

**A Distinctive, Low Grade Oncocytic Fumarate Hydratase-Deficient Renal Cell Carcinoma, Morphologically Reminiscent of SDH-deficient Renal Cell Carcinoma**

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**HISTOP-10-16-0633-R2****A Distinctive, Low Grade Oncocytic Fumarate Hydratase-Deficient Renal Cell Carcinoma, Morphologically Reminiscent of SDH-deficient Renal Cell Carcinoma**

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**Short Running Title:** Oncocytic FH-deficient RCC

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## ABSTRACT

### Aims

Fumarate hydratase-deficient renal cell carcinoma (FH-deficient RCC) is a high grade, aggressive tubulopapillary carcinoma, arising predominantly in the setting of the hereditary leiomyomatosis-renal cell carcinoma syndrome of familial uterocutaneous leiomyomatosis and deficiency of fumarate hydratase. In contrast, succinate dehydrogenase (SDH)-deficient RCC is a lower grade oncocytic carcinoma with cytoplasmic flocculence/vacuolation and inclusions, arising mostly in individuals harboring germline mutations of subunit B of the SDH complex (*SDHB*). Recently, we have identified four cases of a novel type of FH-deficient RCC, where the morphology observed was that of an *oncocytic carcinoma*, reminiscent of SDH-deficient RCC.

### Methods and Results

These distinctive, low grade oncocytic neoplasms, with solid, nested, and focally tubular architecture (0.2-9cm) arose in four males (11-41y). Uniform cytology of polygonal cells with flocculent, vacuolated eosinophilic cytoplasm with scattered inclusions, fine chromatin, and inconspicuous nucleoli was apparent. Despite these features suggestive of SDH-deficient RCC, each tumor was confirmed as an FH-deficient carcinoma with retained SDHB expression. One case showed a synchronous, anatomically separate, **typical** high grade FH-deficient RCC; one other showed such a tumor at nephrectomy 4 years later. No progression has been noted at 3 and 7 years in the cases with only the SDH-like lesions; the two cases with separate, **typical** FH-deficient RCCs progressed.

### Conclusions

In summary, we characterize a novel oncocytic type of FH-deficient RCC with striking resemblance to SDH-deficient RCC, posing a diagnostic challenge and raising concerns for sampling and multifocality for syndrome-associated cases under surveillance protocols.

**Key Words:** renal cell carcinoma; hereditary leiomyomatosis-renal cell carcinoma syndrome; succinate dehydrogenase-deficient renal cell carcinoma; fumarate hydratase-deficient renal cell carcinoma, oncocytic carcinoma.

## INTRODUCTION

Recent series<sup>1, 2</sup> have shed much light on the clinicopathologic features of succinate dehydrogenase (SDH)-deficient renal cell carcinoma (RCC)<sup>3, 4</sup>. Proceeding from the initial observation of kidney tumors in kindreds with germline *SDHB* mutation and predisposition to epithelioid gastrointestinal stromal tumors (GISTs) and multiple pheochromocytomas and paragangliomas<sup>5</sup>, greater experience suggests these RCCs are morphologically distinctive<sup>4</sup>, are under-recognized but rare, arise with slight male predominance in middle aged adults, and are frequently bilateral<sup>1, 2</sup>. The morphology is quite characteristic, of tumors composed of small nests and sheets of oncocytic polygonal cells, unencapsulated, and sometimes entrapping peripheral benign tubules. Cytologically they show vacuolated pink cytoplasm and variably prominent, pale eosinophilic inclusions, thought to represent dysfunctional mitochondria<sup>6</sup>. The nuclear features are usually low grade and monotonous, with what we have called a neuroendocrine-like chromatin pattern<sup>2</sup>. A subset of cases, however, have shown sarcomatoid transformation with overt nuclear atypia and aggressive course. While most SDH-deficient RCCs that have been tested have been shown to harbor *SDHB* mutations, scattered reports have identified *SDHA* and *SDHC* mutations as well<sup>7-9</sup>. In any case, to assist in their recognition, SDHB immunohistochemistry (IHC) has been used for detection of tumors with SDH subunit mutations based on consistent loss of SDHB expression by IHC, whether there is *SDHA*, *SDHB*, *SDHC* or *SDHD* mutation, due to mitochondrial complex II instability<sup>3</sup>.

In contrast, hereditary leiomyomatosis-RCC syndrome (HLRCC) represents another RCC syndrome, related to germline mutation of the gene fumarate hydratase (*FH*), consisting of highly penetrant cutaneous (>90% of males and females) and uterine (~70% of females) leiomyomatosis with less prevalent (10-30%)<sup>10-12</sup> "type 2 papillary" RCC<sup>13</sup> arising in both males and females over a diverse age range. Adrenocortical adenomas have been reported in ~10% of affected subjects<sup>14</sup>. The RCCs arising in HLRCC show a much more variable, high grade morphology, including papillary, tubulopapillary, cribriform, cystic, and infiltrative collecting-duct like patterns<sup>15, 16</sup>. Prospective recognition of these tumors is emphasized due to their remarkable aggression, for which immunohistochemistry for FH (lost in most cases with mutations<sup>17</sup>) and for aberrant succination of nuclear and cytoplasmic proteins (2-succinylcysteine, 2SC<sup>16</sup>) has been used. While most cases are thought to occur with germline rather than somatic *FH* mutations<sup>18</sup>, recent data identify apparently somatic mutations in sporadic uterine leiomyomas<sup>19</sup> and even high grade papillary renal cell carcinomas<sup>20</sup>. In any case, a great many cases showing FH-deficiency by immunohistochemistry do not show stigmata of this syndrome. For this reason, we have recently proposed<sup>21</sup> a term, *FH-deficient RCC*, for provisional use, with recommendation of

genetic consultation, in morphologically and immunohistochemically suggestive cases, without apparent syndromic features and unavailable genotyping<sup>17, 22</sup>.

In a recent interinstitutional review of FH-deficient RCCs<sup>22</sup>, we noted several morphologic outliers. Based on prior experience with SDH-deficient RCC<sup>2</sup>, we noted tumors with a low grade oncocytic morphology, suggestive of SDH-deficiency, among cases with clinical, morphologic, immunohistochemical, or molecular evidence suggestive of FH-deficiency. Careful review of published cases of HLRCC identified another case with such morphology<sup>23</sup>, which we also reviewed. Intrigued by the morphologic overlap of these carcinomas across syndromes defined by disparate classic phenotypes but shared metabolic alterations, we present the findings herein.

## METHODS

### Cases, Immunohistochemistry and Molecular Studies

In accordance with the ethical principles of the Declaration of Helsinki, under IRB-approved protocols (VCU IRB #HM20002545, valid through 4/26/2017; CSMC IRB # Pro00027348; valid through 1/31/2018), granting waivers of consent, cases of renal cell carcinoma arising in the setting of known or suspected HLRCC were identified retrospectively from the files of the authors. All sections of every case were obtained and reviewed, and deidentified clinicopathologic data collected. One case has been reported previously by Alrashdi *et al.*<sup>23</sup>, which was also re-reviewed with extended follow-up. Routine immunohistochemistry (IHC), using protocols and evaluation as reported previously for FH<sup>22</sup> and 2SC<sup>16</sup>, and SDHB and SDHA<sup>2</sup> was employed. For one case, multiplexed PCR-based next-generation sequencing (NGS) of tumor genomic DNA was performed using the Ion Ampliseq Comprehensive Cancer Panel (CCP) targeting 409 cancer-related genes, including *FH* (and *SDHB*), with validation and analysis pipeline, and copy number alteration detection as reported<sup>24-26</sup> using cutoffs for variant detection exactly as reported<sup>22</sup>.

## RESULTS

### Clinicopathologic Features

**Table 1** details the clinicopathologic findings per case. The tumors arose in four males aged 11-41. Presenting stimuli included from left back and abdominal pain (Case 1), imaging findings performed under syndromal surveillance (Case 2), abdominal pain and anemia with

history of polycystic kidneys by imaging (Case 3), and incidental enlarged kidney on MRI (Case 4). The oncocytic tumors were multifocal (**Figure 1A**) in two cases (1&3) and unifocal in two (Cases 2&4). Two cases (3&4) showed separate (one anatomically distant from the oncocytic tumor; one presenting 4 years later at completion nephrectomy) high grade FH-deficient RCCs. Two cases provided strong evidence of HLRCC, including concurrent multiple cutaneous leiomyomata (Case 1), and germline mutation-confirmed HLRCC (Case 2). Case 3 also had suggestive history, including personal history of a first-degree relative having succumbed to metastatic RCC and personal history of “multicystic kidneys” by imaging. No data are available regarding HLRCC-associated stigmata for Case 4. In terms of clinical outcome, the two cases with only low grade oncocytic carcinomas are free of disease at nearly 3 years and more than 7 years post-nephrectomy. The two cases with separate high grade tumors showed progression, at presentation in Case 3 (liver metastasis showing morphology of the high grade tumor at frozen) and at 3 months post completion nephrectomy (though after 4 years had elapsed without progression since the partial nephrectomy with the oncocytic RCC) in Case 4.

The specimens evaluated were 3 radical and 1 partial nephrectomy. The nephrectomy of Case 1 harbored an ipsilateral adrenocortical adenoma (2.7cm) (**Figure 1B**), while cutaneous biopsies performed concurrently with nephrectomy for suspected metastasis (**Figure 1C**) proved to be cutaneous, pilar-type leiomyomatosis (**Figure 1D**). The tumors were described as yellow or tan (**Figure 2A**) and measured at gross exam from 0.2cm up to 9cm. In three cases, these were seen adjacent to variable background cystic change (**Figure 3A, Figures 4A&C, Figure 5A**). In each case, the tumors were grossly unencapsulated but appeared non-infiltrative of the adjacent structures, bulging but confined to the kidney.

### ***Histologic Features***

Morphologically, these tumors showed a low grade oncocytic morphology, with confluent solid or nested confluent growth and prominent nodularity, closely juxtaposed to the adjacent renal parenchyma (**Figure 2B, 3A**). At higher power, the architecture showed a mixed pattern, in some areas with tubular morphology (**Figures 2C-D, 3B-C**), sometimes dilated to impart a microcystic appearance with pink luminal contents (**Figure 2C**). One nodule demonstrated prominent stromal edema with dispersed tubular aggregates of cells (**Figure 2D**); in other foci tubular growth was more packed (inset). Cytologically, consistent oncocytic features were seen, with tumors composed of polygonal cells with eosinophilic cytoplasm with scattered vacuoles and predominantly round, regular nuclei (**Figure 2E, 3C, 4B&D, 5B**). The chromatin was finely granular; many examples showed micronucleoli that were not prominent at 10X

(ISUP grade 2). Numerous stromal mast cells were apparent in many areas (**Figure 2E**). All cases demonstrated variable cytoplasmic flocculence or vacuolation; in three cases (2-4), cytoplasmic inclusions of pink hyaline material reminiscent of those that have been described in SDH-deficient RCC were readily identifiable (**Figure 3C, 5B**). No coagulative tumor cell necrosis was identified in any of these tumors, nor were features of vascular invasion present, including in the multifocal cases, which were interpreted histologically as independent neoplastic foci.

Two cases (3&4) demonstrated separate RCCs with features that have been associated with **typical** high grade FH-deficient RCC. Case 3 demonstrated a 3.5cm tumor, in the upper pole of the kidney, distant from the oncocytic tumors, with prominent tubulocystic morphology and poorly differentiated, solid areas with cribriform and syncytial appearance (**Figure 4E-F**); a metastatic liver lesion with this morphology was present at nephrectomy. In Case 4, a completion nephrectomy was performed 4 years after the partial nephrectomy (that had shown the low grade oncocytic RCC), and a 5cm high grade RCC with tubulocystic and infiltrative collecting duct carcinoma-like areas was identified, invasive of renal sinus adipose (**Figure 5C**). In both of these cases the cytology of the high grade tumors was high grade (ISUP 3 to 4) with syncytial appearance and prominent, viral inclusion-like nucleoli with perinuclear halos (**Figure 5D**).

### ***Immunohistochemical and Molecular Features***

In all four cases, the oncocytic tumors showed strong/diffuse retained expression of both SDHB and SDHA, with loss of expression of FH and induction of strong nucleocytoplasmic 2SC positivity (**Figures 2F, 3D, 5B**). PAX8 expression was diffuse in all the oncocytic tumors (not pictured), with variable, patchy expression of pancytokeratin AE1/AE3 and the oncocytic renal neoplasia-associated marker, kidney-specific cadherin (Ksp-cadherin) as detailed in **Table 2**. Of note, the cutaneous leiomyoma seen in Case 1 also demonstrated SDHB/SDHA positivity with loss of FH (**Figure 1D**), while the separate high grade carcinomas seen in Cases 3&4 were both FH-deficient. In Case 3, NGS was performed on the low grade oncocytic RCC, identifying homozygous *FH* frameshifting mutations (p.K80fs). This mutation was identical to the homozygous *FH* mutations observed in the anatomically separate high grade FH-deficient RCC<sup>22</sup>. Nevertheless, comparing genome wide copy number alterations between these two separate carcinomas, the oncocytic tumor lacked the copy number gains (chromosome 8q) and losses (chromosome 18) seen in the high grade FH-deficient carcinoma (**Figure 4H**). Consistent with the IHC findings, no *SDHB* mutations were identified.

## DISCUSSION

Recent years have seen recognition of distinctive morphologic features of subtypes of RCC associated with genetic syndromes, including the succinate dehydrogenase (SDH)-deficient RCCs associated with familial paraganglioma/pheochromocytoma syndromes<sup>3, 4</sup> and the fumarate hydratase (FH)-deficient RCCs associated with hereditary leiomyomatosis-RCC syndrome<sup>15</sup>. From the standpoint of the diagnostic pathologist, recognition of these RCC subtypes is of utmost importance due to the need to risk stratify individual tumors based on increasing published experience<sup>1, 2, 17, 22</sup> and to recommend genetic counseling and testing for patients and their families.

With regards to the present cases, first, we note that the evidence available suggests that three of these cases represent HLRCC, though proof of germline mutation of *FH* (observation of the same constitutional *FH* mutation in two separate generations) is unavailable except for the second case, which arose in a patient from a well-characterized HLRCC family<sup>23</sup>. The first case, arising in an individual showing multiple, histologically confirmed cutaneous leiomyomas, and the third case, with the separate “type 2 papillary” RCC or “collecting duct carcinoma”<sup>27</sup> before the age of 40 and with an affected first degree relative, would meet the criteria reported by Menko *et al.*<sup>28</sup> for *likely HLRCC*. Quite suggestive of a constitutional mutation, this third case also had the same homozygous *FH* mutation detected from the anatomically separate SDH deficient-like and **typical** high grade FH-deficient RCCs. Notably, no data regarding family history of HLRCC or personal or family history of uterocutaneous leiomyomatosis is available for the fourth case. In light of recently reported data that uterine leiomyomata and even FH-mutant RCCs may occur through somatic mutations<sup>19, 20</sup>, we can only regard this case as an *FH-deficient RCC*<sup>21, 22</sup>.

We acknowledge the wisdom of the opinion held by some that explicit observation of a germline *FH* mutation remains the true gold standard for HLRCC diagnosis<sup>15, 16</sup>; in three of our cases, this will remain impossible (not allowed under the retrospective protocols governing the study). Our inability to retrospectively perform genetic testing for constitutional changes in *FH* and *SDHB* remains the most important limitation of this study. Moreover, it underscores the essential role of surgical pathologist in early recommendation of genetic consultation for patients with tumors in in this morphologic and clinical spectrum. Only accumulated, carefully genetically documented clinical experience will answer the question of whether FH-deficient or SDH-deficient RCCs occur sporadically by somatic mutation at any frequency. Indeed, SDH-deficient

paragangliomas<sup>29, 30</sup> and other lesions with only somatic SDH subunit mutations<sup>31</sup> have been described, making this issue all the more salient.

With regard to the oncocytic tumors, we do not argue that these tumors represent perfect morphologic phenocopies of the most characteristic SDH-deficient RCCs. However, in each case, when encountered after our prior studies of SDH-deficient RCCs<sup>2</sup>, we were sufficiently suspicious that we ordered SDH-deficient workup (by IHC). Each case demonstrated oncocytic morphology with stromal mast cells and areas of distinctive cytoplasmic flocculence and vacuolation, with at least focal inclusion-like formations. The predominant architectural pattern was solid or nested, though tubular or pseudoglandular pattern, reported previously in SDH-deficient RCCs<sup>1</sup>, was prominent in areas of two of the cases. Pancytokeratin AE1/AE3 was positive in a variable, patchy manner; Ksp-cadherin was positive in three cases, similar to SDH-deficient RCC<sup>2</sup> and as is characteristic of oncocytic renal neoplasia generally<sup>32</sup>, as was PAX8. Most important, however, was the observation in all three tumors of consistently retained SDHB in a strong diffuse manner, reflective of an intact SDH complex<sup>33</sup>. In contrast, FH expression was lost and 2SC expression strongly, diffusely induced in a nucleocytoplasmic manner, a pattern that has proven in our experience<sup>16, 22, 34</sup> and that of others<sup>17, 35, 36</sup> to be demonstrative of *FH* mutation and loss of function. Thus, we interpret the FH-2SC+ immunophenotype in each of these tumors as confirmatory of their specificity to FH-deficiency and exclusive of their representing an etiologically unrelated epiphenomenon (sporadic-type carcinomas arising in the polycystic background, for instance).

In summary, we describe four intriguing cases, tumors with a low grade oncocytic morphology and variable cytoplasmic vacuolation and flocculence. On morphologic grounds, these tumors were first deemed quite reminiscent of SDH-deficient renal cell carcinoma. However, immunophenotypic workup points to their representing a novel morphologic type of FH-deficient carcinoma, including loss of FH expression, induction of strong diffuse nucleocytoplasmic 2SC immunostaining, and retained expression of SDHB, to say nothing of the clinical features quite suggestive of (or confirmed) HLRCC in three of them. We speculate that these tumors represent as yet another example of an emerging phenomenon of phenotypic “crossover lesions” between FH-deficient and SDH-deficient syndromes, analogous to recently described paragangliomas occurring the setting of germline FH mutation<sup>35, 37, 38</sup>.

Two of these novel cases also raise the precedent that when not associated with a high grade FH-deficient RCC, this oncocytic tumor type could be associated with a more favorable outcome. High grade FH-deficient RCCs have been associated with progression and/or death of disease in 30-80%<sup>15, 17, 22</sup>. Our experience with these low grade oncocytic tumors emphasizes

the importance of morphologic context for interpreting IHC and molecular findings. Despite the shared “FH-deficient RCC” status of both the low grade oncocytic tumors and **typical** high grade FH-deficient RCCs, such a high risk label would have been inappropriate for the low grade tumors given that progression was only seen in cases with a separate high grade tumor. Certainly, much greater study is needed, and prospectively we recommend careful inquiry regarding the nature of any syndromal stigmata and use of SDHB, SDHA, and FH IHC, as well as genetics referral, for concerning cases. Moreover, given the presence of anatomically (or chronologically) separate, disparate lesions of very different grades, we caution careful correlation between imaging, sampling, and histopathology for surveillance or sampling protocols.

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#### AUTHOR CONTRIBUTIONS:

SCS, DS, CO, and MBA designed the studies; SCS, DS, JBM, JLH, JK, SK, KS, SVH, AKC, DH, DJL, GM, Y-BC, SAT, RM and MBA contributed and analyzed data; SCS, JBM, JLH, AKC, DH, DJL, Y-BC, SAT, and RM performed immunohistochemical and molecular studies; SCS and MBA wrote the manuscript; all authors revised and endorse the manuscript.

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## FIGURE LEGENDS

**Figure 1.** Clinical findings at presentation for Case 1 included an enhancing, multinodular, focally calcified left kidney masses arising in the upper, interpolar, and lower poles of the kidney (**A**). An enlarged ipsilateral adrenal gland excised at nephrectomy was confirmed to be an adrenocortical adenoma (**B**). Multiple nodular cutaneous tumors seen on preoperative imaging and interpreted as suspicious for metastasis (**C**, arrows), proved to be cutaneous, pilar-type leiomyomata (**D**). Fumarate hydratase (FH) immunostain was negative in this tumor (**inset**, see internal control positive endothelium), a pattern that has been interpreted as highly suspicious for Hereditary Leiomyomatosis-Renal Cell Carcinoma (HLRCC)-associated cutaneous leiomyomatosis<sup>39</sup>.

**Figure 2.** The kidney tumor resected in Case 1 demonstrated grossly a partly exophytic multinodular mass with striking yellow gross morphology (**A**). At low power, a solid appearance was imparted by a tumor composed of densely packed nests of cells with eosinophilic cytoplasm (**B**). At intermediate power, scattered foci with a more tubular and more microcystic appearance with dense pink luminal contents were noted (**C**). Similarly, one of the many tumor nodules had prominent stromal myxoedematous change (**D**), with occasional hobnail-shaped cells protruding into the center of tubules (**inset**). However, the predominantly solid and nested tumor was composed of cells with oncocyctic morphology, variably flocculent cytoplasm, and nuclei with finely granular chromatin with scattered small nucleoli (**E**); stromal mast cells were very prevalent (arrows). Expression of FH was lost by immunohistochemistry (**F**, note internal control expression in endothelial cells), while expression of 2SC was strongly induced and SDHA and SDHB expression retained (**insets**, each as indicated).

**Figure 3.** Case 2, arising in an 11-year-old male with proven HLRCC showed a dilated cyst, with adjacent cellular proliferation (**A**). At intermediate power (**B**), the carcinoma is seen to be composed of tubules and nests of oncocyctic cells with indistinct nucleoli. At higher power, (**C**) flocculent eosinophilic cytoplasm is readily apparent, as are several examples of large cytoplasmic vacuoles, containing eosinophilic inclusions (arrows). By immunohistochemistry (**D**), the tumor lacked expression of FH (note weak retention in internal control microvasculature) and showed induction of strong nucleocytoplasmic 2SC, and retained SDHB, and SDHA (insets, as indicated).

**Figure 4.** In the third case, the nephrectomy showed a kidney with striking polycystic background (**A**), wherein a 0.4cm solid nodule was apparent. At intermediate power, this tumor show a pattern of dense, nested and solid oncocytic carcinoma (**B**). Another tiny nodule with similar low power appearance was also identified between locules of cysts (**C**), showing a similar cytomorphology (**D**). The same kidney harbored an anatomically separate, synchronous tumor composed of high grade infiltrative, tubular and cribriform carcinoma (**E**) with large nuclei of high (ISUP) nucleolar grade, prominent nucleoli with perinuclear clearing and syncytial appearance (**F**). This starkly contrasted the ISUP nucleolar grade 2 features of the oncocytic tumors (**G**, at the same magnification as F for comparison). Both tumors demonstrated loss of FH expression, induction of 2SC expression, and retained SDHB by IHC (not shown), while targeted next-generation sequencing demonstrated identical homozygous *FH* p.K80fs mutations. Comparing genome wide copy number calls via scatter plot of  $\log_2$  gene-level copy number ratios between the oncocytic tumor (ordinate) and the synchronous high grade tumor (abscissa), the oncocytic tumor lacked gains at 8q (red) and losses of chromosome 18 (blue).

**Figure 5.** In the fourth case, a partial nephrectomy was performed sampling a solid lesion in an enlarged multicystic kidney. A 5cm solid nodule was seen (**A**, arrows) adjacent to a multicystic lesion, composed of nests of oncocytic cells with even chromatin and indistinct nucleoli (**B**). The cytoplasm was variably flocculent, with infrequent vacuoles with eosinophilic inclusion-like bodies (arrow). FH expression was lost, while strong/diffuse nucleocytoplasmic 2SC was identified (insets); SDHA and SDHB expression were retained (not shown). Though completion nephrectomy was recommended, it was performed 4 years later, showing a high grade, infiltrative RCC with glandular, solid, and tubulocystic growth patterns, invasive of renal sinus adipose (**C**). The cytology of this later tumor, which was also FH-deficient (**D**, inset) was markedly different, with large, variably shaped nuclei with prominent, inclusion-like nucleoli with perinuclear halos (**D**), much more in the spectrum described for typical FH-deficient RCCs.

Table 1: Cases and Clinicopathologic Features

Case	Sex	Age	HLRCC* Evidence	Oncocytic Tumor						Other Findings	Status <sup>^</sup>
				Size	Staging#	Grade\$	Encapsulation	Morphology	SDH-deficient-like cytology		
1	M	35	multiple cutaneous leiomyomata	multiple, 0.2-9cm	mpT2bNxMx	2	unencapsulated, non-infiltrative	variable, solid, nested, tubular; microcysts and stromal edema	cytoplasmic flocculence	adrenocortical adenoma, 2.7cm	NED, 34 mo
2	M	11	germline G354R FH mutation	8.5	pT2bNxMx	2	unencapsulated, non-infiltrative	solid, tubular and cystic	cytoplasmic vacuolation, hyaline inclusions		NED, >7y
3	M	40	homozygous p.K80fs FH mutation in separate tumors	0.4 & 0.2	mpT2aNxMx	2	unencapsulated, non-infiltrative	solid, nodular	cytoplasmic vacuolation, hyaline inclusions	anatomically separate, synchronous high grade FH-deficient RCC	liver metastasis, of high grade, at presentation NED, 4 years (oncocytic RCC); metastasis at 3 months after high grade RCC nephrectomy
4	M	41	none available	5	pT2bNxMx	2	unencapsulated, non-infiltrative	solid	cytoplasmic vacuolation, rare hyaline inclusions	multilocular cystic change; later high grade FH-deficient RCC	

\* Hereditary leiomyomatosis-renal cell carcinoma syndrome  
 # AJCC 2010 staging, with respects to the oncocytic RCC  
 \$ ISUP nucleolar grade  
 ^ NED; no evidence of disease; mo, months; y, years

Table 2: Immunohistochemical Findings

Case	PAX8	SDHB	SDHA	FH	2SC	Ksp-Cadherin	PanCK
1	diffuse, nuclear	retained	retained	lost	strong/diffuse nucleocytoplasmic	diffuse, membranous	patchy, variable
2	diffuse, nuclear	retained	retained	lost	strong/diffuse nucleocytoplasmic	negative	patchy, weak
3	diffuse, nuclear	retained	retained	lost	strong/diffuse nucleocytoplasmic	diffuse, membranous	patchy, variable
4	diffuse, nuclear	retained	retained	lost	strong/diffuse nucleocytoplasmic	focal membranous	patchy, weak

Abbreviations: PAX8, paired-box 8; SDHB, succinate dehydrogenase, subunit B; SDHA, succinate dehydrogenase, subunit A; FH, fumarate hydratase; 2SC, 2-succinyl/cysteine; Ksp-Cadherin, kidney specific cadherin; PanCK, pancytokeratin AE1/AE3

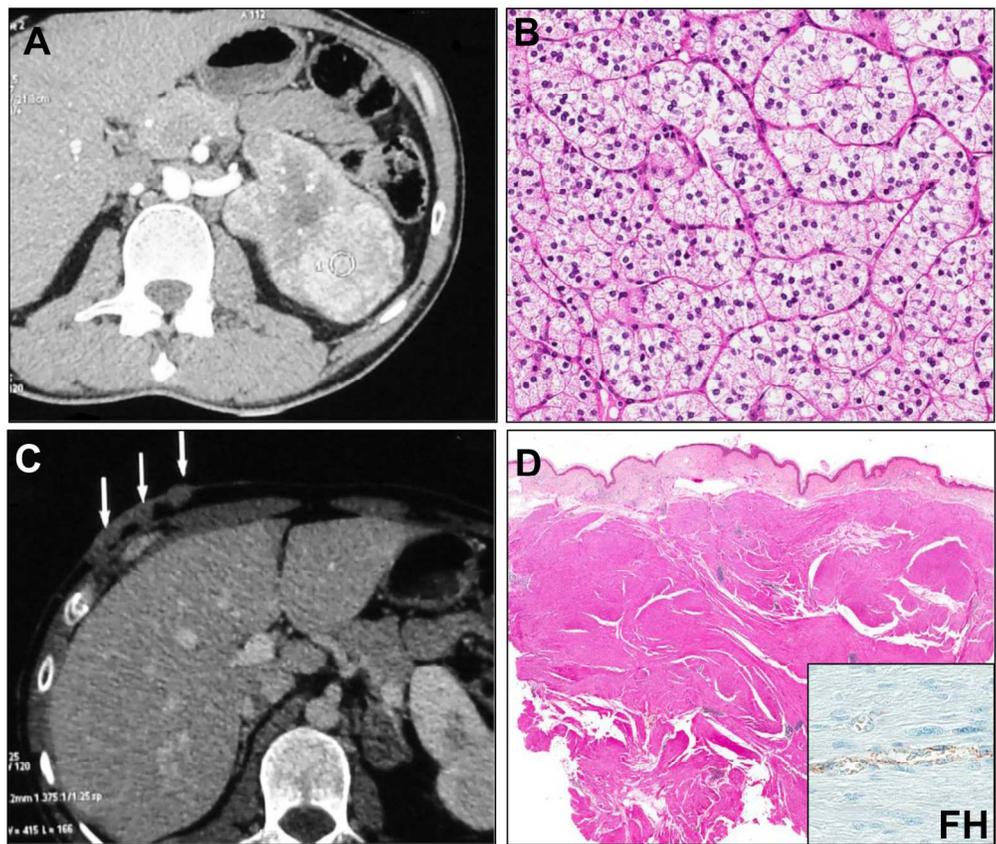


Figure 1

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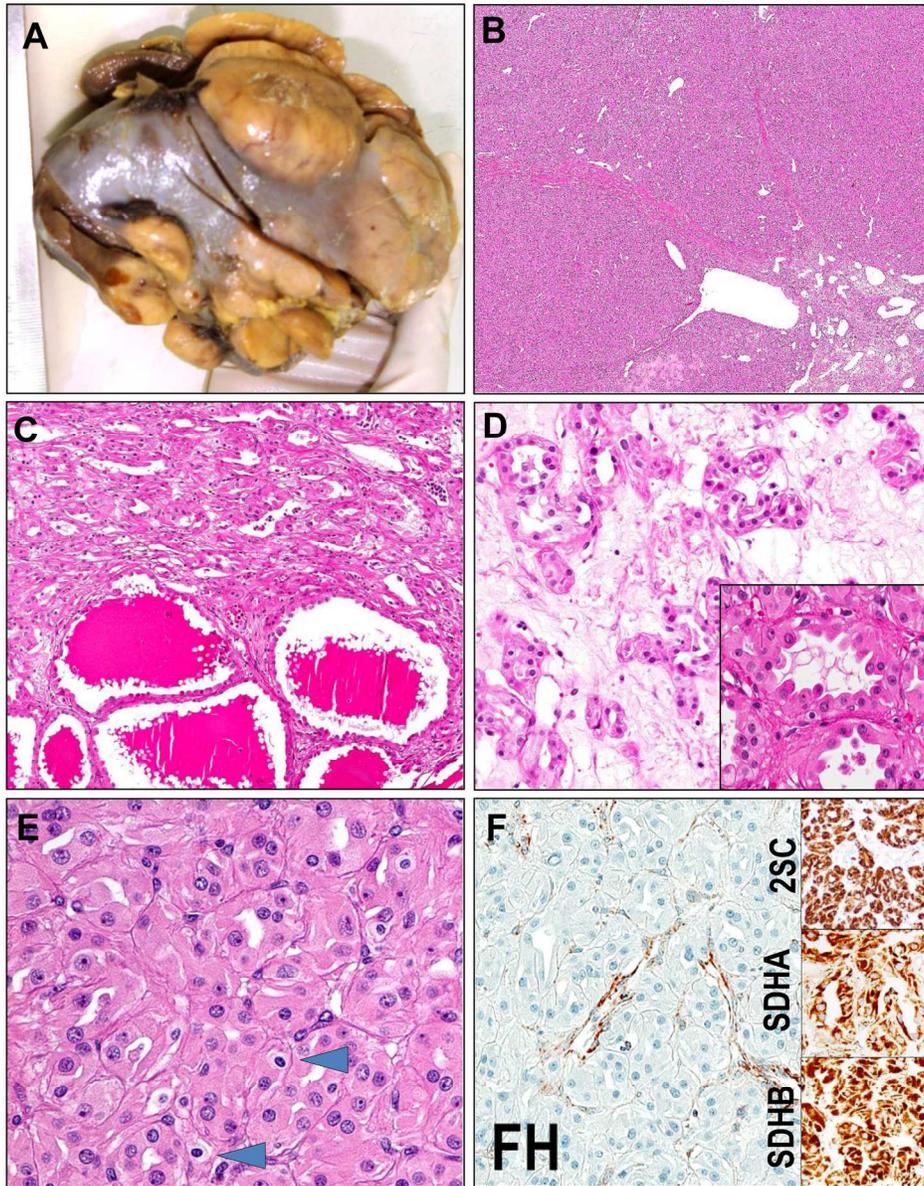


Figure 2

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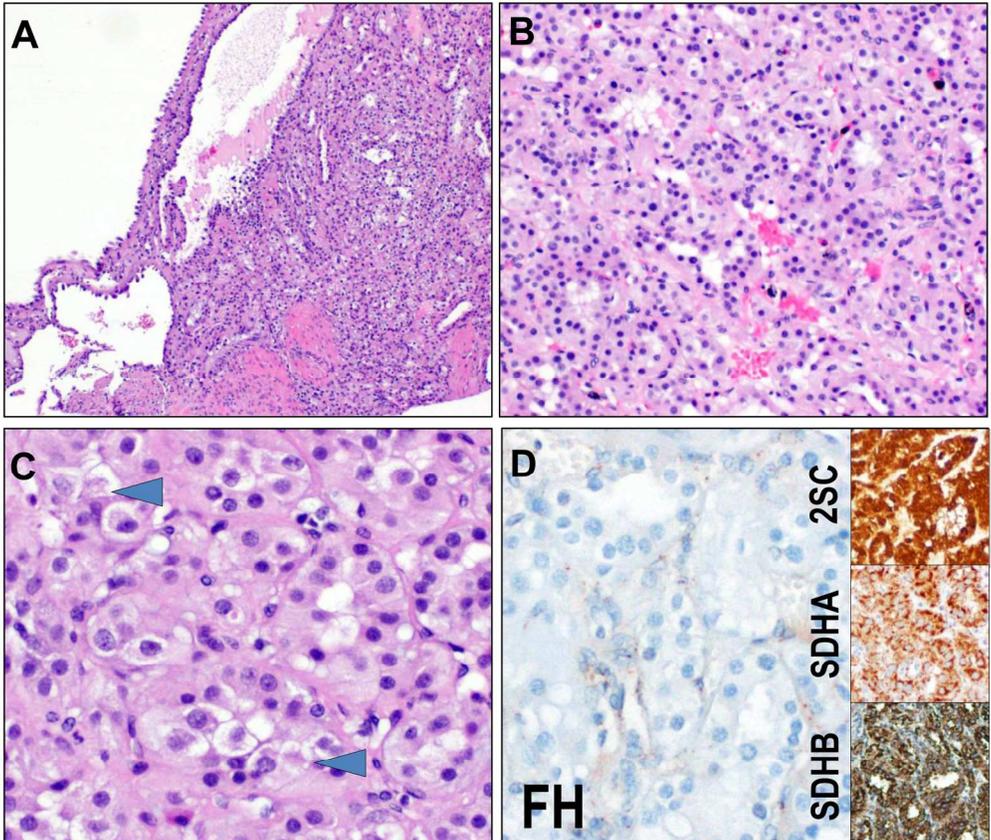


Figure 3

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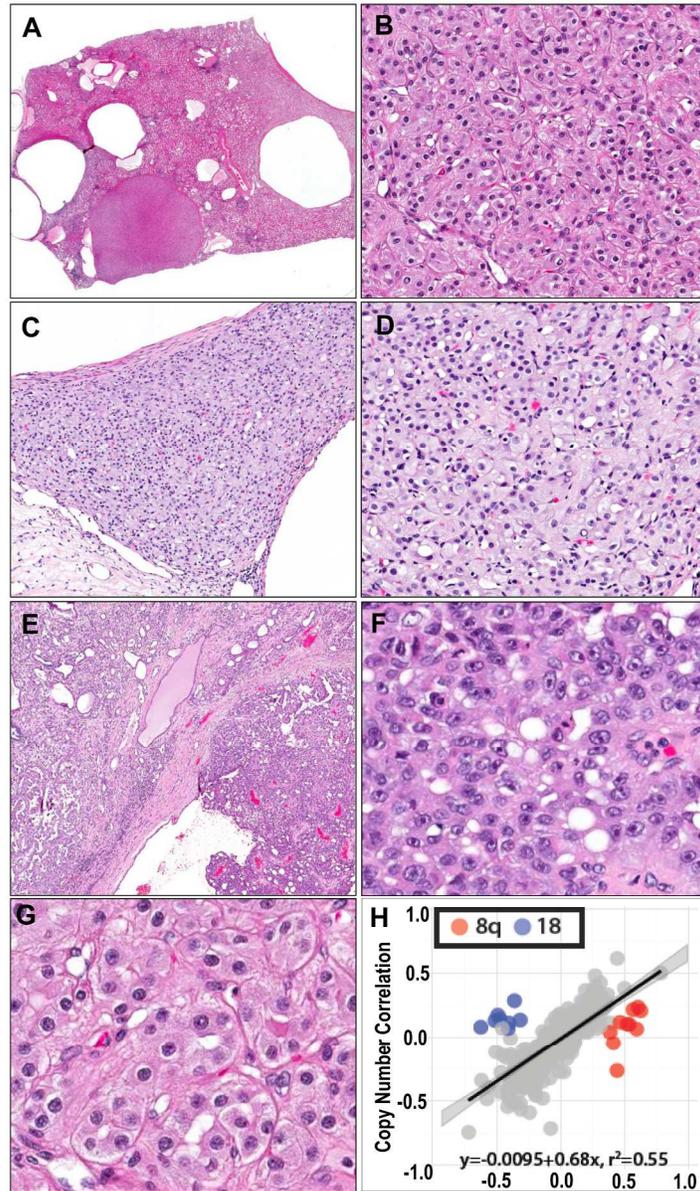


Figure 4

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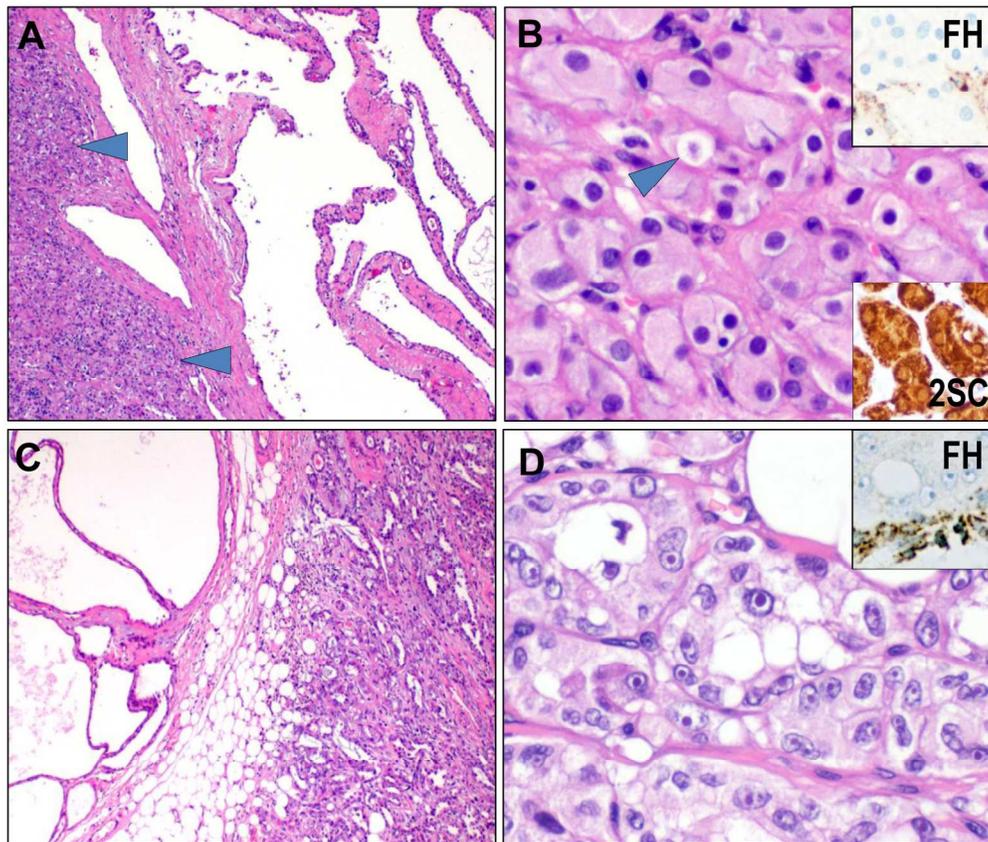


Figure 5

165x142mm (300 x 300 DPI)