



## Original contribution

# Incidence of succinate dehydrogenase and fumarate hydratase–deficient renal cell carcinoma based on immunohistochemical screening with SDHA/SDHB and FH/2SC<sup>☆,☆☆</sup>



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2SC

**Summary** Mutations of the succinate dehydrogenase (*SDHX*) enzyme subunits commonly lead to a loss of function of the holoenzyme complex, and germline *SDHX* mutations lead to a genetic predisposition to SDH-deficient neoplasms, including renal cell carcinomas (RCC). Similarly, loss-of-function alterations of *fumarate hydratase* (*FH*) leads to a genetic predisposition to hereditary leiomyomatosis and renal cell cancer (HLRCC)–associated RCC. Loss of FH leads to an accumulation of fumarate and aberrantly high levels of S-(2-succino)-cysteine (2SC). Subtype-specific consecutively diagnosed renal cell neoplasms were selected for the study and cases were not otherwise selected based on clinicopathologic features. Tissue microarrays were constructed from 1009 renal cell neoplasms (papillary: 400, clear cell: 203, chromophobe: 87, oncocytomas [original diagnosis]: 273, unclassified: 46) and these cases were immunostained for SDHA/SDHB to screen for SDH loss. A smaller subset (n = 730; oncocytomas, papillary and unclassified RCCs) were screened for FH-deficiency using immunohistochemistry for FH/2SC. Loss of SDHA/SDHB was seen in three of 273 tumors originally diagnosed as oncocytomas (1.1%). Diffuse nuclear and cytoplasmic 2SC staining, with retained FH expression was seen in one case (suggestive of dysfunctional FH protein), while absent FH was seen in 3 cases (2/400 papillary RCCs, 0.5% and 2/46 unclassified RCCs, 4.35%). No aberrant FH/2SC

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expression was noted in 273 cases originally diagnosed as oncocytomas. SDH-deficient RCCs were identified only in the cases originally diagnosed as oncocytomas (1.1%), while FH-deficient RCCs were identified in the papillary (0.5%) and unclassified RCC cohorts (4.35%). These results can help guide immunohistochemistry-based screening strategies for these tumors.

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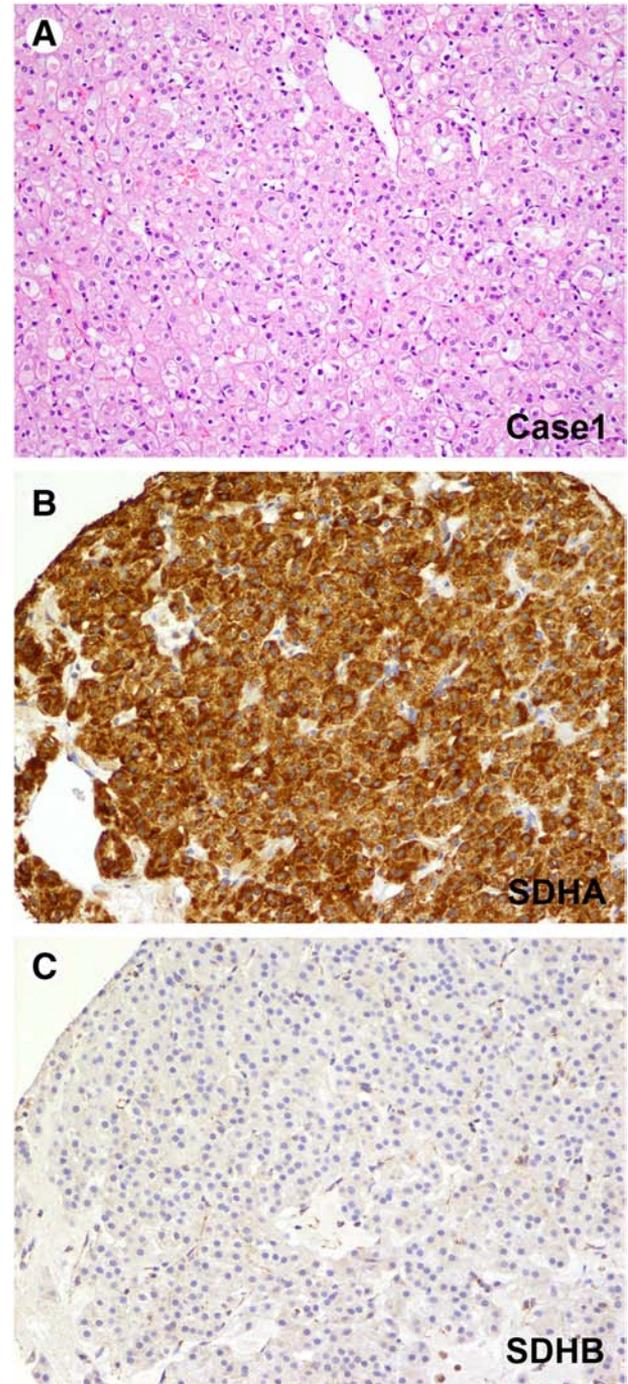
## 1. Introduction

Recently described variants of renal cell carcinomas (RCC) include succinate dehydrogenase (SDH)-deficient RCC, as well as fumarate hydratase (FH)-deficient RCC [1-6].

The SDH holoenzyme subunits (SDHA, SDHB, SDHC, and SDHD) are assembled in association with SDHAF1 and SDHF2 proteins to form complex II which participates as a component of the mitochondrial electron transport system [7]. SDH-deficient RCCs often occur in the setting of germline inactivating mutations of genes encoding SDH subunits and have frequently been reported in patients with a strong hereditary predisposition to SDH-deficient neoplasia [1,2,8]. Immunophenotypically, these tumors are characterized by a loss of SDHB, secondary to molecular alterations of the SDH holoenzyme subunits [1,2,8]. While most SDH-deficient RCCs are morphologically characterized by the presence of cells with eosinophilic cytoplasm and flocculent eosinophilic cytoplasmic inclusions, in a solid growth pattern, a subset of cases with variant histology have been reported [1,2]. Cases with variant histology include clear cell, papillary, chromophobe, and hybrid chromophobe-oncocytoma RCCs [7,9-17].

Germline mutations of *FH* lead to a genetic predisposition to hereditary leiomyomatosis and renal cell cancer-associated RCC (HLRCC) [3-6]. FH-deficient RCC can also occur in a sporadic setting [18]. Inactivation of *FH* leads to an accumulation of its substrate fumarate, which leads to aberrant protein succination, high levels of covalent modifications of cysteine residues [S-(2-succino)-cysteine (2SC)], which may serve as epigenetic modifiers [19,20]. A combination of FH/2SC immunohistochemistry is useful in screening for FH-deficient RCC with a high sensitivity and specificity [3,20]. While the morphologic spectrum of these tumors commonly includes papillary architecture with prominent eosinophilic macronucleoli and perinucleolar halos, the spectrum of reported morphologic patterns has been expanding [3,6,20,21].

In the present study, we sought to assess the incidence of both SDH-deficient and FH-deficient RCCs using an immunohistochemistry-based screening strategy involving the use of SDHA/SDHB (1009 cases screened) and FH/2SC (730 cases screened) in a series of consecutively diagnosed subtype-specific cases. In addition, we sought to assess the morphologic diversity of these tumors in the



**Fig. 1** SDH-deficient renal cell carcinoma. Representative images of an SDH-deficient renal cell carcinoma (Case 1) are depicted. An H&E-stained image (A), corresponding results of SDHA (B) and SDHB (C) immunostaining are shown.

context of previously reported SDH-deficient tumors with variant histology and the expanding morphologic spectrum of FH-deficient tumors [3-7,9-17].

## 2. Materials and methods

### 2.1. Patient specimens

Following approval from the Institutional Review Board at Mayo Clinic, Rochester, Minnesota, 1009 renal neoplasms diagnosed and treated by partial/radical nephrectomy between 1970 and 2012, were retrieved from the archival files. Apart from having carried a diagnosis of renal cell neoplasia and tissue available for analysis, cases were not otherwise selected based on clinicopathologic features. These included 5 cohorts, each of consecutively diagnosed cases of a subtype of renal cell neoplasia, including 400 papillary RCCs, 203 clear cell RCCs, 87 chromophobe RCCs, 273 oncocytomas, and 46 unclassified RCCs. All 1009 renal tumors were screened with SDHA/SDHB immunohistochemistry, while a smaller subset (400 papillary RCCs, 273 oncocytomas, and 46 unclassified RCCs) were screened with FH/2SC immunohistochemistry.

In the cohort of oncocytomas a subset was reclassified on pathologic review as renal cell neoplasms with cytoplasmic eosinophilia. The mean duration of follow up for oncocytomas was 13.3 years (median, 14.1 years; interquartile range, 6.8-19.1 years; minimum, 15 days and maximum, 40.8 years) and only 11 of 273 patients had a follow up of less than 1 year. No recurrences were identified for any of these patients on follow up. Finally, in the cohort of papillary RCCs, the mean size was 5.6 cm (median, 4.5 cm; interquartile range, 3.0-7.0 cm; minimum, 1.5 cm and maximum, 24.5 cm).

Follow-up information for cases with abnormal immunohistochemistry results was obtained by reviewing the medical record and accessing the Mayo Clinic Nephrectomy Registry.

Clinical and pathologic features collected at nephrectomy for this registry include year, age, sex, symptoms, smoking status, Eastern Cooperative Oncology Group performance status, Charlson score, body mass index, tumor size, histologic subtype, the 2018 primary tumor, regional lymph node, and distant metastases classifications (TNM), coagulative tumor necrosis, rhabdoid differentiation, and sarcomatoid differentiation. All clinical information by chart abstraction and yearly follow-up communications with patients is provided by a nurse abstractor and all pathology related data is provided by a slide review by a designated urologic pathologist using 2016 WHO/ISUP classification and grading criteria [22].

### 2.2. Immunohistochemistry

Four, 1.0 mm cores of representative formalin-fixed, paraffin-embedded tissues from each tumor were used to construct tissue microarrays (TMA) for immunophenotyping. Confirmation of aberrant SDHA/SDHB, FH/2SC immunohistochemistry results was performed on representative whole-slide sections. One case each of a papillary renal cell carcinoma and clear cell renal cell carcinoma with equivocal staining for SDHB were excluded from follow up analysis as they lacked appropriate internal control (vascular endothelial) staining. SDHA/SDHB antibodies (Abcam; clones 2E3GC12FB2AEZ and 21A11AE7) were used at a dilution of 1:200. A granular pattern of immunostaining for SDHA/SDHB in adjacent renal tubules and vascular endothelium was considered a positive internal control. FH/2SC IHC was performed as previously described [3,20,23]. Briefly, IHC was conducted by an automated Ventana Discovery system using Optiview detection system (Ventana), with 3,3'-diaminobenzidine (DAB) visualization and counterstained with hematoxylin. FH antibody (Clone J-13, Santa Cruz Biotechnology) was used at a dilution of 1:1000. 2SC polyclonal antibody (Dr. Norma Frizzell, Univ. of South Carolina) was used at a dilution of 1:2000 [20]. FH staining was scored

**Table 1** Renal cell carcinoma with aberrant SDHA/SDHB expression

Case no.	Age (y)	Gender	Follow-up (mo)	Size (cm)	pT	N	M	SDHA IHC	SDHB IHC	Histologic features	Outcome	Associated neoplasia including pheochromocytoma/ paraganglioma
1	65	F	1	3	pT1a	Nx	M0	Retained	Absent	Eosinophilic with flocculent cytoplasm	Dead of other causes	No
2	34	M	20	10.7	pT2b	Nx	M0	Retained	Absent	Eosinophilic with flocculent cytoplasm	Alive without disease	No
3	28	M	37	Bilateral; Right:9, Left:2.5	pT2a	Nx	M0	Retained	Absent	Eosinophilic with flocculent cytoplasm	Alive without disease	No

qualitatively as negative or positive when compared with internal positive controls (endothelial/stromal cells). 2SC staining was assessed for intensity (1+ to 3+) and staining pattern (nuclear and cytoplasmic *versus* cytoplasmic only), and only 3+ intensity of nuclear-cytoplasmic staining was interpreted as positive, as reported previously [3].

### 2.3. Statistical analysis

Continuous clinicopathologic variables were analyzed with frequency counts and percentages.

## 3. Results

### 3.1. SDH-deficient renal cell carcinoma: immunohistochemistry and clinicopathologic features

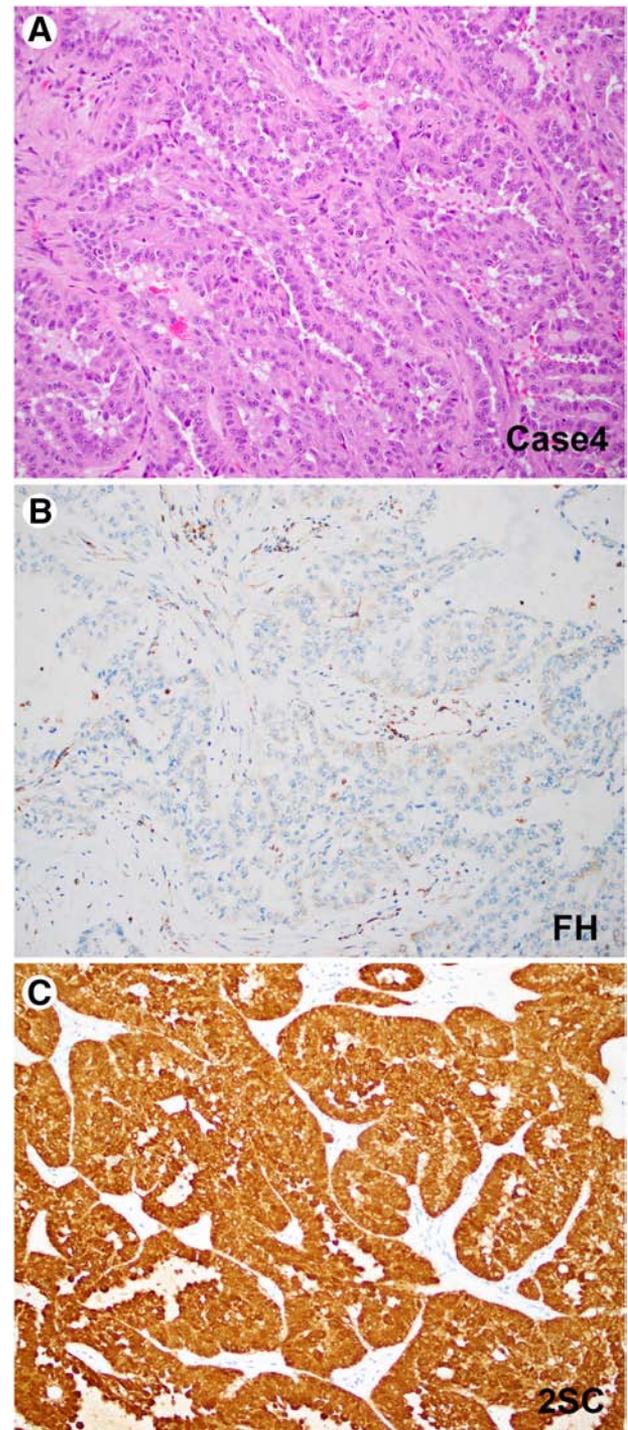
Three tumors originally diagnosed as oncocytomas showed characteristic features of SDH-deficient renal tumors characterized by eosinophilic and flocculent cytoplasm and showed absence of SDHB expression, while SDHA was retained in the same cases (Fig. 1A-C). Clinicopathologic features for these three cases are summarized in Table 1. The average age at diagnosis was 42 years (range, 28-65), the mean size of the tumors was 7.6 cm (range, 2.5-10.7 cm); all cases were pathologic stage pT2 or lower and none of the patients had documented regional or distant metastasis at presentation. One patient presented with bilateral kidney involvement. No disease recurrence, adverse outcomes or germline *SDHX* alteration-associated neoplasia were identified on follow-up.

### 3.2. FH-deficient renal cell carcinoma: immunohistochemistry and clinicopathologic features

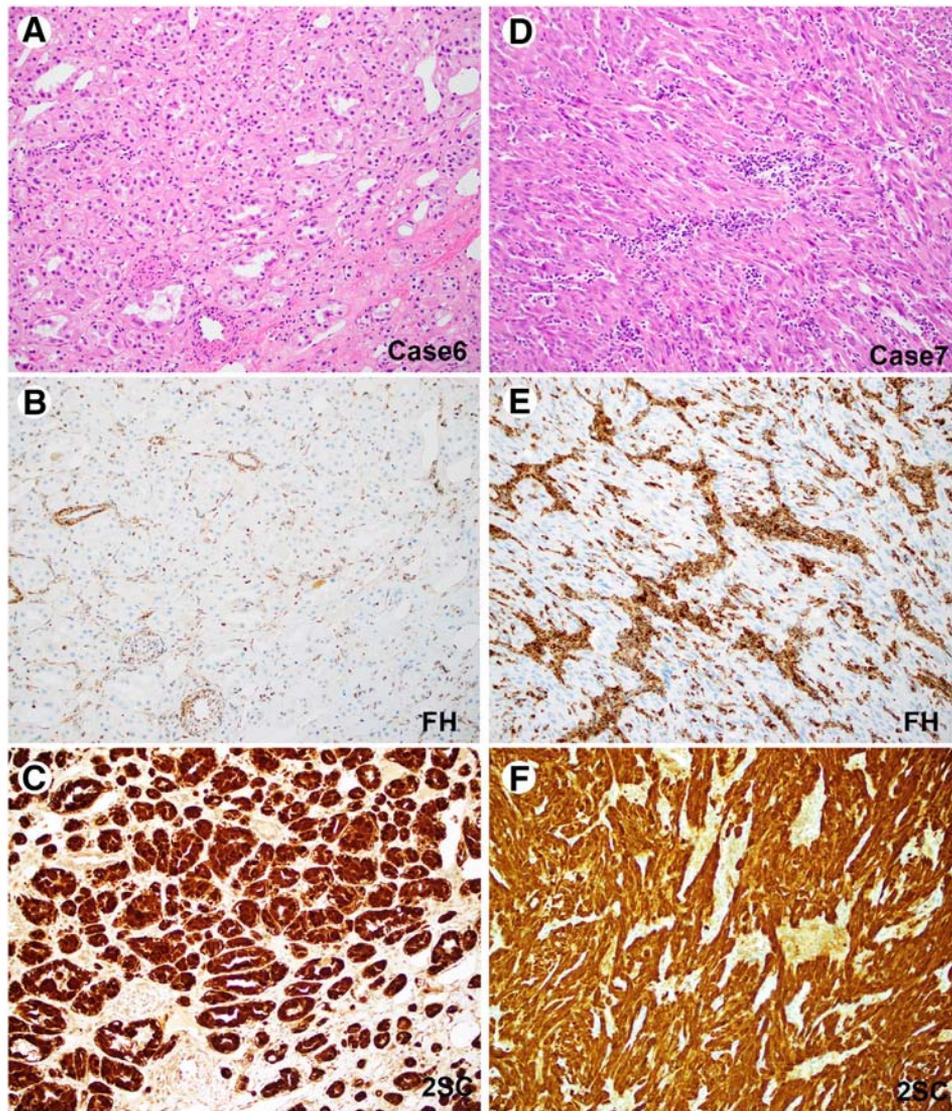
Four tumors with aberrant FH/2SC immunohistochemistry were identified (Figs. 2-4). Clinicopathologic features for all 4 cases are summarized in Table 2. Two cases showed classic morphologic features characterized by papillary architecture and prominent eosinophilic macronucleoli with perinucleolar clear halos [3]. The first case was identified in a 19-year-old female who presented with bilateral renal tumors, with the largest measuring 12 cm, and regional lymph node involvement. This patient showed an absence of fumarate hydratase expression by immunohistochemistry, coupled with an accumulation of 2SC (Case 4, Fig. 2). The second case, which was a 3 cm tumor diagnosed in a 34-year-old male, showed retained FH expression, coupled with an accumulation of 2SC, suggestive of dysfunctional FH protein (Case 5, Fig. 4).

Two additional FH-deficient renal tumors were identified in the unclassified cohort. The first was seen in a 22-year-old male and exhibited low grade oncocytic features with tubular architecture and had documented lymph node

metastasis at presentation (Case 6, Fig. 3A-C). This patient died of disease related complications at 10 months of follow-up. The second unclassified tumor, in a 66-year-old



**Fig. 2** FH-deficient (papillary) renal cell carcinoma. Representative images of an FH-deficient papillary renal cell carcinoma (Case 4) are depicted. An H&E-stained image (A), corresponding results of FH (B) and 2SC (C) immunostaining are shown.



**Fig. 3** FH-deficient (unclassified) renal cell carcinoma. Representative images of two FH-deficient (unclassified) renal cell carcinomas are depicted (Case 6, A-C; Case 7, D-F). H&E-stained images (A and D), corresponding results of FH (B and E) and 2SC (C and F) immunostaining are shown.

man showed a predominant spindle cell component arranged in fascicles without significant cytologic atypia and was also associated with lymph node involvement at presentation (Case 7, Fig. 3D-F). This patient also died of disease related complications at 29 months of follow up.

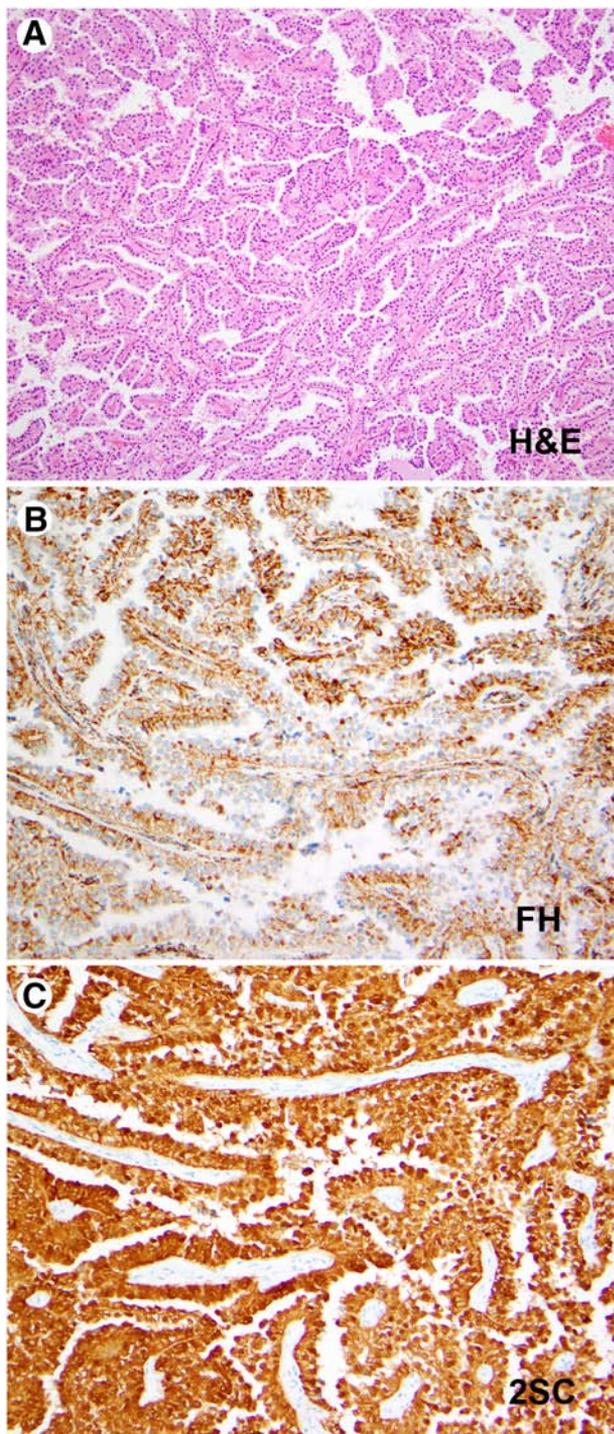
Of note, confirmatory molecular analysis was either not pursued due to lack of consent for germline testing or when pursued, molecular analysis failed. The latter was due to the poor quality of nucleic acids extracted, likely due to the advanced age of the specimens (>30 years).

#### 4. Discussion

Mutations of genes encoding SDH holoenzyme subunits such as SDHB, SDHC, and SDHD lead to a loss of SDHB

immunoreactivity; however, concurrent loss of SDHA immunoreactivity is only seen in tumors with *SDHA* mutations [24]. The vast majority of reported cases of SDH-deficient RCCs are morphologically characterized by sheets of eosinophilic cells with flocculent eosinophilic cytoplasmic inclusions [1,2]. Therefore, diagnostic algorithms used to identify these tumors, for the most part, involve morphology-based screening followed by SDHA/SDHB immunohistochemistry or molecular studies for confirmation. However, multiple reports have documented the presence of SDH alterations in renal tumors with variant histology [7-17]. We therefore sought to determine the incidence of SDH alterations using SDHA/SDHB immunohistochemistry in a large subtype-specific series of renal neoplasia.

In this study, all three cases with SDHB loss by immunohistochemistry were in the oncocytoma cohort (ie, cases



**Fig. 4** FH+/*2SC*+ Immunophenotype. Representative images of an FH-deficient papillary renal cell carcinoma are depicted (Case 5, A-C). An H&E-stained image (A), corresponding results of FH (B) and *2SC* (C) immunostaining are shown.

initially diagnosed as oncocytomas) and had characteristic histologic features of SDH-deficient renal cell carcinomas on retrospective review. Two cases with variant histology (clear cell and papillary RCC) had predominant loss of

SDHB expression and only focal staining, in the absence of a convincing pattern of internal control staining; molecular analysis failed due to the degraded nature of the nucleic acids extracted from both specimens. The presence of a metastatic pheochromocytoma with retained SDHB expression argued against the presence of a germline alteration for one of these cases. Hence, our study recommends caution in the interpretation of negative SDHA/SDHB immunohistochemistry results in cases with variant histology, particularly for clear cell RCCs with optically clear cytoplasm, as has been suggested by others [25]. In addition, SDH-deficient tumors associated with *SDHD* alterations have been reported to show a non-specific cytoplasmic blush when compared to tumors with *SDHA/SDHB/SDHC* pathogenic alterations, which normally demonstrate completely negative cytoplasmic staining [26]. However, it must be noted that the majority of SDH-deficient RCCs harbor alterations of *SDHB*, with only rare reported cases that have documented alterations of *SDHC/SDHA* [26].

In addition, we document a low incidence of SDH-deficient RCCs in the cohort of cases originally diagnosed as oncocytomas (1.1%); in comparison, the estimated reported prevalence in unselected renal cell carcinomas ranges from 0.05% to 0.2% [1]. A similar study by Cornejo et al involving 450 cases of sporadic renal epithelial neoplasia did not identify any cases with SDHB loss [25]. In the study performed by Miettinen et al interrogating 711 RCCs, the investigators identified four cases (of 711), including one clear cell RCC (of 553, 0.2%), one papillary RCC (of 33, 3%) and two unclassified RCCs (of 16, 12.5%), one of which can likely be reclassified as having characteristic morphology of an SDH-deficient RCC [11]. If these three studies are combined, the overall incidence of SDHB loss by immunostaining in clear cell RCCs is 0.1% (1 of 996), papillary RCCs is 0.2% (1 of 517) and no cases were identified in 160 chromophobe RCCs.

The morphologic spectrum of FH-deficient RCCs includes papillary, solid, tubulocystic, cribriform, cystic and the recently described low grade oncocytic forms where the latter are morphologically reminiscent of SDH-deficient RCC [3-5,18,21]. In our series, two FH-deficient RCCs were identified in the papillary RCC cohort (0.5%, 2 of 400) and two additional cases were identified in the unclassified cohort (2 of 46, 4.35%). Interestingly, one of the FH-deficient tumors in the unclassified cohort exhibited morphologic features consistent with what has been described for FH-deficient RCC showing a low grade oncocytic morphology, reminiscent of SDH-deficient RCC, and this patient died of disease at 10 months of follow up [18]. Our results suggest that FH-deficient RCCs with this morphology are likely to be exceptionally rare, given that only 1 such case was identified amongst 319 renal tumors initially classified as either oncocytomas ( $n = 273$ ) or unclassified renal tumors ( $n = 46$ ), with a prevalence of approximately and 0.3% in these groups.

**Table 2** Renal cell carcinoma with aberrant FH/2SC expression

Case no.	Age (y)	Gender	Follow-up (mo)	Size (cm)	pT	N	M	FH IHC	2SC IHC	Tumor type	Outcome	Associated neoplasia including leiomyomata
4	19	F	44	Bilateral; Right:12, Left:2.5	pT2b	N1	M0	Lost	Increased, with nuclear ;and cytoplasmic positivity	Papillary renal cell carcinoma (WHO/ISUP Grade3)	Dead of disease *	No
5	34	M	19	3	pT3a	Nx	M0	Retained	Increased, with nuclear ;and cytoplasmic positivity	Papillary renal cell carcinoma (WHO/ISUP Grade3)	Dead of disease	No
6	22	M	10	3.8	NA	N1	NA	Lost	Increased, with nuclear ;and cytoplasmic positivity	Unclassified renal cell carcinoma	Dead of disease **	No
7	66	M	29	12.5	pT3b	N1	M0	Lost	Increased, with nuclear ;and cytoplasmic positivity	Unclassified renal cell carcinoma	Dead of disease ***	No

Abbreviation: NA, not available.

\* Metastasis to the liver was detected, for Patient 4, at 11 months of follow-up. In addition, family history of maternal death from unspecified cancer at age 40 was documented in this case.

\*\* Patient 6 developed lumbar metastases 1 month after surgery.

\*\*\* Patient 7 developed pulmonary metastases 13 months after surgery.

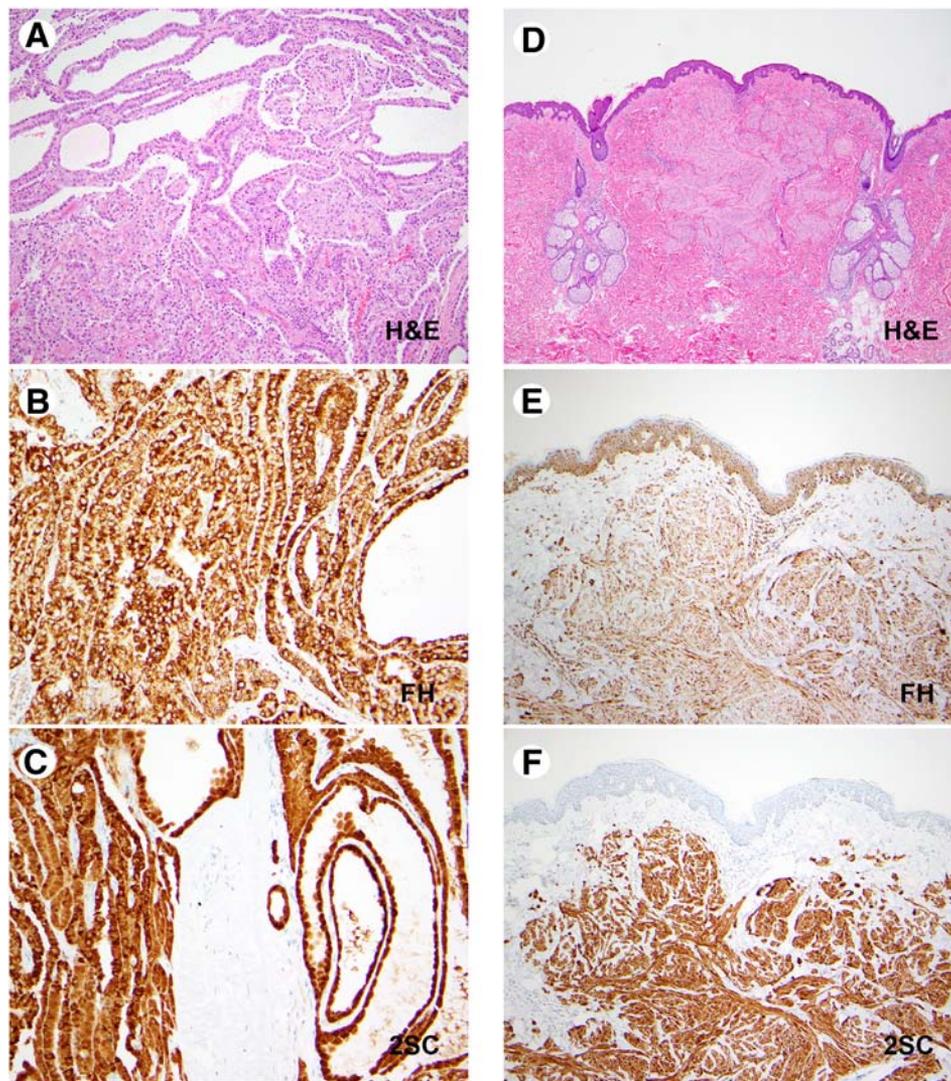
As previously reported, the common immunophenotypic pattern seen in FH-deficient RCCs is a lack of FH, coupled with positive 2SC staining (FH-/2SC+) [3-5]. A positive 2SC immunostaining pattern is defined as diffuse, strong nuclear and cytoplasmic expression and in an initial validation study of 2SC immunohistochemistry in which clear cell RCCs were used as a negative control, this pattern of immunostaining was not appreciated in any of these controls [3]. Follow-up studies have confirmed the lack of *FH* gene alterations by molecular testing in cases with retained FH, which exhibit a FH+/2SC- pattern of immunolabeling [4]. Some FH-deficient RCCs demonstrate variable FH expression, coupled with 2SC positivity (FH±/2SC+) [4]. We identified three tumors with the conventional FH-/2SC+ immunophenotype and a fourth case with a FH+/2SC+ immunophenotype. Of note, we have recently seen a case (not part of the cohort of patients included in the TMA) with an FH+/2SC+ phenotype (Fig. 5) in a patient with confirmed hereditary leiomyomatosis associated RCC, secondary to a germline alteration affecting the *FH* gene (c.1097G > A, p.S366 N). This 48-year-old male had a family history of cutaneous leiomyomas affecting his mother and maternal aunt. He presented with a 26.0 cm renal cell carcinoma with tubulocystic and poorly differentiated foci of infiltrative adenocarcinoma and developed peritoneal carcinomatosis 8 months after surgical resection. The occurrence of the FH+/2SC+ immunophenotype highlights the need for using the 2SC antibody in day-to-day clinical practice, and towards this end, additional efforts

should be implemented to incorporate this antibody into mainstream use. Furthermore, this immunophenotype suggests that the terminology of “FH-deficient RCC” should not be interpreted strictly to only include tumors with negative FH staining and should also include the spectrum of cases with dysfunctional FH expression on immunohistochemistry (FH retained/2SC positive).

In summary, SDH-deficient RCCs were identified only in the cohort of cases originally diagnosed as oncocytomas (1.1%) and FH-deficient RCCs were identified both in the papillary RCC (0.5%) and unclassified RCC cohorts (4.35%). This study helps ascertain the incidence of these tumors in a subtype-specific series of cases to help inform screening strategies for these rare tumors.

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**Fig. 5** FH+/2SC+ immunophenotype: renal cell carcinoma ;and cutaneous leiomyoma. Representative images of an FH-deficient papillary renal cell carcinoma and a cutaneous leiomyoma in a patient with a germline pathogenic *FH* alteration are depicted (*FH* c.1097G>A, p.S366N; renal cell carcinoma: A-C; cutaneous leiomyoma: D-F). H&E-stained images (A and D), corresponding results of FH (B and E) and 2SC (C and F) immunostaining are shown.

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