncocytoma, fat invasion and involvement of the renal vein have been reported in a small subset of tumors (<5%). Several studies have shown that patients with such findings do not develop metastases or recurrence and that these tumors maintain their benign behavior, nonetheless it is likely that many such cases end up in an unclassified or uncertain category. [23-25] Likewise, other recent studies have shown that oncocytic tumors, including oncocytoma and chromophobe RCC, often have a less distinct tumor pseudocapsule, which may correlate with this predilection for oncocytic tumors to intermingle with structures such as perinephric fat or vessels. [26] Therefore, the prognosis of those tumors that are high stage due to fat or vessel invasion, yet without confirmed metastases, remains to be better understood, especially for oncocytic neoplasms.

The oncocytoma/chromophobe RCC-like category also included tumors that are frequently referred to in the literature as “hybrid” tumors due to overlapping features of renal oncocytoma and eosinophilic chromophobe renal cell carcinoma. Such tumors are most often described in the setting of renal oncocytosis and Birt-Hogg-Dubé syndrome but can also rarely occur sporadically. These are not recognized in the current WHO classification as a distinct tumor type and therefore are for the most part placed in the renal cell carcinoma, unclassified category. Some authors believe that in the setting of renal oncocytosis these represent a distinct tumor entity while others have hypothesized that such tumors are a manifestation of Birt-Hogg-Dubé syndrome. [27] The possibility of succinate dehydrogenase-deficient renal cell carcinoma also needs to be considered in these cases. The morphology of this tumor has only recently been described in some detail and up until recently most of these cases would likely have been placed in the RCCU category. [28, 29]

The differential diagnosis for the papillary RCC-like category included papillary RCC, translocation-associated RCC, clear cell papillary RCC, and hereditary leiomyomatosis and RCC. There were several cases which resembled clear cell papillary RCC with low nuclear grade, apically

oriented nuclei, and papillary structures, however complete absence of staining for keratin 7 precluded a definitive diagnosis. [30] While translocation-associated RCC can have a wide range of morphologic appearances, demonstration of compatible immunohistochemical findings or a known genetic aberration is necessary when assigning this diagnosis. Sometimes the lack of appropriate clinical history precluded a definitive diagnosis. For example, one case had tubulopapillary architecture and large nuclei with nuclear clearing and prominent eosinophilic nucleoli, features that raised the possibility of hereditary leiomyomatosis and RCC. However, a definitive diagnosis was not assigned given absence of pertinent clinical history or genetic studies. [31] In cases such as this, a diagnosis of RCCU was assigned and the possibility of a specific diagnosis was addressed in the "note" portion of the pathology report.

The differential diagnosis for cases included in the collecting duct-like category included collecting duct carcinoma, renal medullary carcinoma, urothelial carcinoma, and less commonly papillary RCC. All of the cases had high grade cytologic features and associated desmoplastic stroma. Urothelial carcinoma was excluded in all cases because of the absence of a urothelial component, however this distinction can be difficult if the urothelial component is only focal and because of overlapping immunohistochemical staining for PAX8 in the renal epithelium and upper tract urothelium. [32] In one case, areas which resembled classic collecting duct carcinoma composed the majority of the case, but were juxtaposed with a second area with papillary architecture, which brought up the possibility of hereditary leiomyomatosis and renal cell carcinoma-associated RCC. Furthermore, distinction between renal medullary carcinoma and collecting duct carcinoma can be challenging in the absence of pertinent history and immunohistochemical studies; young age, sickle cell hemoglobinopathy, positive staining for OCT4, and loss of INI1 expression would all support a diagnosis of renal medullary carcinoma. [33]

The morphologic categories did show trends related to AJCC pT and pN categories. We divided these categories into three types and found that the majority of cases in group I (oncocytoma/chromophobe RCC-like) and group II (clear cell RCC-like) were low stage (59% and 63%, respectively). In contrast, most cases in the papillary RCC-like category (58%) and all cases

within the collecting duct-like and pure sarcomatoid categories were high stage (pT3 or pT4), all of which were grouped into group III. While the number of cases is too small to draw definitive conclusions regarding prognosis, it is clear that RCCU is not uniformly high stage. The prognostic significance of these categories may also vary by institution, depending on how stringently the morphologic and immunohistochemical criteria for each subtype of RCC are applied.

We noticed a marked degree of variability in the work-up of tumors diagnosed as RCCU. First and foremost, the cases included in this study were from an approximately 9-year period (2009 to 2016). Therefore, many recently described entities (i.e. succinate dehydrogenase-deficient RCC, eosinophilic, solid, and cystic RCC, hereditary leiomyomatosis and RCC, etc.) were not yet reported. Furthermore, many immunohistochemical stains and FISH studies were not developed and/or were not widely available for clinical use. We also partly attributed the variation in work-up to differing preferences between attending pathologists. Given the number of cases and widely variable array of approaches, we consider quantification of the number of immunohistochemical stains and fluorescence in situ hybridization (FISH) studies performed to be misleading. A truly comprehensive and meaningful assessment of each case would require application of a panel of immunohistochemical markers and FISH studies to every case. Furthermore, we acknowledge that given the circumstances just described, there certainly are cases in this group of tumors that may be able to be further classified if additional work-up could be performed today.

Overall, the variable initial work-up of the cases included in this study argues that a standard approach to these challenging tumors could be useful. In one study of 63 metastases of RCC without an identifiable subtype, 51% (32/63) of cases were able to be classified after the application of a simple panel of immunohistochemical stains (keratin 7, AMACR) and FISH assays (chromosome 3p deletion, trisomy of chromosomes 7 and/or 17). [34] The ideal panel would be an algorithm dependent on the key morphologic features of the tumor, and perhaps multiple standardized approaches could be developed in the future depending on the predominant morphologic category present in a tumor. The "best practices recommendations" from the International Society of Urologic Pathology provide an excellent starting point in their discussion of immunohistochemistry to aid in the classification of

renal tumors since they list recommended stains according to the morphologic pattern. [35] Incorporation of recently characterized immunohistochemical stains (i.e. TFE3, SDHB, fumarate hydratase, 2SC) and molecular assays (i.e. TFE3 FISH) could be useful updates for the future.

Additional studies regarding the morphologic and genetic diversity of the category RCCU and the clinical applications of these findings are needed. Despite not being able to assign these tumors a specific diagnosis, our study highlights the potential of gleaning prognostic information from morphologic patterns. Immunohistochemistry may provide additional prognostic information, as one study reported that RCCU tumors that were positive for TFE3 had a worse 5-year cancer-specific survival (15.6% versus 87.5%). [36] Targetable mutations with directed treatment are continually being discovered, so despite the lack of a specific diagnosis, patients may have viable treatment options. [37-39] Although most trials focus on clear cell RCC, a few have demonstrated that many of the same drugs may still have clinical benefit in non-clear cell RCC to a lesser or unknown degree. [40-44]

The pathogenesis of tumors diagnosed as RCCU is largely unknown. Future studies will likely continue to elucidate the molecular aberrations present in these tumors. Much like in a variety of renal and non-renal primary neoplasms, regardless of diagnosis mutations may serve as prognostic markers or targets for treatment. [38, 39, 45, 46] The only in-depth molecular study of RCCU performed to date described several molecular subtypes (NF2 loss, mTORC1 hyperactive, FH-deficient, *ALK* translocation) with differential clinical outcomes, however the number of cases was small. [11] Another applied comparative genomic hybridization (CGH) and whole exome sequencing to two cases of RCCU with undifferentiated, multinucleated giant cells with eosinophilic cytoplasm. [47] The CGH profiles included 17 gains and 40 losses and whole exome sequencing with Exon BeadChip showed significant differences in the mutational status of 40 genes, implicating a slew of possible biologic pathways. [47] A third manuscript performed single nucleotide polymorphism (SNP) arrays on 21 "morphologically challenging" tumors and were able to assign a "molecular diagnosis" to each case. [48]

Overall, the detailed morphologic features present in this series of RCCU documents the wide range of appearances of these tumors and underlines that this category may be assigned to a case for a variety of reasons (overlapping morphology, ancillary studies, clinical history). This category of tumors is remarkably heterogeneous in its histopathologic findings, nuclear grade, and AJCC TNM stage. Despite the fact that prior publications addressing clinical outcome have been heavily weighted towards high grade, high stage tumors, we found that over half of the tumors in our cohort (56%) were AJCC pT category 1 or 2 and 31% were ISUP grade 2. The most common source of diagnostic difficulty in our cohort was tumors composed of predominantly eosinophilic cells. This manuscript underlines the marked heterogeneity of the category of RCCU by describing the wide variety of morphologic appearances this lesion may have and by showing that these tumors can be high or low grade/stage.

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Figure Legends

Figure 1: Morphologic Features. A. Neoplastic cells with eosinophilic cytoplasm forming tubules with intraluminal blue mucin. B. Psammomatous calcifications within a tumor with papillary architecture. C. Neoplastic cells with eosinophilic cytoplasm on a background of loose, edematous stroma, resembling that seen in an oncocytoma. D. Bands of smooth muscle transecting a tumor composed of cells with clear to eosinophilic cytoplasm. E. Rhabdoid cells with eccentric nuclei and abundant, eosinophilic cytoplasm. F. A tumor with predominant tubulopapillary architecture.

Figure 2: Additional Morphologic Features. A. Neoplastic cells with multinucleated tumor cells and abundant, eosinophilic cytoplasm. B. Cells with abundant clear cytoplasm and intracytoplasmic hyaline globules. C. Abundant foamy macrophages within the stalks of several papillary structures. D. Neoplastic cells lining papillary structures with eosinophilic cytoplasm and punched-out, sieve-like spaces.

Figure 3: Oncotyoma/Chromophobe RCC-Like Phenotype. A and B. Large, polygonal cells with prominent nucleoli and eosinophilic cytoplasm, arranged in sheets and tubules. Large, thick-walled vessels are present (lower right). C. Cells with small to medium, hyperchromatic nuclei, including binucleate forms, and eosinophilic cytoplasm. The cells have perinuclear halos and prominent cells borders. D. Large, polygonal cells with hyperchromatic, wrinkled nuclei, some binucleate forms, and abundant eosinophilic cytoplasm, arranged in sheets.

Figure 4: Clear Cell RCC-Like Phenotype. A and B. Cells with clear cytoplasm arranged in tubules with an intervening delicate, "chicken-wire" vascular network. C and D. A tumor composed of cells with small nuclei and abundant clear cytoplasm, arranged in two distinct architectural patterns. The papillary pattern (left) resembles clear cell papillary RCC, while the solid sheets (right) resemble

conventional clear cell RCC.

Figure 5: Papillary RCC-Like, Collecting Duct-Like, and Pure Sarcomatoid Phenotypes

A and B. Papillary RCC-like phenotype, with thick fibrovascular cores lined by cells with eosinophilic cytoplasm. C and D. Collecting duct-like phenotype, with tubules lined by cells with high grade nuclear features on a background of desmoplastic stroma. E and F. Pure sarcomatoid phenotype, composed of spindled cells resembling a sarcoma.

Figure 6: Distribution of AJCC pT Category According within each Morphologic Group.

Oncocytoma/chromophobe RCC-like and clear cell RCC-like tumors are predominantly low stage (pT1 or pT2). In contrast, papillary RCC-like, collecting duct RCC-like, and pure sarcomatoid tumors are predominantly high stage (pT3 or pT4).

Figure 7: Distribution of AJCC pN Category within each Morphologic Group.

A small proportion of oncocytoma/chromophobe RCC-like (9%, 9/99) and clear cell RCC-like (5%, 1/19) cases had lymph node metastases. In contrast, 44% (8/18) of papillary RCC-like, collecting duct RCC-like, and pure sarcomatoid cases had lymph node metastases.

Tables

Table 1. Clinicopathology Features of Renal Cell Carcinoma, Unclassified (n=136)

Characteristics	No. of Patients (% of total cases)
Gender	
Male	77 (57)
Female	59 (43)
Age (years)	
<50	27 (20)
50-59	36 (26)
60-70	37 (28)
>70	36 (26)
Size (cm)	
≤4 cm	58 (43)
>4 to ≤7 cm	30 (22)
>7 to ≤10 cm	18 (14)
>10 cm	28 (21)
ISUP Nuclear Grade	
1	0 (0)
2	42 (31)
3	66 (49)
4	28 (20)
Pathologic Stage	
T Category	
pT1a	50 (37)
pT1b	14 (11)
pT2a	7 (5)
pT2b	4 (3)
pT3a	50 (37)
pT3b	0 (0)
pT4	9 (7)
N Category	
pN1	18 (13)

*Size and AJCC TNM stage were not available in two cases

Table 2: Morphologic Features of Renal Cell Carcinoma, Unclassified

Morphologic Features	All Tumors n (%)	Eosinophilic Tumors n (%)
Total	136	99
Architectural Patterns		
Tubules	81 (60)	61 (62)
Sheets/solid	77 (57)	55 (56)
Nests	53 (39)	49 (60)
Papillary	53 (39)	28 (29)
Cystic	35 (26)	29 (30)
Sieve-like spaces	5 (4)	5 (5)
Micropapillary	1 (1)	1 (1)
Cytologic Features		
Eosinophilic cytoplasm	113 (83)	99 (100)
Oncocytic	32 (24)	32 (32)
Clear cytoplasm, high volume	28 (21)	10 (10)
Prominent cell borders	23 (17)	20 (20)
Cytoplasmic vacuoles	20 (15)	15 (15)
Perinuclear haloes	14 (10)	14 (14)
Multinucleated tumor cells	12 (9)	12 (12)
Rhabdoid cells	12 (9)	9 (9)
Clear cytoplasm, low volume	10 (7)	3 (3)
Sarcomatoid tumor cells	10 (7)	5 (5)
Cyst lined by hobnail cells	8 (6)	7 (7)
Spindled tumor cells (not sarcomatoid)	6 (4)	2 (2)
Cytoplasmic hyaline globules	5 (4)	3 (3)
Other Features		
Circumscribed border	85 (63)	70 (71)
Hemorrhage	79 (58)	54 (55)
Pigment	45 (33)	33 (33)
Infiltrative border	40 (29)	24 (24)
Loose, hypocellular stroma	31 (23)	28 (28)
Delicate vessels	29 (21)	17 (17)
Necrosis	28 (21)	14 (14)
Dystrophic calcifications	20 (15)	17 (17)
Entrapped nonneoplastic renal tubules	20 (15)	17 (17)
Psammomatous calcifications	11 (8)	8 (8)
Fibrous bands	11 (8)	7 (7)
Fibrous capsule/pseudocapsule	10 (7)	7 (7)
Desmoplastic stroma	9 (7)	3 (3)
Foamy macrophages	7 (5)	6 (6)
Thick-walled vessels	7 (5)	7 (7)
Mucin	3 (2)	1 (1)

Table 3. Morphologic Categories Assigned to Renal Cell Carcinoma, Unclassified (n=136)

Category	No. of Patients (% of total cases)	Pathologic Stage*				Lymph Node Metastasis (% of total cases)
		pT1	pT2	pT3	pT4	
Group 1						
Predominantly oncocytoma/chromophobe RCC-like phenotype	99 (73)	48	10	35	4	9 (12%)
Group 2						
Predominantly clear cell RCC-like phenotype	19 (14)	11	1	7	0	1 (7%)
Group 3	18 (13)	5	0	9	7	8 (44%)
Predominantly papillary RCC-like phenotype	12 (9)	0	0	4	3	
Predominantly collecting duct- like phenotype	4 (3)	5	0	4	3	
Pure sarcomatoid phenotype	2 (1)	0	0	1	1	

*AJCC TNM stage was not available in two cases.