Hereditary leiomyomatosis and Renal Cell Carcinoma Syndrome (HLRCC)

A Contemporary Review and Practical Discussion of the Differential Diagnosis for HLRCC-Associated Renal Cell Carcinoma

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Context.—Hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC), also referred to as Reed syndrome,1–4 is a rare familial cancer disorder caused by a germline mutation in the fumarate hydratase (FH) gene. The kidney cancers that develop in patients with HLRCC are often unilateral and solitary, with a potentially aggressive clinical course; morphologic identification of suspicious cases is of the utmost importance.

Objective.—To review classic morphologic features of HLRCC-associated renal cell carcinoma, the reported morphologic spectrum of these tumors and their mimics, and the evidence for use of immunohistochemistry and molecular testing in diagnosis of these tumors.

Hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC), also referred to as Reed syndrome,1–4 is a rare familial cancer disorder caused by a germline mutation in the fumarate hydratase (FH) gene (Figure 1). In contrast to most other types of hereditary renal cell tumors, HLRCC-associated renal cell carcinoma (RCC) is often unilateral and solitary.1 It is crucial to recognize the importance of renal masses in patients with HLRCC because a significant proportion of lower-stage tumors may metastasize.5,6 Previous reports indicate that many patients die within 5 years of diagnosis.7 Approximately 20% to 35% of patients with germline FH mutations develop RCC.8,9 Given the highly aggressive nature of these tumors, annual surveillance in patients with known HLRCC should begin at age 10 years,2,10,11 and surgical removal of even small renal tumors is recommended.5,12 There is not yet a standard therapy for patients with metastatic RCC associated with germline FH mutations. Patients may be treated with tyrosine kinase inhibitors or mechanistic target of rapamycin (mTOR) inhibitors.13 Yamasaki et al14 reported a case in which treatment with 2-deoxy-D-glucose was administered as a glycolysis inhibitor; however, the treatment was unsuccessful. Only a subset of these tumors has been determined to be positive for programmed death ligand 1 (PD-L1) by polymerase chain reaction and/or immunohistochemistry; targeted immunotherapy may be helpful in such occasional cases.15 The FH-deficient tumor growth has been documented to be reduced in mouse models via lactate dehydrogenase-A inhibition.16 Current phase II clinical trials include vandetanib (kinase inhibitor with activity against vascular endothelial growth factor receptor [VEGFR], epidermal growth factor receptor [EGFR], and RET-tyrosine kinase) in combination with metformin (http://clinicaltrials.gov NCT02495103), guadecitabine (SGI-110, DNA methyl transferase inhibitor) (http://clinicaltrials.gov NCT03165721), and bevacizumab (VEGF inhibitor) and erlotinib (tyrosine kinase inhibitor with activity against EGFR) (http://clinicaltrials.gov NCT01130519); the results from those studies may influence management of HLRCC-associated RCC in the future.

The extrarenal manifestations of HLRCC tend to present clinically in patients younger (during their third decade) than those with HLRCC-associated RCC.7,8 These manifestations include leiomyoma of the skin (Figure 2, A and B) and uterus. The presence of multiple cutaneous leiomyomas is considered a sufficient cause to perform germline molecular testing in diagnosis of these tumors.

Data Sources.—University of Michigan cases and review of pertinent literature about HLRCC and the morphologic spectrum of HLRCC-associated renal cell carcinoma.

Conclusions.—Histologic features, such as prominent nucleoli with perinucleolar halos and multiple architectural patterns within one tumor, are suggestive of HLRCC-associated renal cell carcinoma. However, the morphologic spectrum is broad. Appropriate use of FH immunohistochemistry and referral to genetic counseling is important for detection of this syndrome.

testing for FH mutations.\textsuperscript{17,18} Cutaneous leiomyomata also need to be followed because of the possibility of the tumor transforming into leiomyosarcoma.\textsuperscript{19,20} Although uterine leiomyomata are fairly common in the general population, patients with HLRCC tend to have hysterectomies for symptomatic leiomyomata approximately 10 years (often by the age of 30 years) earlier than other women have them.\textsuperscript{7,21}

Uterine leiomyomata are a highly penetrant feature of HLRCC and may show nuclear features similar to those seen in HLRCC-associated RCC (Figure 2, C and D), as well as increased cellularity, nuclear pleomorphism, and the presence of eosinophilic globular cytoplasmic inclusions.\textsuperscript{22–25} There have been reports of the development of uterine leiomyosarcoma in patients with HLRCC at a younger age.\textsuperscript{26} Various adrenal manifestations have been reported in such patients, including adrenal cortical hyperplasia,\textsuperscript{27–29} pheochromocytoma,\textsuperscript{30} and very rarely, adrenal cortical carcinoma.\textsuperscript{31} Testicular Leydig cell tumors have been reported in patients with HLRCC,\textsuperscript{32} although the specificity of that association needs confirmation by additional studies.

The clinical features currently considered to be definitional for HLRCC include either the presence of multiple biopsy-proven cutaneous piloleiomyomas or the presence of 2 of the following minor criteria: (1) surgical treatment for symptomatic uterine leiomyomata before age 40 years, (2) type 2 papillary RCC before age 40 years, or (3) first-degree family member who meets 1 of these criteria.\textsuperscript{15} Germline mutational testing for FH is necessary for confirmation of HLRCC in a proband or suspected family.

MORPHOLOGICAL FEATURES OF HLRCC-ASSOCIATED RCC

The HLRCC-associated RCC was originally described as papillary RCC or, less commonly, collecting duct carcinoma (CDC).\textsuperscript{7,8,20,29,33} The initial series\textsuperscript{8} of 4 high-grade kidney tumors in a family with HLRCC demonstrated areas of papillary architecture, abundant cytoplasm, and large nuclei with inclusion-like eosinophilic nucleoli. One tumor had admixed tubulopapillary architecture (similar to that shown in Figure 2, E), and another had admixed solid, cystic, and sarcomatoid areas. It was later proposed that a notable morphologic feature of these tumors was the large nucleus with a prominent inclusion-like nucleolus and perinucleolar halo (Figure 2, F).\textsuperscript{34} Although helpful, those nuclear features may be present only in scattered cells in some cases (Figure 3, A and B). In a more recent series of renal tumors from 9 patients with germline FH mutations, papillary architecture predominated in only one-third of cases.\textsuperscript{35} Fibrovascular cores in these tumors were often edematous or hyalinized, and micropapillary structures were often present. All tumors showed mixed architectural patterns, including tubulopapillary, solid (Figure 3, C and D), and cystic (often with intracyctic papillary/tubulopapillary structures). Morphologic overlap with collecting duct carcinoma was common (Figure 3, E and F), with infiltrative carcinoma and inflammation involving desmoplastic stroma. In tubulocystic regions of HLRCC-associated RCC, prominent nucleoli were sometimes seen. Sarcomatoid growth has been described in at least 2 HLRCC-associated RCCs. The widely metastatic HLRCC-associated RCC previously described in a “rapid autopsy” case report\textsuperscript{36} from our institution showed the classic nuclear features even in the sarcomatoid and rhabdoid components (Figure 4, A through D). A large subset of RCC cases with tubulocystic and associated dedifferentiated collecting duct carcinoma-like areas (“tubulocystic carcinoma with poorly differentiated foci,” similar to those shown in Figure 5, A and B) have been demonstrated to show somatic FH deficiency, and some cases have been confirmed to be associated with HLRCC.\textsuperscript{37,38} A low-grade oncocytic variant of HLRCC-associated RCC with morphologic resemblance to succinate dehydrogenase (SDH)-deficient RCC (similar to that shown in Figure 5, C and D) has been recently reported.\textsuperscript{39} Rarely, HLRCC-associated RCCs may be entirely cystic (Figure 5, E and F). A recent study comparing 24 renal tumors from known carriers of the FH mutation to 12 type 2 papillary RCCs from patients with wild-type FH demonstrated that a multiplicity of architectural patterns (including papillary, tubulopapillary, tubulocystic, sarcomatoid, and rhabdoid) within the same tumor was more specific for HLRCC-associated RCC than was the presence of prominent nucleoli with perinucleolar halos.\textsuperscript{40} Selected entities in the differential diagnosis for HLRCC-associated RCC are presented in the Table. Based on the current literature, the uninvolved kidney in patients with HLRCC may show cysts lined by eosinophilic to clear epithelium with somewhat similar nuclear features.\textsuperscript{2,40,41}

Overall, HLRCC-associated RCC is relatively enriched for renal tumors, which morphologically demonstrate a mixture of different growth patterns within the same tumor, including tubulopapillary, cystic, and/or solid areas, as well as those with sarcomatoid and/or poorly differentiated components; such tumors, when encountered within

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**Figure 1.** Germline fumarate hydratase mutations. Schematic representation of fumarate hydratase (FH) protein, indicating its functional Pfam (protein families) Database (European Molecular Biology Laboratory, Heidelberg, Germany) domains lyase (green) and fumarase (Fum; red). The FH germline mutation positions and types reported in ClinVar (National Center for Biotechnology Information, Bethesda, Maryland) as pathogenic or likely pathogenic are indicated as lollipops. Of the 46 mutation calls, 18 are nonsense mutations (black) and 28 are missense events (purple). Schematic representation was generated with the mutation mapper tool in cBioPortal (cBioPortal for Cancer Genomics, http://www.cbioportal.org).
Figure 2. Manifestations of hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC). The cutaneous leiomyomata (A) seen in patients with HLRCC usually do not show distinct morphologic features but often demonstrate loss of fumarate hydratase (FH) expression by immunohistochemistry (B). The epidermis and adnexal structures serve as positive internal controls. C, Uterine leiomyomata in HLRCC may show atypical features and often show nuclear features reminiscent of HLRCC-associated renal cell carcinoma (RCC), although usually not as well developed as those depicted here. D, The FH expression is lost in neoplastic cells. E, The HLRCC-associated RCC often show papillary and tubular growth patterns. F, Classically, HLRCC-associated RCC show foci with prominent inclusion-like nucleoli and perinucleolar halos (hematoxylin-eosin, original magnifications ×100 [A and E], ×200 [F], and ×400 [C and F-inset]; original magnifications ×100 [B] and ×400 [D]).
Figure 3. Morphologic spectrum of hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)–associated renal cell carcinoma (RCC). A and B, In some cases of HLRCC-associated RCC, the nuclear features are poorly developed. C, Some cases of HLRCC-associated RCC show papillary and solid growth patterns reminiscent of solid papillary RCC. D, This example shows the prominent inclusion-like nucleoli with prominent perinucleolar halos that are classic for HLRCC-associated RCC. In addition, HLRCC-associated RCC may mimic collecting duct carcinoma with neoplastic tubules demonstrating an infiltrative growth pattern (E); however, fumarate hydratase expression is lost in HLRCC-associated RCC (F) but retained in healthy kidney (F, inset) (hematoxylin-eosin, original magnifications ×200 [A and E], ×400 [B and D], and ×100 [C]; fumarate hydratase, original magnifications ×400 [F] and ×200 [F, inset]).
Immunohistochemical Evaluation/Markers

The 2 immunohistochemical biomarkers that show a high correlation with the diagnosis of HLRCC-associated RCC are fumarate hydratase (FH) and S-(2-succino)-cysteine (2SC). Inactivating mutations in the FH gene lead to loss of fumarate hydratase expression within tumor cells. Cytoplasmic and granular (mitochondrial) expression is considered a positive result; neoplastic cells can be considered to have absent FH expression if there is an appropriate positive internal control in blood vessels, inflammatory cells, or other nonneoplastic cells. The high level of fumarate in tumor cells leads to aberrant succination of cellular proteins, which is a stable chemical modification that can be detected with the 2SC antibody (available only in a research setting at the time of this publication). Neoplastic cells that stain positively for 2SC expression generally show strong cytoplasmic and nuclear expression, with negative staining in the background healthy renal parenchyma. A FH/2SC immunophenotype in a renal tumor morphologically suspicious for HLRCC-associated RCC should be regarded as a strong trigger to perform further clinical workup and germline mutational testing in an index patient without a previously established diagnosis of HLRCC. Cytokeratin 7 (CK7) and Ulex europaeus agglutinin-1 are generally reported to be negative in HLRCC-associated RCC, but these observations are considered to be relatively nonspecific.

Although a renal tumor with FH/2SC immunophenotype carries a strong correlation with the presence of the FH mutation at the germline level, the type of FH mutation itself may determine whether FH protein loss can be detected by immunohistochemical evaluation. A small subset of patients with HLRCC-associated RCC may demonstrate equivocal results or retain FH expression within the tumor; a correlated finding reported in the literature is that tumors from patients with FH missense mutations may show...
Figure 5. Morphologic spectrum of hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)-associated renal cell carcinoma (RCC). A. The HLRCC-associated RCC may show prominent tubulocystic and microcystic growth pattern, with poorly differentiated areas (red arrow). Although there is some morphologic resemblance to tubulocystic carcinoma in focal areas, presence of poorly differentiated foci (B) should prompt consideration of HLRCC and evaluation of fumarate hydratase (FH) status (B-inset, loss of FH expression by IHC). Rare cases of HLRCC-associated RCC have low-grade oncocytic morphology (C) resembling succinate dehydrogenase-deficient RCC; however, FH expression is lost in such HLRCC-associated RCC (D). The HLRCC-associated RCC may have an entirely cystic architecture (E) with patchy prominent nucleoli (F) and loss of FH expression in the neoplastic cells (F-inset) (hematoxylin-eosin, original magnifications ×20 [A], ×200 [B and C], ×10 [E], and ×400 [F]; FH, original magnifications ×200 [B-inset and D] and ×400 [F-inset]).
equivocal or retained FH expression because of alteration in protein-antibody interactions.25,43

Immunohistochemical evaluation of FH and 2SC on cutaneous or uterine leiomyoma specimens may be helpful in identifying patients who should undergo further clinical workup or germline mutation testing for HLRCC.23,38,43–45 Another group has suggested molecular screening of uterine leiomyoma specimens for FH mutations in patients with a hereditary predisposition.44 Although absent FH expression aids in the detection of patients with HLRCC, uterine leiomyomata may be FH deficient in both syndromic and sporadic settings.47

Molecular Underpinnings of HLRCC

The autosomal-dominant HLRCC syndrome has germ-line-inactivating mutations in the FH gene (1q42.3–q43).3,7,29,46 The loss of FH function leads to increased levels of intracellular fumarate, which is considered an oncometabolite and has been shown to mediate various proteomic and epigenetic events. Competitive inhibition of prolyl-hydroxylase domain-containing proteins ultimately affects the stability of proteins such as the transcription factor hypoxia-induced factor 1 (HIF1).49,50 Such events lead to downregulation of several proteins, including KEAP1, a component of the cullin 3 E3 ubiquitin ligase, thereby disrupting its regulation of nuclear factor erythroid 2-related factor 2 (NRF2). NRF2 is a key regulator of antioxidant response genes, such as AKR1B10, whose overexpression has been previously shown in HLRCC.31–50 Competitive inhibition of multiple α-ketoglutarate–dependent dioxygenases, including histone demethylases, and the TET family of 5-methylcytosine hydroxylases results in decreased histone and DNA demethylation.57 Inhibition of TET has been attributed to the DNA-hypermethylation phenotype of HLRCC. The Cancer Genome Atlas RCC cases with hypermethylation included a subset with loss of FH expression.58 Although molecular aberrations in HLRCC

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<tr>
<td>HLRCC-associated RCC</td>
<td>Broad spectrum, often with multiple architectural patterns within the same tumor; classic nuclear features; prominent inclusion-like nuclei with perinucleolar halos</td>
<td>Absent FH expression; positive for 2SC and negative for CK7 and AMACR expression</td>
<td>Germline FH testing</td>
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<tr>
<td>Papillary RCC, type 2</td>
<td>Papillary architecture with pseudostratified high-grade epithelium; abundant eosinophilic cytoplasm and nuclear anaplasia</td>
<td>Positive for PAX8, pancytokeratin, CK7, EMA, and AMACR; FH retained</td>
<td>Chromosome 7/17 FISH</td>
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<td>TFE3-translocation RCC</td>
<td>Broad spectrum, but classically papillary with nested architecture and high-grade cells with dual (eosinophilic and clear) cytoplasmic tones and psammoma bodies</td>
<td>May or may not show decreased/absent pancytokeratin expression; FH retained</td>
<td>TFE3 FISH</td>
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<td>TFEB-amplified RCC</td>
<td>Papillary architecture with high-grade oncocytic cells</td>
<td>Often positive for Melan-A and pancytokeratin expression; FH retained</td>
<td>TFEB FISH</td>
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<td>Collecting duct carcinoma</td>
<td>Infiltrative growth pattern with high-grade cells, predominant tubular morphology, desmoplastic stromal reaction, and medullary involvement</td>
<td>Positive for CK7 expression (relatively nonspecific immunophenotype); FH retained</td>
<td>None</td>
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<tr>
<td>“Pure” tubulocystic carcinoma</td>
<td>Small- to intermediate-sized, sometimes dilated, tubules with a single layer of flat, hobnail, cuboidal, or columnar epithelium and prominent nuclei</td>
<td>Positive for CK7 and AMACR; FH retained</td>
<td>None</td>
</tr>
<tr>
<td>Tubulocystic carcinoma with poorly differentiated foci</td>
<td>Same as “pure” type above, with the addition of infiltrative adenocarcinoma or collecting duct-type features</td>
<td>Positive for CK7 and AMACR; FH often lost (with some cases representing HLRCC-associated RCC)</td>
<td>None</td>
</tr>
<tr>
<td>SDH-deficient RCC</td>
<td>Nested to solid architecture, low-grade cells with monotonous nuclei, fine chromatin, and flocculent/vacuolated eosinophilic cytoplasm with occasional inclusions</td>
<td>Absent SDH subunit B expression; FH retained</td>
<td>Germline SDH testing</td>
</tr>
<tr>
<td>Clear cell RCC</td>
<td>Nested, with or without pseudopapillary architecture; cells with clear cytoplasm and delicate (“racemose”) vascular network; high-grade areas often show eosinophilic cytoplasm</td>
<td>Positive for PAX8, pancytokeratin, EMA, and CAIX (diffuse strong membranous expression); negative for CK7; FH retained</td>
<td>3p25 FISH</td>
</tr>
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Abbreviations: 2SC, 5-(2-succino)-cysteine; AMACR, α-methylacyl-coenzyme A racemase; CAIX, carbonic anhydrase IX; CK7, cytokeratin 7; EMA, epithelial membrane antigen; FH, fumarate hydratase; FISH, fluorescence in situ hybridization; PAX8, paired box gene 8; SDH, succinate dehydrogenase.
continue to unfold, methods for translating those findings to the clinical practice still need to be determined. Although immunohistochemical assessment can be helpful in classifying RCC, FH-deficient RCC is rarely seen sporadically. Definitive confirmation of HLRCC can be achieved by testing the patient for a germline FH mutation.

Differential Diagnosis

Type 2 Papillary RCC

Many examples of HLRCC-associated RCC demonstrate enrichment for papillary architecture; therefore, HLRCC-associated RCC was frequently regarded as type 2 papillary RCC in the past.34,59 Type 2 papillary RCC is characterized by pseudostratified high-grade epithelium with abundant eosinophilic cytoplasm and nuclear anaplasia (Figure 6, A and B). Up to 58% of type 2 papillary RCC may have prominent nuclei with perinuclear halos.40 Papillary architecture is the dominant pattern in papillary RCC, although tubular and solid architecture are not uncommon and glomerulations may be seen in approximately 20% of cases, especially common with type 2 papillary architecture.50 Papillary RCC often has a well-defined tumor capsule and lacks extensive infiltration. There is clear prognostic importance in accurately classifying these tumors because papillary RCC is generally considered to have a more-favorable prognosis than clear cell RCC or HLRCC-associated RCC has, and metastatic disease is fairly uncommon at presentation in papillary RCC.63–64

Although HLRCC-associated RCC and type 2 papillary RCC demonstrate extensive morphologic overlap, some features help discriminate between those 2 entities. The HLRCC-associated RCC, when compared with papillary RCC, more commonly shows a variety or a mixture of diverse architectural patterns within the same tumor.40 In contrast to papillary RCC, HLRCC-associated RCC is markedly infiltrative and lacks the relative circumscription generally associated with true papillary RCC. Immunohistochemical evaluation can aid in the distinction as well. Papillary RCC is usually stains diffusely positive for CK7 and AMACR expression, whereas those markers are commonly negative in HLRCC-associated RCC. Sporadic papillary RCC often demonstrates trisomy of chromosomes 7 and 17, with loss of chromosome Y. Hereditary papillary renal carcinoma syndrome–associated cases, as well as a minor subset of sporadic papillary RCC, have been demonstrated to harbor activating mutations of the MET oncogene.68 Although the hypoxia pathway–associated genes can be somewhat turned on and overexpressed in HLRCC-associated RCC (see the narrative above in the molecular section), HLRCC-associated RCC in general shows limited to negative carbonic anhydrase IX expression. However, significantly, in contrast to papillary RCC, HLRCC-associated RCC demonstrates a loss of FH expression by IHC in most cases.

Melanogenesis-Associated Transcription Factor Family

Aberration–Associated RCC

Similar to HLRCC–associated RCC, both TFE3-translocation RCC and TFE3-amplified RCC tend to be high-grade tumors with mixtures of architectural patterns, including papillary. The classic appearance of TFE3-translocation RCC is a tumor with a papillary architecture and cells with voluminous clear to eosinophilic cytoplasm and numerous psammoma bodies (Figure 6, O).58,67 Much like HLRCC-associated RCC, TFE3-translocation RCC has been reported to have morphologic features overlapping with several other RCC subtypes.58–73 High-grade RCCs, which demonstrate subnuclear clearing, with linear nuclear array and nuclear pseudo-inclusions, have been reported to be enriched in TFE3 genomic aberrations.75,77 Although not yet included in the World Health Organization classification of renal tumors, TFE3-amplified RCC has been recognized as an emerging entity. Most reported cases of TFE3-amplified RCC show predominant papillary architecture with high-grade features and an oncocyctic phenotype (Figure 6, D).98,75,77 Although the diagnosis of translocation-associated carcinoma should always be entertained in a renal tumor that demonstrates a relative underexpression of cytokeratins or epithelial markers by immunohistochemistry, cytokeratin expression (focal or diffuse) can be present in these tumors, and lack of cytokeratin expression generally does not serve as a very faithful tool in delineating these entities diagnostically. Immunohistochemical stains for evaluating TFE3 and TFE3 protein expression exist but are technically challenging to perform and suffer from fixation and other issues78,79; fluorescence in situ hybridization (FISH), in contrast, is a useful tool for accurate classification of these tumors.70,71 TFE3-translocation RCC and TFE3-amplified RCC, like HLRCC–associated RCC and clear cell RCC, are potentially aggressive subtypes of RCC.68,74–77,80–82

The melanogenesis-associated transcription factor (MITF) aberration–associated RCC and HLRCC–associated RCC have been reported to show broad and overlapping morphologic spectrums. Helpful features to aid in the diagnosis of TFE3-translocation RCC include the presence of dual (eosinophilic and clear) cytoplasmic tones and psammoma bodies, as well as identification of TFE3 rearrangement by FISH. TFE3-amplified RCC generally demonstrates high-grade oncocyctic cells with papillary architecture and will show TFE3 amplification by FISH. At the morphologic level, HLRCC–associated RCCs are enriched for the features described in the preceding sections including a spectrum or mixture of diverse morphologic patterns within the same tumor. When MITF aberration–associated RCCs and HLRCC–associated RCCs are in the differential diagnosis for high-grade tumors with prominent nuclei and papillary architecture, immunohistochemical and molecular assessment can be helpful because HLRCC–associated RCC, unlike the MITF aberration–associated RCC, demonstrates loss of FH expression by IHC and does not show MITF aberrations by FISH.

Collecting Duct Carcinoma

CDC comprises one of the major differential diagnoses for HLRCC–associated RCC at the clinical, morphologic, and immunohistochemical levels. Collecting duct carcinoma, like HLRCC–associated RCC, tends to occur in younger patients.1 Approximately 50% of patients with CDC have metastatic disease at the time of presentation, and the clinical course can be rapid with many patients dead of disease within 2 years.83 Collecting duct carcinoma is a medullary–centered tumor, although large tumors can involve the cortex secondarily and mimic cortical tumors. Similar to HLRCC–associated RCC, CDC shows multiple architectural patterns with an infiltrative growth pattern and occasional multinodularity.5,84 Per the 2016 World Health Organization (Geneva, Switzerland) classification of renal tumors,1 diagnostic criteria supporting the diagnosis of CDC include medullary involvement, predominant tubular morphology,
Figure 6. Differential diagnosis. Type 2 papillary renal cell carcinoma (RCC) can show a solid growth pattern with prominent nucleoli (A), which may raise concern for hereditary leiomyomatosis and RCC syndrome (HLRCC)–associated RCC; however, fumarate hydratase (FH) expression is retained in papillary RCC (B). Both TFE3-translocation RCC (C) and TFE3-amplified RCC (D) can show prominent, papillary architecture with prominent nucleoli. E, Collecting duct carcinoma is an infiltrative tumor that can show prominent nucleoli with poorly developed halos that may raise concern for HLRCC-associated RCC. F, Clear cell RCC (F) can show prominent pseudopapillary growth and prominent nucleoli similar to HLRCC-associated RCC; however, carbonic anhydrase IX shows diffuse membranous staining in clear cell RCC (inset) (hematoxylin-eosin, original magnifications x200 [A, C, D, and F] and x400 [E]); original magnification x200 [B]; original magnification x200 [F-inset]).
desmoplastic stromal reaction, high-grade cytology, an infiltrative growth pattern, and the absence of other RCC or urothelial carcinoma. Growth patterns described in CDC include tubular, solid, acinar, papillary, cribriform, and signet ring. Collecting duct carcinoma tends to demonstrate a desmoplastic stroma and associated inflammation (Figure 6, E). Tubular dysplasia is often seen in the kidney parenchyma adjacent to CDC. At the immunohistochemical level, CDC generally stains positive for PAX8, high-molecular-weight cytokeratin, CK7, and carcinoembryonic antigen and has negative p63 expression. Interestingly, a recent Foundation Medicine (Cambridge, Massachusetts) study demonstrated that nearly one-third of CDCs have genomic alterations in NF2, indicating that mTOR inhibitors may be beneficial for treatment of a subset of CDCs.

Because of the immense morphologic overlap, some examples of HLRCC-associated RCC were originally classified as CDC in the literature; however, FH immunohistochemistry and certain morphologic features coupled with genetic testing (as necessary) can aid in the accurate classification of these tumors. Morphologically, HLRCC-associated RCC often demonstrates hyalinized fibrovascular cores, rather than the delicate or absent fibrovascular cores of CDC. Although a tubulopapillary growth pattern is common in both HLRCC-associated RCC and CDC, intracytic papillary and tubulocystic growth is more often seen in HLRCC-associated RCC. The presence of prominent nucleoli with classic perinucleolar halos is also more indicative of the possibility of an association with HLRCC. In both entities, cytologic features are high grade, with marked pleomorphism and brisk mitotic activity. In contrast to CDC, HLRCC-associated RCC is usually negative for CK7 expression, and FH expression is lost in most HLRCC-associated RCCs.

One can hypothesize that a small subset of CDCs might demonstrate the loss of FH protein expression because of a somatic FH genomic aberration. Hence, clinical evaluation and germline testing is very important in patients who demonstrate CDC with CDC-like morphology and FH protein loss as assessed by immunohistochemistry. Germ-line mutational testing in such cases should correctly categorize the patients into the HLRCC subgroup versus those with a somatic FH mutation.

**High-Grade Clear Cell RCC**

Despite its name, high-grade clear cell RCC tends to have eosinophilic cytoplasm and may demonstrate papillary or pseudopapillary architecture (Figure 6, F), thus, presenting itself as a mimic to HLRCC-associated RCC and other high-grade renal tumor subtypes. Although pseudopapillary architecture and focal true papillary architecture are acceptable for the diagnosis of clear cell RCC, a prominent papillary architecture should prompt consideration of other diagnoses, including papillary RCC and HLRCC-associated RCC. Even high-grade clear cell RCC usually retains the delicate (‘racemose’) vascular network, at least focally. Clear cell RCC is typically positive for pancytokeratin and carbonic anhydrase IX (CAIX, diffuse membranous), and negative for AMACR and CD117. Often, CAIX expression can be appreciated in perinecrotic areas in other tumors, but diffuse membranous reactivity is generally not seen in HLRCC-associated RCC. CK7 can be focally positive in cystic and/or fibrotic areas of clear cell RCC but is usually negative in HLRCC-associated RCC. Clear cell RCC is the most common subtype of RCC, as well as the most aggressive of the common subtypes of RCC, with an overall 5-year survival of 75%. Most clear cell RCCs show band 3p copy number losses at the molecular level. von Hippel-Lindau syndrome confers predisposition to the development of clear cell RCC.

To summarize, clear cell RCC and HLRCC-associated RCC are both aggressive neoplasms that can show a papillary or pseudopapillary architecture. Clear cell RCC often shows diffuse membranous positivity for CAIX and retains FH expression. Most examples of clear cell RCC retain the characteristic, delicate vascular network, at least focally, and do not show a significant proportion of true papillary architecture. In contrast, HLRCC-associated RCC shows the loss of FH expression and lacks diffuse positivity for CAIX expression.

**Tubulocystic Carcinoma With Poorly Differentiated Foci**

In 2016, Smith and colleagues reported a series of 29 tubulocystic carcinomas with poorly differentiated foci of infiltrative adenocarcinoma and demonstrated that RCCs with that morphology are enriched for FH deficiency. Tubulocystic RCC (when “pure”) is a relatively rare and well-circumscribed tumor, classically composed of small- to intermediate-sized, sometimes dilated, tubules (Figure 7, A) with a single layer of flat, hobnail, cuboidal, or columnar epithelium with uniform, large nuclei and prominent nucleoli (Figure 7, B) and frequently fibrotic stroma; no solid or papillary areas are present. Renal cell carcinoma with tubulocystic tumors with poorly differentiated foci of infiltrative adenocarcinoma morphology, in contrast, has a component of classic tubulocystic carcinoma-like morphology, in addition to poorly differentiated foci with infiltrative adenocarcinoma or collecting-duct type features and focal papillary growth. Tubulocystic RCC, when pure, is thought to have low malignant potential; however, patients with tubulocystic carcinomas with poorly differentiated foci of infiltrative adenocarcinoma morphology have more aggressive disease than those with pure tubulocystic RCC.

Renal cell carcinoma that demonstrates a tubulocystic growth pattern with associated poorly differentiated foci should prompt consideration of HLRCC-associated RCC. The HLRCC-associated RCC, in contrast to the more-indolent pure tubulocystic RCC, often shows an overtly invasive phenotype with a mixture of different growth patterns. From an immunohistochemical perspective, pure tubulocystic RCC demonstrates positive CK7, AMACR, and FH expression, whereas tubulocystic RCC with poorly differentiated foci of infiltrative adenocarcinoma morphology is negative for FH expression in most cases.

**SDH-Deficient RCC**

Succinate dehydrogenase-deficient RCC may show some overlapping morphologic features with the low-grade oncocytic variant of HLRCC-associated RCC in a few cases. The SDH-deficient RCC is classically described as an oncocyti  carcinoma with predominantly solid or nested architecture (Figure 7, C) and sometimes having a minor component of tubular or microcystic growth pattern. The neoplastic cells show uniform cytology, polygonal shape, fine chromatin and inconspicuous nucleoli (“neuroendocrine features”), and flocculent/vacuolated eosinophilic cytoplasm with occasional inclusions of pink hyaline material. Intratumoral mast cells are commonly seen, and benign renal tubules may be entrapped at the periphery of the tumor. The neoplastic cells are positive for PAX8.
Figure 7. Differential diagnosis. Tubulocystic carcinoma is a well-circumscribed tumor composed of tubules and cystic spaces (A), lined by cuboidal cells with prominent central nucleoli (B), which may prompt consideration of hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)-associated renal cell carcinoma (RCC), particularly if areas of poor differentiation are present. Succinate dehydrogenase (SDH)-deficient RCC (C) shows cells with uniform nuclei, fine chromatin, and flocculent eosinophilic cytoplasm, with occasional inclusions arranged in nests (similar to a rare, low-grade, oncocytic variant of HLRCC-associated RCC) but demonstrates loss of SDH subunit B expression by immunohistochemistry (D) and retained fumarate hydratase (FH) expression (not shown). Renal cell carcinoma, type unclassified (E), may show morphologic overlap with high-grade RCCs, including HLRCC-associated RCC and translocation RCC; however, this case showed retained FH expression by immunohistochemistry (F) and lacked TFE3 or TFEB aberrations by fluorescence in situ hybridization (not shown) (hematoxylin-eosin, original magnifications ×100 [A], ×400 [B], and ×200 [C and E]; original magnification ×100 [D]; original magnification ×200 [F]).
expression, variably positive for EMA (delicate membranous pattern), CAM 5.2, and AE1/AE3 and are generally negative for CK7 and CD117 expression. Characteristically, the neoplastic cells show loss of SDHB protein expression by immunohistochemistry (Figure 7, D) because of mitochondrial complex II instability resulting from mutation of SDHA, SDHB, SDHC, or SDHD. A recent study reported a series of 4 RCCs with similar morphology but strong and diffuse SDHB expression, consistent with an intact SDH complex. Those cases were negative for FH expression and positive for 2SC by immunohistochemistry. Such data indicate that a subset of HLRCC-associated RCC might demonstrate a relatively lower-grade morphology that is also enriched for oncogenic features. Such FH-deficient, relatively low-grade oncogenic tumors may have a more-favorable outcome, based on the current (albeit limited) data.

Although some instances of HLRCC-associated RCC show a morphology that is nearly identical to that of SDH-deficient RCC, based on the limited data presented in the literature, the distinction should be faithfully resolved by immunohistochemical evaluation. The HLRCC-associated RCCs interrogated so far demonstrate retained SDHB expression and absent FH expression, compared with the absent SDHB expression and retained FH expression in SDH-deficient RCC.

Together, FH and SDH are involved in pathways that respond to metabolic stress within the kidney and are known to have a central role in the mitochondrial tricarboxylic acid cycle, which is coupled to energy production through oxidative phosphorylation. Mutations in both FH and SDH can result in dysregulation of metabolic pathways involved in oxygen or energy sensing, suggesting that kidney cancers associated with such genomic aberrations result from a dysfunctional metabolic state within the cell. In that context, the morphologic overlap of low-grade oncogenic RCC shared by SDH-deficient carcinomas and FH-deficient HLRCC-associated RCC in a few cases can otherwise be defined by disparate classic phenotypes. It is interesting (but maybe not surprising) that metabolic insults resulting from mutational dysfunction of 2 distinct but mutually cooperative genes (FH and SDH in this context) can uncommonly result in tumors with a common morphologic phenotype.

**RCC, Type Unclassified**

If a primary RCC does not fit well into a well-described World Health Organization category based on morphologic, immunophenotypic, and/or molecular grounds, it is best to assign it to the RCC, type unclassified, category for more-accurate prognostication and guidance of therapy. Figure 7, E and F, demonstrates an example of a high-grade RCC with retained FH expression, absent CAIX expression, and the absence of MITF aberrations by FISH (not shown), which falls best under the RCC, type unclassified, category. Recent literature highlights the fact that even RCCs correctly assigned to the type unclassified category demonstrate distinct molecular subsets with implications for potential targeted therapy. The Chen et al molecular analysis of 62 high-grade primary RCCs with unclassified histology demonstrated that approximately three-quarters of cases fell into molecularly distinct subsets. One subset of RCC with unclassified histology demonstrated NF2 loss and dysregulated Hippo-YAP signaling, and was associated with a poor prognosis; potential future therapies in such cases may interfere with YAP activity. Another subset of RCC with unclassified histology demonstrated mTORC1 hyperactivity and more-favorable prognosis; patients with such tumors may benefit from treatment with mTOR inhibitors. The worst prognosis in the cohort was observed in the 4 FH-deficient RCCs with unclassified histology. Of note, although 3 patients were confirmed to have germline FH mutations, the fourth had a somatic FH alteration. Further studies are needed to elucidate the clinical behavior of RCC with somatic FH alterations in comparison to HLRCC-associated RCC.

**A Practical Surgical Pathology Diagnostic Approach**

In general, HLRCC-associated RCC has an aggressive clinical course, with frequent metastases and subsequent death. As such, the identification of patients with HLRCC-associated RCC is important because those patients and their families should undergo regular clinical assessment and genetic evaluation and counseling.

The morphologic spectrum of HLRCC-associated RCC is broad and overlaps with multiple other entities including clear cell RCC, papillary RCC, tubulocystic RCC with poorly differentiated foci, CDC, SDH-deficient RCC, and translocation RCC. Histologic features in a high-grade RCC, such as prominent nucleoli with perinucleolar halos (particularly when diffuse), are considered suggestive of HLRCC-associated RCC. Appropriate use of FH immunohistochemistry coupled with a clinical workup and referral to genetic counseling, as outlined above, is important for detection of this syndrome. Until a germline FH mutation has been confirmed, the diagnosis of FH-deficient RCC is most appropriate for renal tumors with loss of FH protein expression upon immunohistochemistry.

On a day-to-day basis, for a pathologist who is presented with a renal tumor with some or all of the morphologic features described above, FH immunohistochemical evaluation along with other markers (especially CK7 and CAIX) can be a good starting point for a diagnostic workup. For patients with a previously unestablished diagnosis, communication with a patient’s urologist and/or medical oncologist about the clinical stigmata and the patient’s personal and family history is extremely important because these data might further indicate clues to an association with HLRCC syndrome. Loss of FH protein expression in renal tumors upon immunohistochemistry is considered a good trigger for launching germline mutational testing and a thorough clinical evaluation for patients who might harbor HLRCC that has not otherwise been proven. In that context, a small subset of patients with HLRCC-associated RCC may demonstrate preserved or reduced FH protein expression (instead of the completely absent immunoeexpression); hence, thorough communication with the clinician about strongly suspicious RCC morphology (for HLRCC, even with retained or equivocal FH loss) is paramount, so that appropriate clinical and/or genetic testing may be performed.

The above discussion underscores the essential role of the surgical pathologist in the early recommendation of genetic consultation for patients harboring tumors in the morphologic and clinical spectrum described above. There is ample evidence now that the genomic/molecular classification of renal tumors is becoming increasingly important because it may allow for a clinically useful, algorithmic subdivision based on predicted response to treatment and a more-accurate risk assessment for small renal masses. Recognition and correct classification of these RCC subtypes are
very important for accurate risk stratification and therapeutic management.

References

2. World Health Organization Classification of Tumours. 3rd ed. vol 8.
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