Histopathology

Histopathology 2017, **71**, 42–52. DOI: 10.1111/his.13183

A distinctive, low-grade oncocytic fumarate hydratase-deficient renal cell carcinoma, morphologically reminiscent of succinate dehydrogenase-deficient renal cell carcinoma

Steven C Smith,[1] Deepika Sirohi,[2] Chisato Ohe,[2] Jonathan B McHugh,[3] Jason L Hornick,[4] Jigna Kalariya,[5] Sushil Karia,[5] Katie Snape,[6] Shirley V Hodgson,[6] Andi K Cani,[3] Daniel Hovelson,[3] Daniel J Luthringer,[2] Guido Martignoni,[7,8] Ying-Bei Chen,[9] Scott A Tomlins,[3,10] Rohit Mehra[3,10] & Mahul B Amin[2,11]

[1]*Departments of Pathology and Urology, VCU School of Medicine, Richmond, VA,* [2]*Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA,* [3]*Department of Pathology, University of Michigan Medical School, Ann Arbor, MI,* [4]*Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA,* [5]*BT Savani Kidney Hospital, Rajkot, India,* [6]*St Georges Hospital, London, UK,* [7]*Department of Pathology and Public Health, University of Verona,* [8]*Department of Pathology, Pederzoli Hospital, Peschiera del Garda, Verona, Italy,* [9]*Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY,* [10]*Michigan Center for Translational Pathology, Department of Urology, Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, and* [11]*Department of Pathology and Laboratory Medicine, University of Tennessee Health Science Center, Memphis, TN, USA*

Date of submission 10 October 2016
Accepted for publication 1 February 2017
Published online *Article Accepted* 6 February 2017

Smith S C, Sirohi D, Ohe C, McHugh J B, Hornick J L, Kalariya J, Karia S, Snape K, Hodgson S V, Cani A K, Hovelson D, Luthringer D J, Martignoni G, Chen Y-B, Tomlins S A, Mehra R & Amin M B (2017) *Histopathology* **71**, 42–52. DOI: 10.1111/his.13183

A distinctive, low-grade oncocytic fumarate hydratase-deficient renal cell carcinoma, morphologically reminiscent of succinate dehydrogenase-deficient renal cell carcinoma

Aims: Fumarate hydratase (FH)-deficient renal cell carcinoma (RCC) is a high-grade, aggressive tubulopapillary carcinoma, arising predominantly in the setting of the hereditary leiomyomatosis–RCC syndrome of familial uterocutaneous leiomyomatosis and deficiency of FH. In contrast, succinate dehydrogenase (SDH)-deficient RCC is a lower-grade oncocytic carcinoma with cytoplasmic flocculence/vacuolation and inclusions, arising mostly in individuals harbouring germline mutations of subunit B of the SDH complex (SDHB). Herein we aim to report the clinicopathologic features of a novel form of FH-deficient RCC showing a low grade oncocytic morphology, reminiscent of SDH-deficient RCC.

Methods and results: These distinctive, low-grade oncocytic neoplasms, with solid, nested and focally tubular architecture (2–90 mm), arose in four males (aged 11–41 years). 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 tumour 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 tumour 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 a striking

Address for correspondence: M B Amin, Department of Pathology and Laboratory Medicine, University of Tennessee Health Sciences, 930 Madison Avenue; Suite 531, Memphis, TN 38163, USA. e-mail: mamin5@uthsc.edu

resemblance to SDH-deficient RCC, posing a diagnostic challenge and raising concerns about sampling and multifocality for syndrome-associated cases under surveillance protocols.

Keywords: fumarate hydratase-deficient renal cell carcinoma, hereditary leiomyomatosis–renal cell carcinoma syndrome, oncocytic carcinoma, renal cell carcinoma, succinate dehydrogenase-deficient renal cell carcinoma

Introduction

Recent series[1,2] have shed much light on the clinicopathological features of succinate dehydrogenase (SDH)-deficient renal cell carcinoma (RCC).[3,4] Proceeding from the initial observation of kidney tumours in kindreds with germline *SDHB* mutations and a predisposition to develop epithelioid gastrointestinal stromal tumours and multiple pheochromocytomas and paragangliomas,[5] greater experience suggests that these RCCs are morphologically distinctive,[4] are under-recognized but rare, arise with a slight male predominance in middle-aged adults, and are frequently bilateral.[1,2] The morphology is quite characteristic, with tumours 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.[6] The nuclear features are usually low-grade and monotonous, with what we have called a neuroendocrine-like chromatin pattern.[2] A subset of cases, however, have shown sarcomatoid transformation with overt nuclear atypia and an aggressive course. Although most SDH-deficient RCCs that have been tested have been shown to harbour *SDHB* mutations, scattered reports have identified *SDHA* and *SDHC* mutations as well.[7–9] In any case, to assist in their recognition, SDHB immunohistochemistry (IHC) has been used for the detection of tumours with SDH subunit mutations based on consistent loss of SDHB expression by IHC, whether there is *SDHA*, *SDHB*, *SDHC* or *SDHD* mutation, owing to mitochondrial complex II instability.[3]

In contrast, hereditary leiomyomatosis–RCC (HLRCC) syndrome represents another RCC syndrome, related to germline mutation of the fumarate hydratase (FH) gene, consisting of highly penetrant cutaneous (>90% of males and females) and uterine (~70% of females) leiomyomatosis with less prevalent (10–30%)[10–12] 'type 2 papillary' RCC[13] arising in both males and females over a diverse age range. Adrenocortical adenomas have been reported in ~10% of affected subjects.[14] The RCCs arising in HLRCC show a much more variable, high-grade morphology, including papillary, tubulopapillary, cribriform, cystic and infiltrative collecting-duct like patterns.[15,16] Prospective recognition of these tumours is emphasized, owing to their remarkable aggression, for which IHC for FH (lost in most cases with mutations[17]) and for aberrant succination of nuclear and cytoplasmic proteins [2-succinylcysteine (2SC[16])] has been used. Although most cases are thought to occur with germline rather than somatic *FH* mutations,[18] recent data have identified apparently somatic mutations in sporadic uterine leiomyomas[19] and even high-grade papillary RCCs.[20] In any case, a great many cases showing FH deficiency by IHC do not show signs of this syndrome. For this reason, we have recently proposed[21] 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.[17,22]

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

Marerials and methods

In accordance with the ethical principles of the Declaration of Helsinki, under Institutional Review Board (IRB)-approved protocols (VCU IRB no. HM20002545, valid to 26 April 2017; CSMC IRB no. Pro00027348; valid to 31 January 2018), granting waivers of consent, cases of RCC 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 clinicopathological data were collected. One case has been reported previously by Alrashdi *et al.*,[23] and this was also re-reviewed with extended

follow-up. Routine IHC, with protocols and evaluation as reported previously for FH[22] and 2SC,[16] and SDHB and SDHA,[2] was employed. For one case, multiplexed polymerase chain reaction-based next-generation sequencing (NGS) of tumour genomic DNA was performed with the Ion Ampliseq Comprehensive Cancer Panel targeting 409 cancer-related genes, including *FH* (and *SDHB*), with a validation and analysis pipeline, and copy number alteration detection as previously reported,[24–26] with cutoffs for variant detection exactly as previously reported.[22]

Results

CLINICOPATHOLOGICAL FEATURES

Table 1 shows the clinicopathological findings per case. The tumours arose in four males aged 11–41 years. Reasons for presentation included left back and abdominal pain (Case 1), imaging findings performed under syndromal surveillance (Case 2), abdominal pain and anaemia with a history of polycystic kidneys by imaging (Case 3), and incidental enlarged kidney on magnetic resonance imaging (Case 4). The oncocytic tumours were multifocal (Figure 1A) in two cases (Cases 1 and 3) and unifocal in two cases (Cases 2 and 4). Two cases (Cases 3 and 4) showed separate (one anatomically distant from the oncocytic tumour; 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 a suggestive history, including a personal history of a first-degree relative having succumbed to metastatic RCC, and a personal history of 'multicystic kidneys' by imaging. No data are available regarding HLRCC-associated signs 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 >7 years post-nephrectomy. The two cases with separate high-grade tumours showed progression, at presentation in Case 3 (liver metastasis showing morphology of the high-grade tumour at frozen section) and at 3 months after completion nephrectomy (although after 4 years had elapsed without progression since the partial nephrectomy with the oncocytic RCC) in Case 4.

The specimens evaluated were three radical nephrectomies and one partial nephrectomy. The nephrectomy of Case 1 harboured an ipsilateral adrenocortical adenoma (27 mm) (Figure 1B), whereas cutaneous biopsies performed concurrently with nephrectomy for suspected metastasis (Figure 1C) proved to be cutaneous, pilar-type leiomyomatosis (Figure 1D). The tumours were described as yellow or tan (Figure 2A), and measured, on gross examination, from 2 mm up to 90 mm. In three cases, these were seen adjacent to variable background cystic change (Figures 3A, 4A,C, and 5A). In each case, the tumours were grossly unencapsulated but appeared not to have infiltrated the adjacent structures, bulging but confined to the kidney.

HISTOLOGICAL FEATURES

Morphologically, these tumours showed low-grade oncocytic morphology, with confluent solid or nested confluent growth and prominent nodularity, closely juxtaposed to the adjacent renal parenchyma (Figures 2B and 3A). At higher power, the architecture showed a mixed pattern, in some areas with tubular morphology (Figures 2C,D and 3B,C), sometimes dilated to impart a microcystic appearance with pink luminal contents (Figure 2C). One nodule showed prominent stromal oedema with dispersed tubular aggregates of cells (Figure 2D); in other foci, tubular growth was more packed (inset). Cytologically, consistent oncocytic features were seen, with tumours being composed of polygonal cells with eosinophilic cytoplasm with scattered vacuoles and predominantly round, regular nuclei (Figures 2E, 3C, 4B,D, and 5B). The chromatin was finely granular; many examples showed micronucleoli that were not prominent at ×10 [International Society of Urological Pathology (ISUP) grade 2]. Numerous stromal mast cells were apparent in many areas (Figure 2E). All cases showed variable cytoplasmic flocculence or vacuolation; in three cases (Cases 2–4), cytoplasmic inclusions of pink hyaline material reminiscent of those that have been described in SDH-deficient RCC were readily identifiable (Figures 3C and 5B). No coagulative tumour cell necrosis was identified in any of these tumours, and nor were features of vascular invasion present, including in the multifocal cases, which were interpreted histologically as independent neoplastic foci.

Two cases (Cases 3 and 4) showed separate RCCs with features that have been associated with typical high-grade FH-deficient RCC. Case 3 showed a 35-mm tumour, in the upper pole of the kidney, distant from the oncocytic tumours, with prominent tubulocystic morphology and poorly differentiated, solid areas with a cribriform and syncytial appearance (Figure 4E,F); a metastatic liver lesion with this morphology was present at nephrectomy. In Case 4,

Table 1. Cases and clinicopathological features

				Oncocytic tumour							
Case	Sex	Age (years)	HLRCC evidence	Size (mm)	Staging*	Grade†	Encapsulation	Morphology	SDH-deficient-like cytology	Other findings	Status
1	M	35	Multiple cutaneous leiomyomata	Multiple, 2–90	mpT2bNxMx	2	Unencapsulated, non-infiltrative	Variable, solid, nested, tubular, microcysts, and stromal oedema	Cytoplasmic flocculence	Adrenocortical adenoma, 27 mm	NED, 34 months
2	M	11	Germline G354R *FH* mutation	85	pT2bNxMx	2	Unencapsulated, non-infiltrative	Solid, tubular, and cystic	Cytoplasmic vacuolation, hyaline inclusions		NED, >7 years
3	M	40	Homozygous p.K80 fs *FH* mutation in separate tumours	4 and 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
4	M	41	None available	50	pT2bNxMx	2	Unencapsulated, non-infiltrative	Solid	Cytoplasmic vacuolation, rare hyaline inclusions	Multilocular cystic change; later high-grade FH-deficient RCC	NED, 4 years (oncocytic RCC); metastasis at 3 months after high-grade RCC nephrectomy

FH, Fumarate hydratase; HLRCC, Hereditary leiomyomatosis–renal cell carcinoma; M, Male; NED, No evidence of disease; RCC, Renal cell carcinoma; SDH, Succinate dehydrogenase.

*American Joint Committee on Cancer 2010 staging, with respect to the oncocytic RCC.

†International Society of Urological Pathology nucleolar grade.

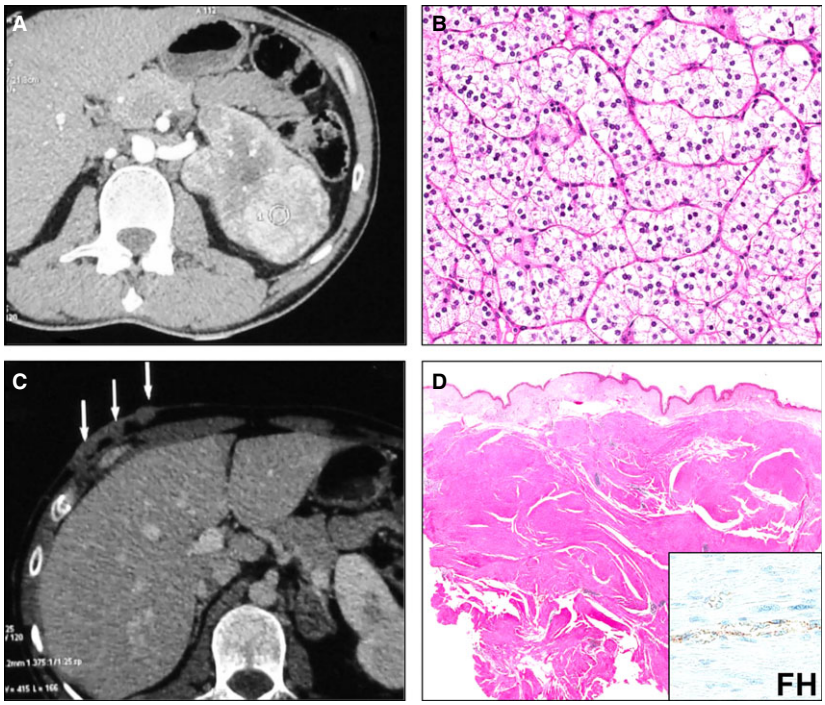

Figure 1. Clinical findings at presentation for Case 1 included enhancing, multinodular, focally calcified left kidney masses arising in the upper, interpolar and lower poles of the kidney (**A**). An enlarged ipsilateral adrenal gland excised at nephrectomy was confirmed to be an adrenocortical adenoma (**B**). Multiple nodular cutaneous tumours seen on preoperative imaging and interpreted as suspicious for metastasis (**C**, arrows) proved to be cutaneous, pilar-type leiomyomata (**D**). Fumarate hydratase immunostaining was negative in this tumour (inset: see internal control positive endothelium), a pattern that has been interpreted as highly suspicious for hereditary leiomyomatosis–renal cell carcinoma-associated cutaneous leiomyomatosis.[39] [Colour figure can be viewed at wileyonlinelibrary.com]

a completion nephrectomy was performed 4 years after the partial nephrectomy (which had shown the low-grade oncocytic RCC), and a 50-mm 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 tumours was high-grade (ISUP grade 3–4), with a syncytial appearance and prominent, viral inclusion-like nucleoli with perinuclear halos (Figure 5D).

IMMUNOHISTOCHEMICAL AND MOLECULAR FEATURES

In all four cases, the oncocytic tumours 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, and 5B). PAX8 expression was diffuse in all of the oncocytic tumours (not pictured), with variable, patchy expression of pancytokeratin AE1/AE3 and the oncocytic renal neoplasia-associated marker kidney-specific cadherin (Ksp-cadherin), as shown in Table 2. It is of note that the cutaneous leiomyoma seen in Case 1 also showed SDHB/SDHA positivity with loss of FH (Figure 1D), whereas the separate high-grade carcinomas seen in Cases 3 and 4 were both FH-deficient. In Case 3, NGS was performed on the low-grade oncocytic RCC, and identified homozygous *FH* frameshift mutations (p.K80 fs). This mutation was identical to the homozygous *FH* mutations observed in the anatomically separate high-grade FH-deficient RCC.[22] Nevertheless, on comparison of genome-wide copy number alterations between these two separate carcinomas, the oncocytic tumour 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 the recognition of distinctive morphological features of subtypes of RCC associated with genetic syndromes, including the SDH-deficient

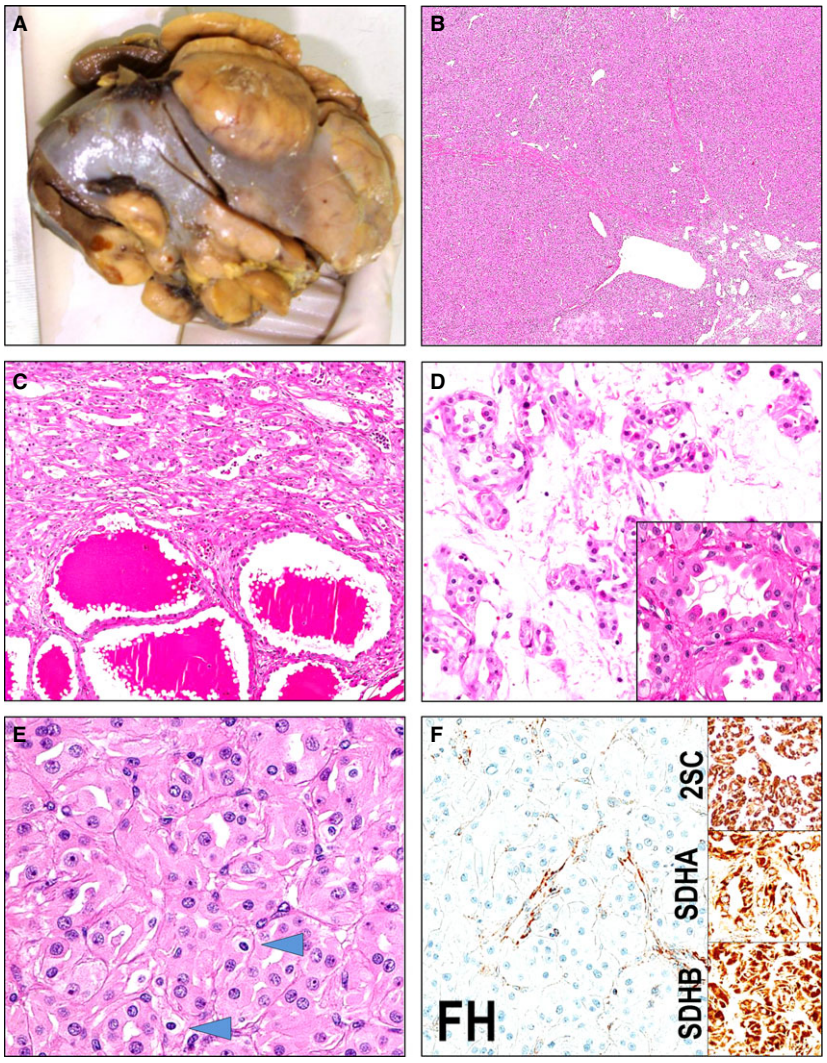

Figure 2. The kidney tumour resected in Case 1 showed grossly a partly exophytic multinodular mass with striking yellow gross morphology (**A**). At low power, a solid appearance was imparted by a tumour composed of densely packed nests of cells with eosinophilic cytoplasm (**B**). At intermediate power,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

RCCs associated with familial paraganglioma/pheochromocytoma syndromes[3,4] and the FH-deficient RCCs associated with HLRCC syndrome.[15] From the standpoint of the diagnostic pathologist, recognition of these RCC subtypes is of the utmost importance, owing to the need to risk-stratify individual tumours on the basis of increasing published experience[1,2,17,22] and to recommend genetic counselling and testing for patients and their families.

With regard to the present cases, we first note that the evidence available suggests that three of these cases represent HLRCC, although 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.[23] The first case, arising in an individual showing multiple, histologically confirmed cutaneous

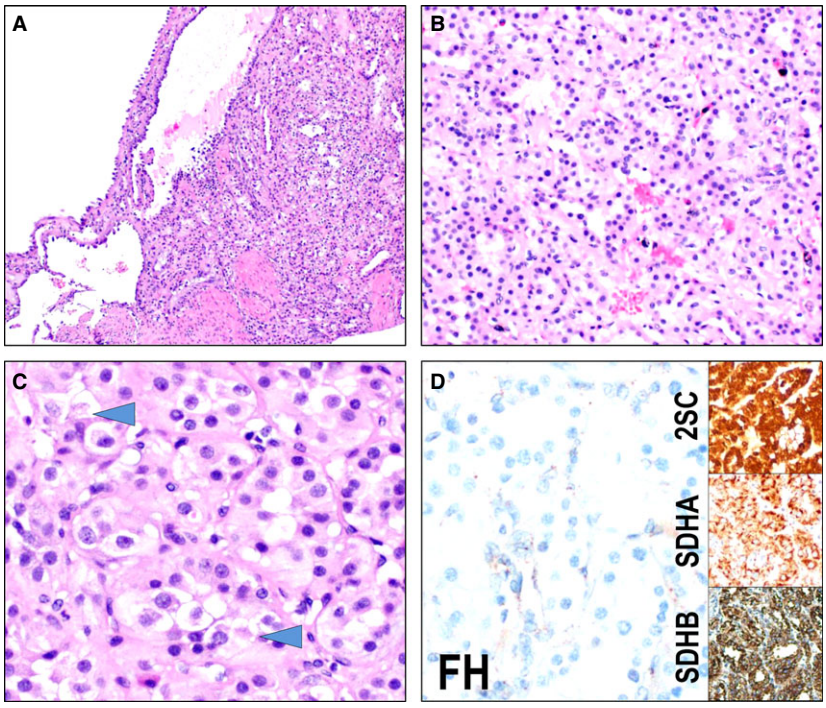

Figure 3. Case 2, arising in an 11-year-old male with proven hereditary leiomyomatosis–renal cell carcinoma, showed a dilated cyst, with adjacent cellular proliferation (**A**). At intermediate power (**B**), the carcinoma was seen to be composed of tubules and nests of oncocytic cells with indistinct nucleoli. At higher power (**C**), flocculent eosinophilic cytoplasm was readily apparent, as were several examples of large cytoplasmic vacuoles, containing eosinophilic inclusions (arrows). By immunohistochemistry (**D**), the tumour lacked expression of fumarate hydratase (note weak retention in internal control microvasculature) and showed induction of strong nucleocytoplasmic 2-succinylcysteine expression, and retained SDHB and SDHA expression (insets: as indicated). SDH, Succinate dehydrogenase. [Colour figure can be viewed at wileyonlinelibrary.com]

leiomyomas, and the third case, with the separate 'type 2 papillary' RCC or 'collecting duct carcinoma'[27] before the age of 40 years and with an affected first-degree relative, would meet the criteria reported by Menko *et al.*[28] for likely HLRCC. Quite suggestive of a constitutional mutation, this third case also had the same homozygous *FH* mutation detected in the anatomically separate SDH deficient-like and typical high-grade FH-deficient RCCs. Notably, no data regarding a family history of HLRCC or a personal or family history of uterocutaneous leiomyomatosis are available for the fourth case. In light of recently reported data showing that uterine leiomyomata and even FH-mutant RCCs may occur through somatic mutations,[19,20] we can only regard this case as an FH-deficient RCC.[21,22]

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;[15,16] 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 the surgical pathologist in the early recommendation of genetic consultation for patients with tumours in this morphological 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[29,30] and other lesions with only somatic SDH subunit mutations[31] have been described, making this issue all the more salient.

With regard to the oncocytic tumours, we do not argue that these tumours represent perfect morphological phenocopies of the most characteristic SDH-deficient RCCs. However, in each case, when encountered after our prior studies of SDH-deficient RCCs,[2] we were sufficiently suspicious that we ordered SDH-deficient workup (by IHC). Each case showed 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, although a tubular or pseudoglandular

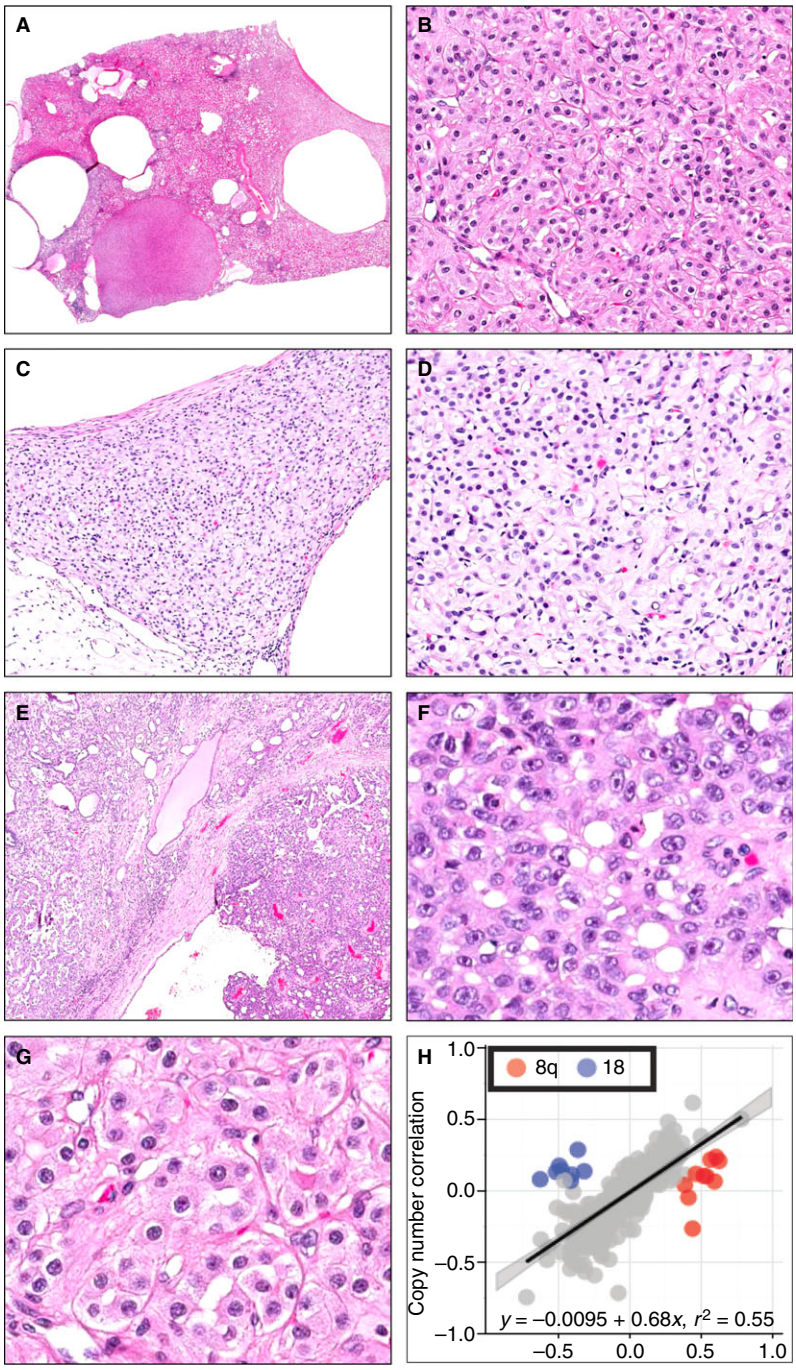

Figure 4. In the third case, the nephrectomy showed a kidney with a striking polycystic background (**A**), wherein a 4-mm solid nodule was apparent. At intermediate power, this tumour showed a pattern of dense, nested and solid oncocytic carcinoma (**B**). Another tiny nodule with a similar low-power appearance was also identified between locules of cysts (**C**), showing a similar cytomorphology (**D**). The same kidney harboured an anatomically separate, synchronous tumour composed of high-grade infiltrative, tubular and cribriform carcinoma (**E**) with large nuclei of high [International Society of Urological Pathology (ISUP)] nucleolar grade, prominent nucleoli with perinuclear clearing, and a syncytial appearance (**F**). This starkly contrasted with the ISUP nucleolar grade 2 features of the oncocytic tumours (**G**, at the same magnification as **F** for comparison). Both tumours showed loss of fumarate hydratase (FH) expression, induction of 2-succinylcysteine expression and retained SDHB expression as determined by immunohistochemistry (not shown), and targeted next-generation sequencing demonstrated identical homozygous *FH* p.K80 fs mutations. On comparison of genome-wide copy number calls via a scatter plot of $\log_2$ gene-level copy number ratios between the oncocytic tumour (ordinate) and the synchronous high-grade tumour (abscissa), the oncocytic tumour lacked gains at 8q (red) and losses of chromosome 18 (blue). SDH, Succinate dehydrogenase.

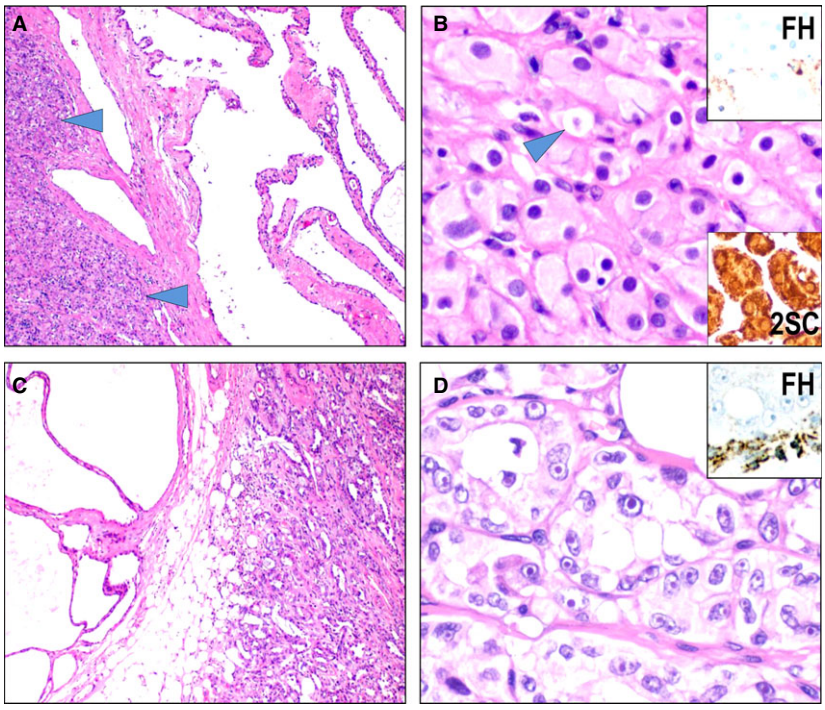

Figure 5. In the fourth case, a partial nephrectomy was performed, sampling a solid lesion in an enlarged multicystic kidney. A 50-mm solid nodule was seen (**A**, arrows) adjacent to a multicystic lesion, composed of nests of oncocytic cells with even chromatin and indistinct nucleoli (**B**). The cytoplasm was variably flocculent, with infrequent vacuoles with eosinophilic inclusion-like bodies (arrow). fumarate hydratase (FH) expression was lost, whereas strong/diffuse nucleocytoplasmic 2-succinylcysteine expression was identified (insets); SDHA and SDHB expression was retained (not shown). Although completion nephrectomy was recommended, it was performed 4 years later, showing a high-grade, infiltrative renal cell carcinoma (RCC) with glandular, solid and tubulocystic growth patterns, invasive of renal sinus adipose (**C**). The cytology of this later tumour, 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. SDH, Succinate dehydrogenase. [Colour figure can be viewed at wileyonlinelibrary.com]

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

FH, Fumarate hydratase; Ksp-cadherin, Kidney-specific cadherin; PanCK, Pancytokeratin AE1/AE3; PAX8, Paired-box 8; SDHA, Succinate dehydrogenase, subunit A; SDHB, Succinate dehydrogenase, subunit B; 2SC, 2-Succinylcysteine.

pattern, as reported previously in SDH-deficient RCCs,[1] 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, similarly to SDH-deficient RCC,[2] as is characteristic of oncocytic renal neoplasia generally,[32] as was PAX8. Most important, however, was the observation in all three tumours of consistently retained SDHB expression in a strong, diffuse manner, reflective of an intact SDH complex.[33] In contrast, FH expression was lost and 2SC expression was strongly, diffusely induced in a nucleocytoplasmic manner, a pattern that has proven, in our experience[16,22,34] and that of others,[17,35,36] to be demonstrative of *FH* mutation and loss of function. Thus, we interpret the FH-negative/2SC-positive immunophenotype in each of these tumours as being confirmatory of their specificity to FH deficiency and exclusive of their representing an

aetiologically unrelated epiphenomenon (sporadic-type carcinomas arising in the polycystic background, for instance).

In summary, we describe four intriguing cases: tumours with low-grade oncocytic morphology and variable cytoplasmic vacuolation and flocculence. On morphological grounds, these tumours were first deemed to be quite reminiscent of SDH-deficient RCC. However, immunophenotypic workup points to their representing a novel morphological 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 that are quite suggestive of (or confirmed) HLRCC in three of them. We speculate that these tumours represent yet another example of an emerging phenomenon of phenotypic 'crossover lesions' between FH-deficient and SDH-deficient syndromes, analogous to recently described paragangliomas occurring in the setting of germline *FH* mutation.[35,37,38]

Two of these novel cases also raise the possibility that, when not associated with a high-grade FH-deficient RCC, this oncocytic tumour type could be associated with a more favourable outcome. High-grade FH-deficient RCCs have been associated with progression and/or death from disease in 30–80% of cases.[15,17,22] Our experience with these low-grade oncocytic tumours emphasizes the importance of morphological context for interpreting IHC and molecular findings. Despite the shared 'FH-deficient RCC' status of both the low-grade oncocytic tumours and typical high-grade FH-deficient RCCs, such a high-risk label would have been inappropriate for the low-grade tumours, given that progression was only seen in cases with a separate high-grade tumour. Certainly, much more study is needed, and, prospectively, we recommend careful inquiry regarding the nature of any syndromal signs and the 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 recommend careful correlation between imaging, sampling and histopathology for surveillance or sampling protocols.

Acknowledgements

The authors would like to thank Ann Bialik (MSKCC) and Jorge Almenara (VCU) for technical assistance with immunohistochemistry. S. A. Tomlins is supported by the A. Alfred Taubman Medical Research Institute. C. Ohe has fellowship training support from the Uehara Memorial Foundation.

Conflicts of interest

S. A. Tomlins had a prior sponsored research agreement with and has received travel support from Thermo Fisher Scientific. A preliminary version of this project was presented at the 2015 Annual Meeting of the United States and Canadian Academy of Pathology.

Author contributions

S. C. Smith, D. Sirohi, C. Ohe and M. B. Amin designed the studies. S. C. Smith, D. Sirohi, J. B. McHugh, J. L. Hornick, J. Kalariya, S. Karia, K. Snape, S. V. Hodgson, A. K. Cani, D. Hovelson, D. J. Luthringer, G. Martignoni, Y.-B. Chen, S. A. Tomlins, R. Mehra and M. B. Amin contributed and analysed data. S. C. Smith, J. B. McHugh, J. L. Hornick, A. K. Cani, D. Hovelson, D. J. Luthringer, Y.-B. Chen, S. A. Tomlins and R. Mehra performed immunohistochemical and molecular studies. S. C. Smith and M. B. Amin wrote the manuscript. All authors revised and endorse the manuscript.

References

1. Gill AJ, Hes O, Papathomas T *et al.* Succinate dehydrogenase (SDH)-deficient renal carcinoma: a morphologically distinct entity: a clinicopathologic series of 36 tumors from 27 patients. *Am. J. Surg. Pathol.* 2014; **38**; 1588–1602.
2. Williamson SR, Eble JN, Amin MB *et al.* Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. *Mod. Pathol.* 2015; **28**; 80–94.
3. Gill AJ, Pachter NS, Clarkson A *et al.* Renal tumors and hereditary pheochromocytoma-paraganglioma syndrome type 4. *N. Engl. J. Med.* 2011; **364**; 885–886.
4. Gill AJ, Pachter NS, Chou A *et al.* Renal tumors associated with germline SDHB mutation show distinctive morphology. *Am. J. Surg. Pathol.* 2011; **35**; 1578–1585.
5. Vanharanta S, Buchta M, McWhinney SR *et al.* Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am. J. Hum. Genet.* 2004; **74**; 153–159.
6. Housley SL, Lindsay RS, Young B *et al.* Renal carcinoma with giant mitochondria associated with germ-line mutation and somatic loss of the succinate dehydrogenase B gene. *Histopathology* 2010; **56**; 405–408.
7. Jiang Q, Zhang Y, Zhou YH *et al.* A novel germline mutation in SDHA identified in a rare case of gastrointestinal stromal tumor complicated with renal cell carcinoma. *Int. J. Clin. Exp. Pathol.* 2015; **8**; 12188–12197.
8. Ozluk Y, Taheri D, Matoso A *et al.* Renal carcinoma associated with a novel succinate dehydrogenase a mutation: a case

report and review of literature of a rare subtype of renal carcinoma. *Hum. Pathol.* 2015; **46**; 1951–1955.
9. Yakirevich E, Ali SM, Mega A *et al.* A novel SDHA-deficient renal cell carcinoma revealed by comprehensive genomic profiling. *Am. J. Surg. Pathol.* 2015; **39**; 858–863.
10. Alam NA, Barclay E, Rowan AJ *et al.* Clinical features of multiple cutaneous and uterine leiomyomatosis: an underdiagnosed tumor syndrome. *Arch. Dermatol.* 2005; **141**; 199–206.
11. Srigley JR, Delahunt B, Eble JN *et al.* The International Society of Urological Pathology (ISUP) Vancouver classification of renal neoplasia. *Am. J. Surg. Pathol.* 2013; **37**; 1469–1489.
12. Toro JR, Nickerson ML, Wei MH *et al.* Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am. J. Hum. Genet.* 2003; **73**; 95–106.
13. Launonen V, Vierimaa O, Kiuru M *et al.* Inherited susceptibility to uterine leiomyomas and renal cell cancer. *Proc. Natl Acad. Sci. USA* 2001; **98**; 3387–3392.
14. Shuch B, Ricketts CJ, Vocke CD *et al.* Adrenal nodular hyperplasia in hereditary leiomyomatosis and renal cell cancer. *J. Urol.* 2013; **189**; 430–435.
15. Merino MJ, Torres-Cabala C, Pinto P, Linehan WM. The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. *Am. J. Surg. Pathol.* 2007; **31**; 1578–1585.
16. Chen YB, Brannon AR, Toubaji A *et al.* Hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cancer: recognition of the syndrome by pathologic features and the utility of detecting aberrant succination by immunohistochemistry. *Am. J. Surg. Pathol.* 2014; **38**; 627–637.
17. Trpkov K, Hes O, Agaimy A *et al.* Fumarate hydratase-deficient renal cell carcinoma is strongly correlated with fumarate hydratase mutation and hereditary leiomyomatosis and renal cell carcinoma syndrome. *Am. J. Surg. Pathol.* 2016; **40**; 865–875.
18. Barker KT, Bevan S, Wang R *et al.* Low frequency of somatic mutations in the FH/multiple cutaneous leiomyomatosis gene in sporadic leiomyosarcomas and uterine leiomyomas. *Br. J. Cancer* 2002; **87**; 446–448.
19. Harrison WJ, Andrici J, Maclean F *et al.* Fumarate hydratase-deficient uterine leiomyomas occur in both the syndromic and sporadic settings. *Am. J. Surg. Pathol.* 2016; **40**; 599–607.
20. Cancer Genome Atlas Research Network, Linehan WM, Spellman PT, Ricketts CJ *et al.* Comprehensive molecular characterization of papillary renal-cell carcinoma. *N. Engl. J. Med.* 2016; **374**; 135–145.
21. Smith SC, Trpkov K, Mehra R *et al.* Is tubulocystic carcinoma with dedifferentiation a form of HLRCC/fumarate hydratase-deficient RCC? *Mod. Pathol.* 2015; **28**; 260A.
22. Smith SC, Trpkov K, Chen YB *et al.* Tubulocystic carcinoma of the kidney with poorly differentiated foci: a frequent morphologic pattern of fumarate hydratase-deficient renal cell carcinoma. *Am. J. Surg. Pathol.* 2016; **40**; 1457–1472.
23. Alrashdi I, Levine S, Paterson J *et al.* Hereditary leiomyomatosis and renal cell carcinoma: very early diagnosis of renal cancer in a paediatric patient. *Fam. Cancer* 2010; **9**; 239–243.
24. Grasso C, Butler T, Rhodes K *et al.* Assessing copy number alterations in targeted, amplicon-based next-generation sequencing data. *J. Mol. Diagn.* 2015; **17**; 53–63.
25. Warrick JI, Hovelson DH, Amin A *et al.* Tumor evolution and progression in multifocal and paired non-invasive/invasive urothelial carcinoma. *Virchows Arch.* 2015; **466**; 297–311.
26. Kadakia KC, Tomlins SA, Sanghvi SK *et al.* Comprehensive serial molecular profiling of an 'n of 1' exceptional non-responder with metastatic prostate cancer progressing to small cell carcinoma on treatment. *J. Hematol. Oncol.* 2015; **8**; 109.
27. Lehtonen HJ. Hereditary leiomyomatosis and renal cell cancer: update on clinical and molecular characteristics. *Fam. Cancer* 2011; **10**; 397–411.
28. Menko FH, Maher ER, Schmidt LS *et al.* Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. *Fam. Cancer* 2014; **13**; 637–644.
29. van Nederveen FH, Korpershoek E, Lenders JW, de Krijger RR, Dinjens WN. Somatic SDHB mutation in an extraadrenal pheochromocytoma. *N. Engl. J. Med.* 2007; **357**; 306–308.
30. Giubellino A, Lara K, Martucci V *et al.* Urinary bladder paragangliomas: how immunohistochemistry can assist to identify patients with SDHB germline and somatic mutations. *Am. J. Surg. Pathol.* 2015; **39**; 1488–1492.
31. Gill AJ, Toon CW, Clarkson A *et al.* Succinate dehydrogenase deficiency is rare in pituitary adenomas. *Am. J. Surg. Pathol.* 2014; **38**; 560–566.
32. Kuehn A, Paner GP, Skinnider BF *et al.* Expression analysis of kidney-specific cadherin in a wide spectrum of traditional and newly recognized renal epithelial neoplasms: diagnostic and histogenetic implications. *Am. J. Surg. Pathol.* 2007; **31**; 1528–1533.
33. Gill AJ, Benn DE, Chou A *et al.* Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes. *Hum. Pathol.* 2010; **41**; 805–814.
34. Chen YB, Kong MX, Bialik A *et al.* Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)-associated renal cancer: a comparison of fumarate hydratase (FH) and S-(2-succino)-cysteine (2SC) immunohistochemistry as ancillary tools. *Mod. Pathol.* 2015; **28**; 211A.
35. Castro-Vega LJ, Buffet A, De Cubas AA *et al.* Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum. Mol. Genet.* 2014; **23**; 2440–2446.
36. Joseph NM, Solomon DA, Frizzell N, Rabban JT, Zaloudek C, Garg K. Morphology and immunohistochemistry for 2SC and FH aid in detection of fumarate hydratase gene aberrations in uterine leiomyomas from young patients. *Am. J. Surg. Pathol.* 2015; **39**; 1529–1539.
37. Clark GR, Sciacovelli M, Gaude E *et al.* Germline FH mutations presenting with pheochromocytoma. *J. Clin. Endocrinol. Metab.* 2014; **99**; E2046–E2050.
38. Letouze E, Martinelli C, Loriot C *et al.* SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* 2013; **23**; 739–752.
39. Llamas-Velasco M, Requena L, Kutzner H *et al.* Fumarate hydratase immunohistochemical staining may help to identify patients with multiple cutaneous and uterine leiomyomatosis (MCUL) and hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome. *J. Cutan. Pathol.* 2014; **41**; 859–865.