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SMARCA4-deficient undifferentiated uterine sarcoma (malignant rhabdoid tumor of the uterus): a clinicopathologic entity distinct from undifferentiated carcinoma

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Abstract

Small cell carcinoma of the ovary, hypercalcemic type is a rare, aggressive malignancy which usually occurs in young women and is characterized by mutations in *SMARCA4*, with few other alterations. We recently encountered uterine tumors with morphologic, immunohistochemical, and genetic similarities to small cell carcinoma of the ovary, hypercalcemic type. Herein we report the clinicopathologic and molecular features (using a targeted massively parallel sequencing [MPS] assay) of these tumors. The cases were diagnosed on cervical and endometrial biopsies (*n* = 2, 34, and 29 years) or hysterectomy and bilateral salpingo-oophorectomy (*n* = 3, 25, 33, and 58 years). The tumors were composed of sheets of large atypical epithelioid cells with prominent rhabdoid morphology, indistinguishable from the "large cell" variant of small cell carcinoma of the ovary, hypercalcemic type. In three cases, the ovaries were pathologically examined to exclude a primary ovarian malignancy. Immunohistochemically, four of four cases showed SMARCA4 loss, and were negative or only focally positive for keratins, EMA, and claudin-4. One of three cases was positive for WT-1. Targeted MPS was successfully performed on 4 of 5 tumors, and showed recurrent mutations in *SMARCA4*, with few other alterations. Of the cases diagnosed on hysterectomy, all had extensive lymphovascular invasion, extra-uterine spread, and marked infiltrative growth. These tumors were uniformly aggressive; all patients died of disease (median survival 7 months, range 1–43 months). We propose this entity be called "SMARCA4-deficient undifferentiated uterine sarcoma (malignant rhabdoid tumor of the uterus)", a term which describes both the tumor's underlying molecular abnormality and its morphology. Its unique clinicopathologic and molecular features differentiate it from other related malignancies, including undifferentiated endometrial carcinoma, small cell carcinoma of the ovary (hypercalcemic type), and epithelioid sarcoma. We review and discuss previously reported "rhabdoid tumors of the uterus;" while they are a heterogenous group of tumors, some of them are likely examples of this entity. Correctly identifying cases of SMARCA4-deficient uterine sarcoma from histologic mimics is important as it may have prognostic, predictive, and germline implications.

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Introduction

Small cell carcinoma of the ovary, hypercalcemic type is a rare, aggressive ovarian tumor that occurs in young females [1]. Recently, germline and/or somatic mutation in *SMARCA4*, a member of the SWI/SNF chromatin remodeling complex, was found to be its main underlying molecular alteration [2–4]. This has facilitated its diagnosis by using widely available immunohistochemistry for SMARCA4 (also known as BRG1), which shows loss of expression in tumor cells. In the ovary, loss of SMARCA4 is a relatively sensitive and specific marker for small cell carcinoma of the ovary, hypercalcemic type [5]. Since the discovery of *SMARCA4* mutations as the defining molecular

feature of small cell carcinoma of the ovary, hypercalcemic type, some have proposed a shift in nomenclature to "malignant rhabdoid tumor of the ovary" given its similarity to malignant rhabdoid tumors associated with loss of SMARCB1 (also known as INI-1), a related protein [6]. SWI/SNF-deficient sarcomas have been reported in other anatomic sites, including atypical teratoid/rhabdoid tumors of the CNS, and the recently described SMARCA4-deficient thoracic sarcoma [7]. However, a uterine variant has not been previously characterized.

Herein, we provide the first unified description, including immunophenotypic and molecular characterization, of a new member of the SWI/SNF family of tumors: SMARCA4-deficient undifferentiated uterine sarcoma/ malignant rhabdoid tumor of the uterus. These aggressive tumors have distinct clinicopathologic and molecular features which differentiate them from histologic mimics including undifferentiated endometrial carcinomas, high-grade endometrial stromal sarcoma, leiomyosarcoma, rhabdomyosarcoma, PEComa, Müllerian adenosarcoma, melanoma, and epithelioid sarcoma. Differentiating cases of SMARCA4-deficient uterine sarcoma from other histologically similar tumors may have prognostic, predictive, and genetic implications for patients.

Materials and methods

An index case of a SMARCA4-deficient uterine sarcoma was identified, which prompted reevaluation of two others cases seen by one of the authors (BH) over a period of 4 years that had similar clinicopathologic features. Two additional cases were identified for inclusion in the study by reviewing pathology reports containing the term "undifferentiated carcinoma," and then reviewing their morphology and immunohistochemistry, for a total of five cases.

Immunohistochemistry

Immunohistochemistry was performed on 4 µm thick sections from formalin-fixed paraffin embedded tissue. The clone, dilution, and supplier used for each antibody are listed in Table 1. Appropriate positive and negative controls were examined for each immunohistochemical stain.

Targeted massively parallel sequencing (Oncopanel)

The methodology of MPS implementation has been previously reported in detail [8, 9]. In brief, tumor DNA was isolated from formalin-fixed, paraffin embedded tissue and enriched for exonic regions of 447 tumor suppressor genes and oncogenes (Supplemental Material) by solution-based hybrid capture. Sequencing was performed using Illumina HiSeq 2500, and analysis was performed via a custom informatics pipeline.

Results

Five cases for study were identified, three of which had sufficient tissue for complete characterization. Their clinicopathologic features are summarized in Table 2, and presented in greater detail below.

Clinicopathologic features

Case 1

The patient was a 25-year-old female who presented with a large mass protruding through the cervix. A biopsy at the outside hospital was reported as most consistent with a high-grade adenosarcoma, associated with large round cell epithelioid areas, hemorrhage, and necrosis.

The patient underwent a hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and pelvic lymph node dissection. Grossly, a 9 cm mass arose from the endomyometrium, invaded through >50% of the myometrial thickness extending to within 0.5 cm of the serosa, involved the cervix and extended to the parametrial and paracervical resection margins. Macroscopic tumor also involved the omentum, and bilateral fallopian tube serosa. Microscopically, the tumor was composed of epithelioid cells displaying a prominent "rhabdoid" morphology reminiscent of epithelioid sarcoma that infiltrated around normal glands that had a vague leaf-like architecture (Fig. 1). The mitotic rate reached 11 per 10 high power fields (HPFs). There was extensive lymphovascular space invasion, involving uterine and adnexal vessels. Although tumor was present in ovarian lymphovascular spaces, there was no tumor in the ovarian parenchyma. Tumor was metastatic to 3 of 4 pelvic lymph nodes, with extranodal extension. Endometriosis was present in parametrial soft tissue. An extensive immunohistochemical work-up at the outside hospital showed that the tumor cells were positive for vimentin, and negative for pan-keratin, EMA, CK7, CD45, HMB-45, S100, and germ cell markers. Subsequently, immunohistochemistry was performed at BWH, and the tumor demonstrated loss of SMARCA4 expression, had retained/intact SMARCB1, MSH6, and PMS2 expression, and was negative for claudin-4, WT-1, SMA, desmin, and caldesmon (Fig. 2). The case was received in consultation and a diagnosis of adenosarcoma with extensive undifferentiated epithelioid component was favored by the submitting pathologist. The patient died of disease 7 months after hysterectomy.

Table 1 Antibody dilutions, clones, and suppliers used for immunohistochemical stains

Antibody	Clone	Dilution	Supplier
SMARCA4	ERP3912	1:50	Abcam, Cambridge, MA, USA
Cyclin D1	SP4	1:50	ThermoScientific, Waltham, MA, USA
Inhibin	R1	1:20	Bio-Rad Antibodies, Hercules, CA, USA
Caldesmon	h-CD	1:300	Dako, Carpinteria, CA, USA
NUT	C52B1	1:200	Cell Signaling Technology, Danvers, MA, USA
BerEP4	BerEP4	1:100	Dako, Carpinteria, CA, USA
SMARCB1	25	1:250	BD Biosciences, Woburn, MA, USA
Claudin-4	3E2C1	1:500	Invitrogen, Carlsbad, CA, USA
CAM5.2	CAM5.2	1:50	BD Biosciences, Woburn, MA, USA
EMA	E29	1:200	Dako, Carpinteria, CA, USA
SALL4	6E3	1:4000	Abnova, Walnut, CA, USA
WT-1	6F-H2	1:75	Dako, Carpinteria, CA, USA
MLH1	NCL-L-MLH1	1:100	Leica Biosystems, Buffalo Grove, IL, USA
MSH2	FE11	1:200	Oncogene Research Products, La Jolla, CA, USA
MSH6	PU29	1:50	Leica Biosystems, Buffalo Grove, IL, USA
PMS2	MRQ-28	1:50	Cell Marque, Rocklin, CA, USA
Pan-keratin	MNF116	1:700	Dako, Carpinteria, CA, USA
SOX10	EP 268	1:2000	Cell Marque, Rocklin, CA, USA
S100	Polyclonal	1:3000	Dako, Carpinteria, CA, USA
HMB-45	HMB-45	1:400	Dako, Carpinteria, CA, USA
PTEN	Polyclonal	1:200	Cell Signaling Technology, Danvers, MA, USA
PAX-2	Polyclonal	1:75	Invitrogen, Carlsbad, CA, USA
ERG	EPR3864	1:3000	Abcam, Cambridge, MA, USA
PAX8	Polyclonal	1:1000	Proteintech Group, Rosemont, IL, USA
CD30	Ber-H2	1:75	Dako, Carpinteria, CA, USA
CD34	M7165	1:150	Dako, Carpinteria, CA, USA

Case 2

The patient was a 33-year-old female with a history of anemia, endometriosis, and urinary tract infections. She was brought to the operating room for a pre-operative diagnosis of fibroids. A frozen section performed on a uterine mass was reported as malignant, and a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and omentectomy was performed.

Grossly, the hysterectomy showed a 25.5 cm mass involving her uterus and left ovary. The bulk of tumor was in the uterus, with full thickness involvement of the tumor with nodules in the cervix, and endomyometrium, extending through to the serosa and left ovary. Cut sections of the tumor showed tan-pink to gray-white soft, hemorrhagic tissue.

Microscopically, the tumor appeared to be arising in the endometrium and extending through the myometrium directly into the left ovary. Tumor was metastatic to the cervix, left tubal serosa, bilateral ovarian surfaces, and omentum. Lymphovascular invasion was present in the myometrium and cervix. The tumor showed compressed benign glands with a leaf-like architecture. Most of the tumor cells were large and epithelioid, though monomorphic in appearance (Fig. 3). In some regions, the cells were smaller, lacked rhabdoid morphology and had inconspicuous nucleoli.

Immunohistochemistry performed at the outside institution and reviewed showed the tumor cells were positive for vimentin, p16, synaptophysin (focal), and EMA (scattered cells). The cells were negative for chromogranin, p53, PAX8, HMB-45, S100, pan-keratin, CD45, inhibin, CD30, HMWCK, LMWCK, CK7, WT-1, CD10, SMA, and desmin. Immunohistochemistry performed at BWH demonstrated that SMARCA4 was lost, SMARCB1 was intact, and mismatch repair proteins MLH1, MSH2, MSH6, and PMS2 had intact nuclear staining. There was patchy positivity for SALL4 and cyclin D1. The tumor cells were negative for claudin-4, WT-1, inhibin, PAX8, CD34, ERG, BerEP4, NUT and caldesmon. A reticulin stain highlighted a nested growth pattern.

The case was received in consultation and the submitting pathologist favored a diagnosis of a high-grade endometrial

Table 2 Clinicopathologic features of cases of SMARCA4-deficient undifferentiated uterine sarcoma/malignant rhabdoid tumor of the uterus

Case	Age (years)	Follow-up	MMR IHC	SMARCA4 IHC	Claudin-4 IHC	SMARCB1 IHC	WT-1 IHC	HMB-45 IHC	Genomic alterations
1	25	DOD at 7 months	Intact	Lost	Negative	Intact	Negative	Negative	Failed MPS
2	33	DOD at 9 months	Intact	Lost	Negative	Intact	Negative	Negative	*SMARCA4* c.3426delC frameshift *SMARCA4* c.4759G>T nonsense *TERT* promoter mutation
3	34	DOD at 1 month	Intact	Lost	Rare cells	Intact	Positive	ND	*SMARCA4* c.2554A>T nonsense *ASXL1* c.1205G>A, p.R402Q missense
4	29	DOD at 4 months	ND	Lost	Negative	ND	ND	Negative	*SMARCA4-PSG8* inversion *ARID1B* c.5687G>A, p.R1896Q missense
5	58	DOD at 43 months	ND	ND	ND	Intact	ND	Negative	*SMARCA4* c.582_587GCCCCT>G frameshift *NPRL2* c.428G>A *SH2B3* c.1666G>A *SMARCA4* c.598C>G, p.L200V (VUS) *ZNF217* c.2061_2061A>TTA frameshift

MMR mismatch repair, *DOD* dead of disease, *VUS* variant of uncertain significance, *ND* not done

stromal sarcoma. The patient died of disease 9 months after hysterectomy.

Case 3

The patient was a 34-year-old female who underwent endocervical curettage and endometrial biopsy. Both samples showed abundant tumor composed of diffuse, solid sheets of atypical epithelioid cells with rhabdoid morphology with extensive necrosis, and no distinct differentiation or architectural pattern (Fig. 4). The tumor involved endocervical tissue in both specimens.

Immunohistochemical stains showed that SMARCA4 was lost in tumor cells, while SMARCB1 was retained. Epithelial markers, including claudin-4, pan-keratin, CAM5.2, and EMA were positive in <1% of tumor cells. SALL4 was weakly positive in scattered cells. WT-1 was diffusely positive. Mismatch repair protein immunohistochemistry for MSH6 and PMS2 was intact. The tumor was negative for SOX10, S100, PTEN, PAX-2, CD30, and CD34. A reticulin stain highlighted both nests of tumor and individual cells.

Immunohistochemical stains performed at the outside institution and reviewed showed the tumor was positive for vimentin, ER (scattered cells), and negative for CK5/6, p16, pCEA, desmin, CD45, synaptophysin, chromogranin, and cytokeratin. p53 showed a patchy, wild-type pattern of expression. Ki-67 expression was increased (~60–70%). The submitting pathologist believed the tumor was a sarcoma but was unable to further classify it. The patient died of disease 1 month after the biopsy.

Case 4

This patient was a 29-year-old female, with a past medical history significant for cervical squamous cell carcinoma, treated 3 years previously with chemoradiation. She presented with vaginal bleeding. A laparoscopy was performed, and biopsies showed tumor involving the cervix, uterine serosa, bilateral ovaries, and omentum. Morphologically, it was composed of sheets of large rhabdoid cells, similar to the other cases (Fig. 5). Immunohistochemistry showed loss of SMARCA4, and negativity for claudin-4, HMB-45, p63 CD45, S100, pan-keratin, and AE1/AE3. CAM5.2 was focally positive. The patient died from disease 4 months after the onset of vaginal bleeding.
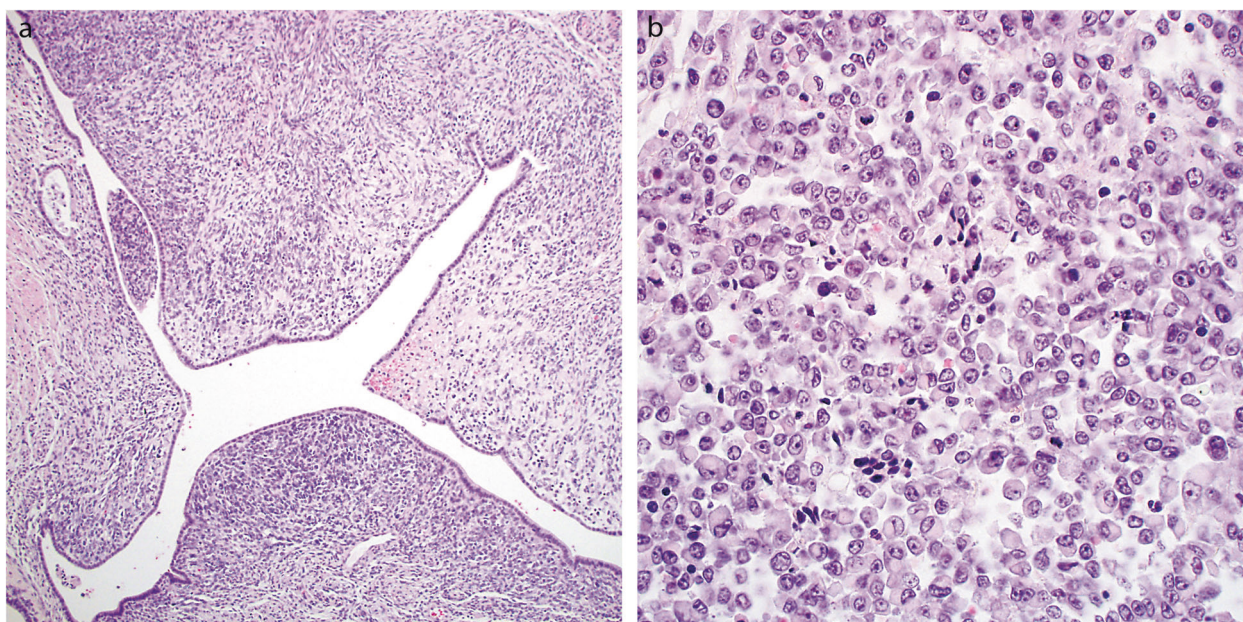
Fig. 1 Case 1. **a** Low power shows a vaguely phyllodiform architecture with tumor infiltrating around benign endometrial glands. **b** The tumor cells are large, epithelioid, with prominent nucleoli and abundant mitotic activity

Case 5

This case was a 58-year-old female who presented with post-menopausal bleeding and back pain. An abdominal CT scan showed a 13 cm solid-cystic uterine mass, carcinomatosis, and inguinal lymphadenopathy. Her serum CA-125 was 185. An endometrial biopsy was performed which showed an undifferentiated malignant neoplasm. The patient underwent a hysterectomy, bilateral salpingo-oophorectomy, appendectomy, and omentectomy. Tumor involved nearly the complete thickness of the endomyometrium and cervical stroma. Tumor was metastatic to the uterine serosa, bilateral ovaries, one fallopian tube, omentum, sigmoid colon, and appendix. There was extensive lymphovascular and perineural invasion. Microscopically, the tumor was composed of sheets of large epithelioid cells with prominent nucleoli (Fig. 6). There was brisk mitotic activity (up to 23/10 HPFs). In some areas, the cells were embedded in a fibrous stroma. Immunohistochemistry performed at the outside institution showed that the tumor cells were positive for vimentin, CD10 (weak, focal), and negative for AE1/AE3, desmin, cyclin D1, ER, PR, and c-kit. Immunohistochemistry performed at BWH showed that the tumor was positive for CAM5.2 (rare isolated cells), EMA (focal, weak), S100 (scattered cells), SMA (scattered cells), and CD34 (focal, weak). The tumor was negative for AE1/AE3, pan-keratin, HMB-45, desmin, caldesmon, and LCA. SMARCB1 expression was retained in the tumor cells. No tissue was available for SMARCA4

immunohistochemistry. The patient died from disease 43 months after diagnosis.

Serum calcium levels were not available for any of the cases. Follicle-like spaces and small cell morphology, characteristic of small cell carcinoma of the ovary, hypercalcemic type, were not observed in any of the cases.

Molecular genetics results

MPS quality control metrics

Case 1 failed MPS quality control metrics with poor coverage (mean target coverage was 9×, 0% of the targeted regions had base coverage of at least 30×). Considering cases 2 to 5, the mean number of aligned high quality reads was 9,262,340 (range: 5,766,471–13,048,502). The average mean target coverage was 257× (range: 156×–379×). An average of 98.6% (range: 98%–98.9%) of the targeted regions had base coverage of at least 30×.

SNVs and CNVs identified in SMARCA4-deficient uterine sarcoma

Considering the four cases of SMARCA4-deficient uterine sarcoma with successful sequencing (cases 2–5), case 2 had two truncating *SMARCA4* mutations (c.3426delC, p.F1142Lfs*22 and c.4759G>T, p.E1587*), and a *TERT* promoter mutation. Case 3 harbored a truncating *SMARCA4* mutation (c.2554A>T, p.K852*), as well as one missense
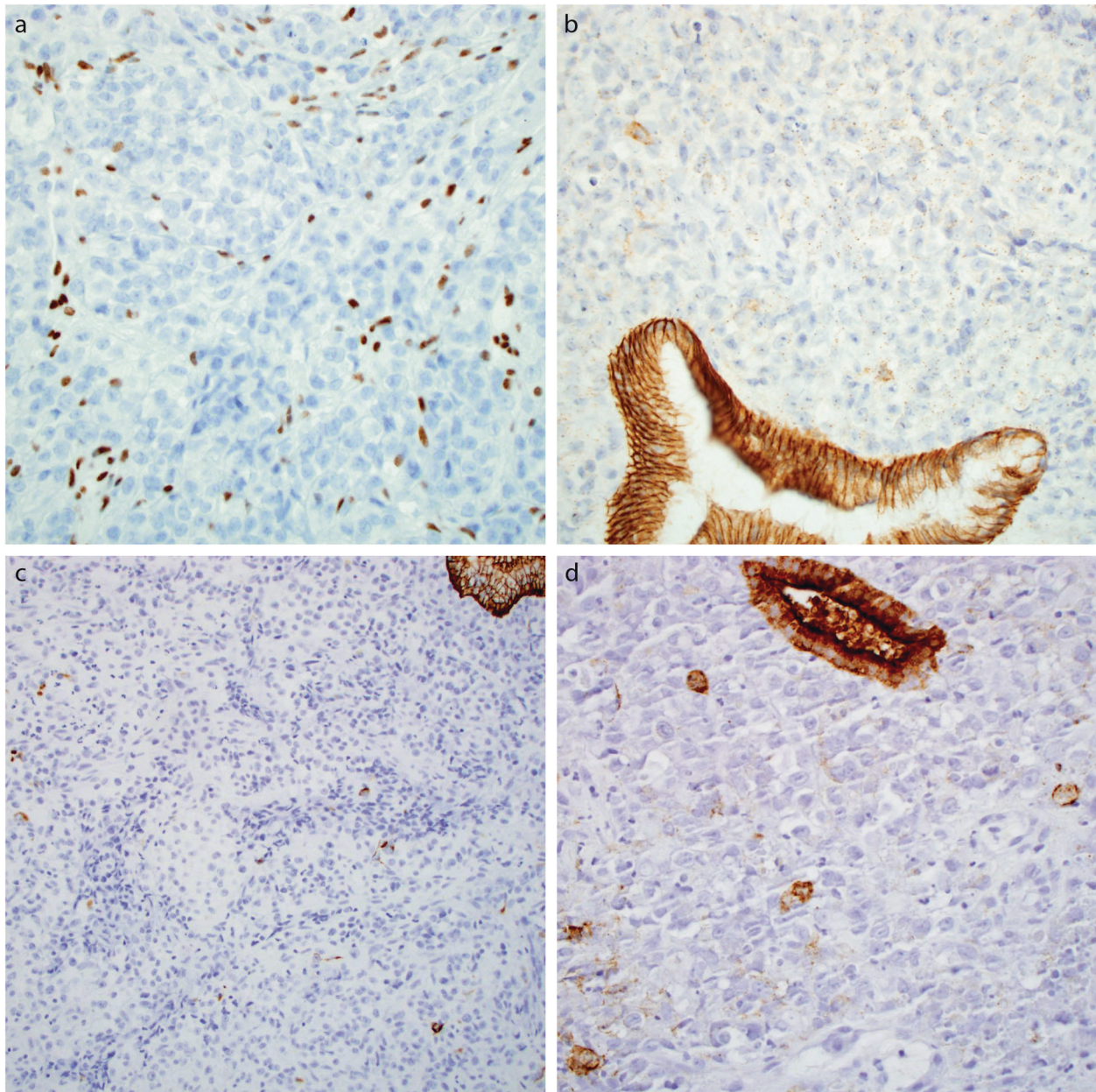
Fig. 2 Immunohistochemistry shows that SMARCA4-deficient sarcomas show loss of expression of SMARCA4 (**a**), and at most focal, weak reactivity to claudin-4 (**b**). Cam5.2 (**c**) and EMA (**d**) may also show focal reactivity. Benign glands are included as internal controls for claudin-4, Cam5.2, and EMA stains

mutation in *ASXL1* (c.1205G>A, p.R402Q). Case 4 had a *SMARCA4-PSG8* inversion, and one missense mutation of uncertain significance in *ARID1B* (c.5687G>A, p.R1896Q). Case 5 harbored two mutations in *SMARCA4*: a frameshift mutation (c.582_587GCCCCT>G, p.Q194fs) and a missense mutation (c.598C>G, p.L200V) of uncertain significance. MPS did not show evidence of hypermutation or enrichment of mutations at microsatellite sites in any case. Copy number variations included gain of 1q in case 2, single copy loss of *CDKN2A* in case 3, and scattered low level alterations in case 4. Case 5 did not have any copy number alterations.

Discussion

We have presented the clinical, morphologic, immunophenotypic, and molecular features of a tumor, which we propose be called "SMARCA4-deficient undifferentiated uterine sarcoma (malignant rhabdoid tumor of the uterus)."
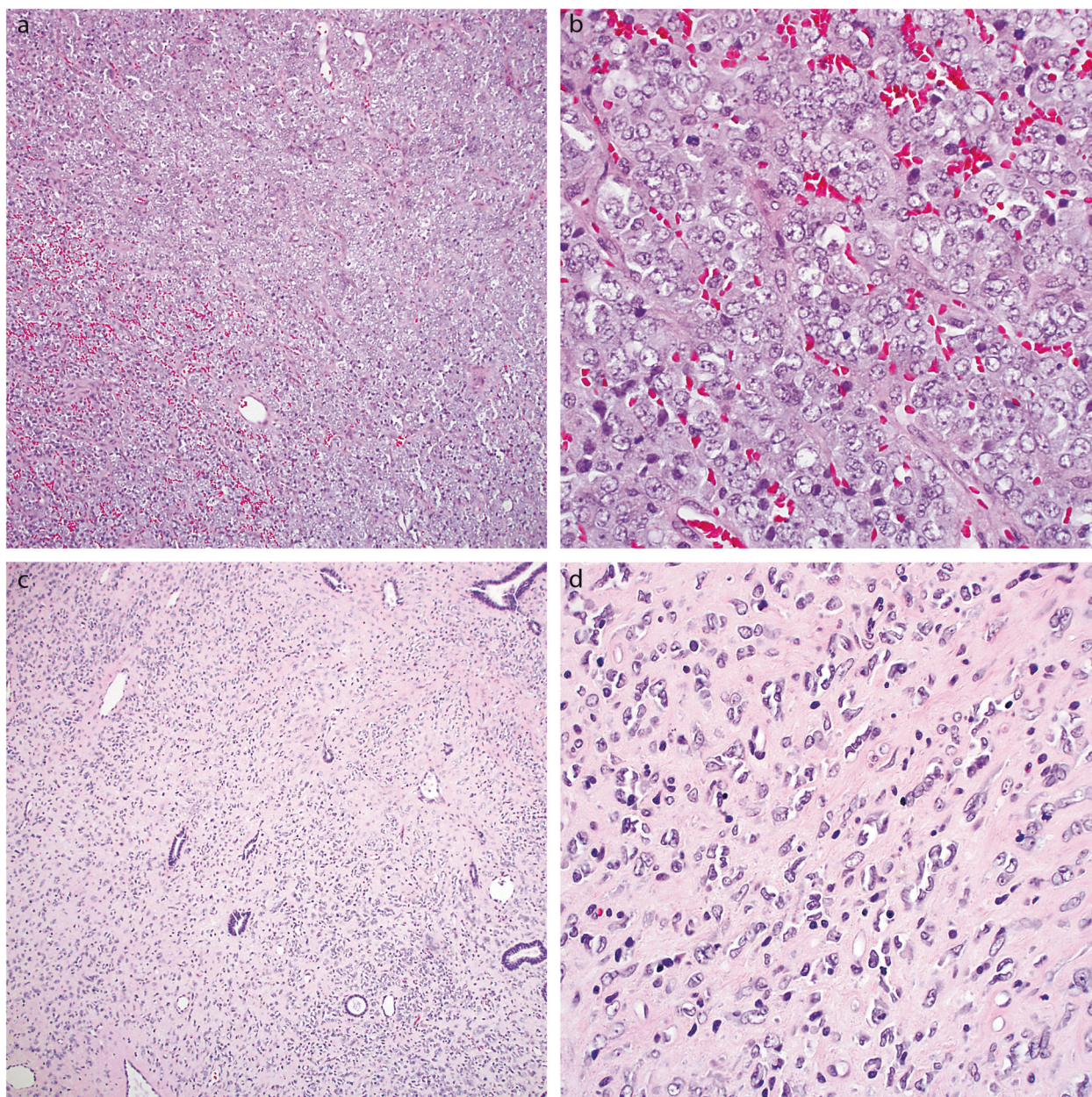
Fig. 3 Case 2. The tumor is composed of solid sheets of cells (**a**), with prominent nucleoli, vesicular chromatin, and scant cytoplasm (**b**). In some areas, the tumor cells are surrounded by hyalinized stroma (**c**, **d**)

Morphologically, it is characterized by diffuse sheets of cells with rhabdoid morphology, with large, epithelioid cells with vesicular chromatin, eosinophilic inclusions, and prominent nucleoli. Immunohistochemically, it shows loss of SMARCA4 expression, retained/intact SMARCB1 and MMR staining, and lack of significant epithelial marker expression including keratin, EMA, and claudin-4. Molecularly, SMARCA4-deficient uterine sarcoma is characterized by truncating mutations (nonsense, frameshift, or inversions) in *SMARCA4*. The tumor is clinically

aggressive, highly infiltrative, and usually with multiple foci of lymphovascular invasion. From this series, it appears to be uniformly fatal. The most salient clinical, pathologic, and genetic features of SMARCA4-deficient uterine sarcoma are presented in Table 3 with a comparison to several closely related tumors.

In case 4, only one H&E slide was available for review, an incomplete immunohistochemical panel had been performed (notably no WT-1), and the tumor involved the ovaries bilaterally. As well, the patient had the confounding
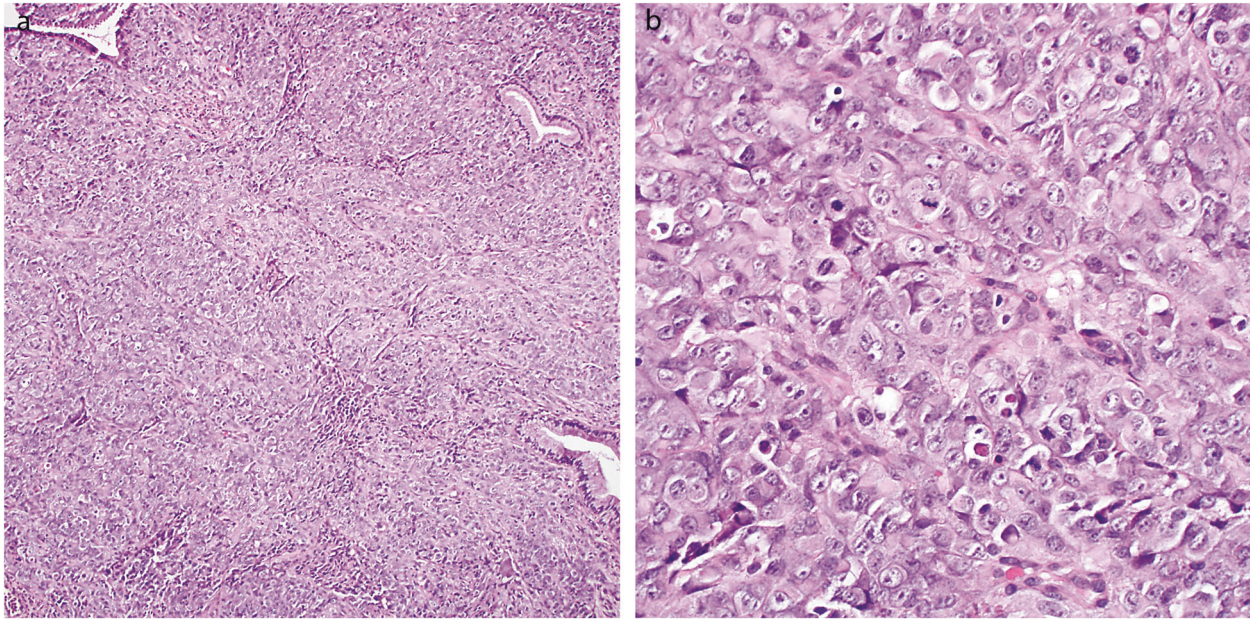
Fig. 4 Case 3. The tumor forms solid sheets of cells with benign entrapped endocervical glands (**a**). The tumor cells are atypical, yet monomorphous, with prominent nucleoli, and large nuclei (**b**). There is abundant mitotic activity

factor of prior cervical carcinoma and radiation therapy. Interestingly, there are reports of radiation-induced malignant rhabdoid tumors and atypical teratoid/rhabdoid tumors [10–13]. The ovarian involvement raises the possibility of small cell carcinoma of the ovary hypercalcemic type. However, small cell carcinoma of the ovary hypercalcemic type is "almost invariably" unilateral [1], and we are not aware of any case presenting with vaginal bleeding, a cervical mass, and bilateral ovarian involvement as in case 4. On balance, we favor this fourth case to be an additional example of SMARCA4-deficient uterine sarcoma.

Case 5 was not available for review in its entirety (only one representative slide), and material was not available for additional immunohistochemical stains, including SMARCA4 and claudin-4. However, given the morphologic features and the molecular profile of the tumor, we believe that this may represent another case of SMARCA4-deficient uterine sarcoma. If so, it suggests that the disease may also occur in older patients, and be associated with a less aggressive course.

Undifferentiated carcinomas of many sites, including the endometrium, may demonstrate discohesive, sheet-like growth with rhabdoid morphology. Like SMARCA4-deficient uterine sarcoma, they may also show loss of SMARCA4 or SMARCB1 expression [14, 15]. However, their clinicopathologic and genetic features are dissimilar to SWI/SNF-deficient sarcomas. For example, SMARCA4-deficient uterine sarcoma, small cell carcinoma of the ovary (hypercalcemic type), and atypical teratoid/rhabdoid tumors occur at a younger age, may be more aggressive, and show

fewer genetic abnormalities compared with carcinomas. Specifically, they have recurrent mutations in *SMARCA4* or *SMARCB1*, and few other somatic protein-coding mutations [16].

SMARCA4-deficient uterine sarcoma shares many clinicopathologic features of small cell carcinoma of the ovary (hypercalcemic type), including young age, aggressive clinical behavior, morphologic resemblance to the large cell variant of small cell carcinoma of the ovary, hypercalcemic type, and loss of SMARCA4 expression. Several issues must be considered in both recognizing SMARCA4-deficient uterine sarcoma and distinguishing it from small cell carcinoma of the ovary, hypercalcemic type. (1) SMARCA4-deficient uterine sarcoma arises in the uterus and is frequently negative for WT-1. (2) When there is significant tumor burden in an ovary, the large cell variant of small cell carcinoma of the ovary, hypercalcemic type, involving the uterus, must be considered. However, small cell carcinoma of the ovary, hypercalcemic type is usually a large (mean 15 cm), unilateral mass, whereas SMARCA4-deficient uterine sarcoma may involve both adnexa with smaller deposits [1]. (3) In keeping with the different sites of origin, the clinical presentations also differ: all five cases of SMARCA4-deficient uterine sarcoma presented with a cervical mass or vaginal bleeding. In one series of 124 patients with small cell carcinoma of the ovary, hypercalcemic type, the most common presentations were abdominal pain or swelling, and none presented with a cervical mass or vaginal bleeding [1]. (4) There may be therapeutic implications in recognizing SMARCA4-deficient uterine
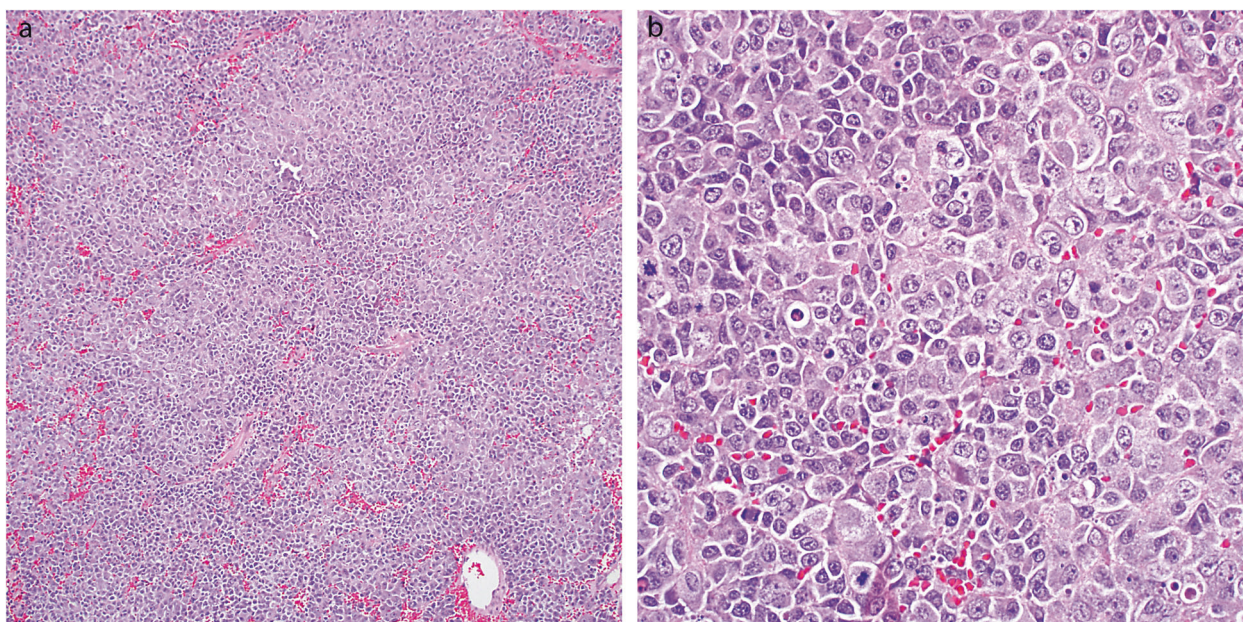
Fig. 5 Case 4. Similar to the other cases, the tumor forms solid sheets of cells (**a**) and is composed of discohesive rhabdoid cells with prominent nucleoli (**b**)

sarcoma. Recent in vitro studies with small cell carcinoma of the ovary, hypercalcemic type cell lines and in vivo mouse xenograft models have shown antiproliferative and antitumor effects when treated with EZH2 inhibitors [17]. A phase 2 clinical trial is now treating patients with small cell carcinoma of the ovary, hypercalcemic type with the EZH2 inhibitor tazemetostat. Given the aggressive nature of SMARCA4-deficient uterine sarcoma and the shared genetics with small cell carcinoma of the ovary, hypercalcemic type, consideration should be given to including patients with SMARCA4-deficient uterine sarcoma in EZH2 inhibitor trials. There is also a report of four patients with small cell carcinoma of the ovary, hypercalcemic type responding to anti-PD-1 immunotherapy, despite the tumors' low mutational burden [18]. Similarly, there may be a role for PD-1 blockade in SMARCA4-deficient uterine sarcomas.

Given the above information, the distinction between undifferentiated endometrial carcinoma and SMARCA4-deficient uterine sarcoma is important. Lack of SWI/SNF complex expression by immunohistochemical staining is a necessary but not sufficient requirement for the diagnosis of SMARCA4-deficient uterine sarcoma, because dedifferentiated and undifferentiated carcinomas may commonly also show lack of expression of SMARCA4 or SMARCB1 [14, 19]. SMARCA4-deficient uterine sarcoma is uniformly fatal (albeit with small numbers in this study) and may be even more aggressive than undifferentiated endometrial carcinoma. However, given the rarity of these tumors, a direct comparison of survival is difficult. The average age of

patients with SMARCA4-deficient uterine sarcoma in this limited series is 36 years old (median 33 years), which is far closer to the average age of small cell carcinoma of the ovary, hypercalcemic type (24 years) [1] than undifferentiated endometrial carcinoma (59 years, range 40–69) [20]. Further work is needed to determine the full age range of affected patients. Morphologically, undifferentiated carcinomas may also show rhabdoid morphology, albeit usually only focally when present [20], and distinguishing them SWI/SNF-deficient sarcomas can be challenging. Undifferentiated carcinomas may show increased nuclear pleomorphism, characterized by occasional large, highly atypical cells. In contrast, SMARCA4-deficient uterine sarcoma is composed of a population of uniformly atypical cells. The reticulin staining in SMARCA4-deficient uterine sarcoma highlights a nested pattern, which also may overlap with reticulin patterns in undifferentiated carcinomas. Immunohistochemistry may be helpful in this regard. SMARCA4-deficient uterine sarcomas are microsatellite stable and lack significant expression of epithelial markers. Undifferentiated carcinomas usually express claudin-4 [21]. Claudin-4 has recently been shown to be a marker of epithelial differentiation, which may remain expressed in undifferentiated and poorly differentiated carcinomas which have rhabdoid morphology, but is negative in small cell carcinoma (hypercalcemic type), SWI/SNF-deficient sarcomas, and in our series of SMARCA4-deficient uterine sarcomas [7, 21]. Four of the cases presented here were negative for claudin-4, suggesting that it may be an effective marker to distinguish them from rhabdoid carcinomas
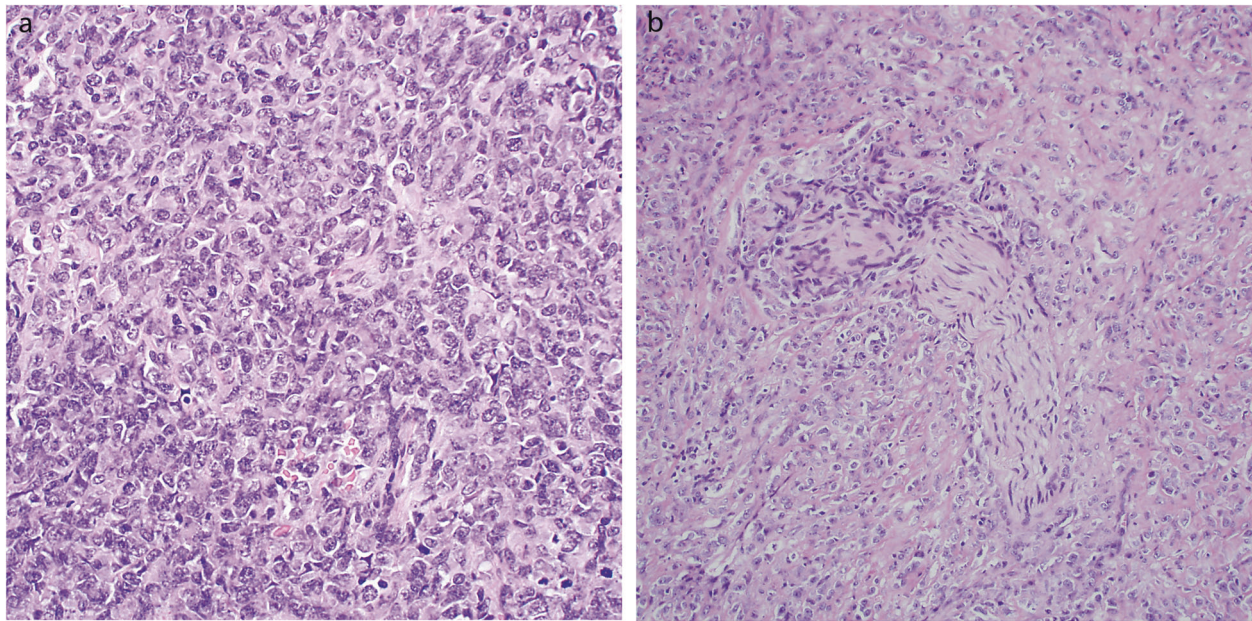
Fig. 6 Case 5. The tumor is composed of solid sheets of tumor cells (**a**). In other areas, the tumor cells are embedded in a hyalinized stroma (**b**), as can be seen in some areas of small cell carcinoma of the ovary, hypercalcemic type. Perineural invasion is also present

and indicating that the term "sarcoma" is more appropriate than "carcinoma" to describe these tumors. However, claudin-4 has not been extensively studied in undifferentiated endometrial carcinomas, so its specificity in this setting is unknown. Cyclin D1 immunohistochemistry may also be helpful; it is diffuse and strong in half of undifferentiated endometrial carcinomas [22], but is negative or patchy in SMARCA4-deficient uterine sarcoma. We suggest that when faced with a poorly differentiated uterine neoplasm with rhabdoid cells, if genomic studies are unavailable, claudin-4, cyclin D1, mismatch repair, and SMARCA4 immunohistochemistry may be used, in combination with histology and the appropriate clinical setting, to differentiate SMARCA4-deficient uterine sarcoma from mimics, including undifferentiated carcinomas with rhabdoid morphology.

Undifferentiated uterine carcinomas have many mutations, some of which occur in genes frequently mutated in endometrioid carcinoma (e.g., *KRAS, PTEN, CTNNB1, PIK3CA*). They may also have mutations in SWI/SNF complex members. In contrast, *SMARCA4* is the sole driver mutation in SMARCA4-deficient uterine sarcoma.

Although proximal-type epithelioid sarcoma and SMARCA4-deficient uterine sarcoma share SWI/SNF complex abnormalities and a common age range, they have several differences. (1) SMARCA4-deficient uterine sarcoma is a more aggressive disease with dismal prognosis, while epithelioid sarcoma follows a protracted course with frequent local recurrences. (2) The anatomical sites affected also differ: while SMARCA4-deficient uterine sarcoma

occurs in the uterus, proximal-type epithelioid sarcoma is usually in the inguinal region or vulva. CD34 is positive in approximately half of cases of epithelioid sarcoma, but is negative in SMARCA4-deficient uterine sarcoma. (3) Finally, epithelioid sarcoma is typically characterized by loss of *SMARCB1*, but *SMARCA4* is altered in SMARCA4-deficient uterine sarcoma.

The morphologic differential diagnosis of malignant epithelioid tumors in the uterus also includes epithelioid leiomyosarcoma and malignant PEComa. Both entities show immunoreactivity to smooth muscle markers, but PEComas are also immunoreactive to HMB-45 and/or melanA. In contrast, SMARCA4-deficient uterine sarcoma is negative for markers of both smooth muscle and melanocytic differentiation. Loss of SMARCA4 by immunohistochemistry effectively excludes leiomyosarcoma, as it was intact in all cases in one series of 21 leiomyosarcomas [5]. Histologically, leiomyosarcomas and malignant PEComas often demonstrate striking nuclear pleomorphism; in contrast, SMARCA4-deficient uterine sarcoma has a more uniform cytologic atypia without significant pleomorphism. Molecularly, both leiomyosarcoma and PEComa show complex chromosomal abnormalities, whereas SMARCA4-deficient uterine sarcoma usually has a diploid karyotype with a small number of CNVs [23, 24]. Additionally, PEComas often show loss of heterozygosity of the *TSC2* locus; [24] this aberration was not observed in any cases of SMARCA4-deficient sarcoma in this series.

Case 1 showed focal phyllodiform growth (Fig. 1a), raising the possibility of an adenosarcoma. However, no

Table 3 Comparison of clinicopathologic features of SWI/SNF complex-deficient tumors

	Age	Location	Main molecular abnormality	Germline association	Prognosis	Non-germline risk factors
Malignant rhabdoid tumor [31, 32]	Usually infants <1 year, rarely adolescents and adults	Kidney, liver, head and neck	SMARCB1 in 95%, rare cases with SMARCA4	SMARCB1 deletion in 15–30%	Poor; 31% of patients survive 1 year	Low birthweight, preterm birth
Small cell carcinoma of the ovary, hypercalcemic type [1–4, 33–35]	Mean 24 years (range 14 months–71 years)	Ovary	SMARCA4	SMARCA4 mutation in 8%-50%	Poor; almost all patients greater than stage 1A die from disease; 10–20% overall survival	None
Atypical teratoid/rhabdoid tumor [32, 36–38]	Usually under 3 years, rarely adolescents and adults	CNS	Usually SMARCB1, rarely SMARCA4	SMARCB1 mutation in 35%	Poor; median survival 8 months	Low birthweight, increased maternal age, higher parental socioeconomic status
Proximal-type epithelioid sarcoma [39]	Median 40 years (range 13–80 years)	Inguinal region, thigh, vulva	SMARCB1	None	Local recurrences common; median survival 6 years	History of trauma in some cases
SMARCA4-deficient thoracic sarcoma [7, 40]	Median 39 years (27–82 years)	Thorax	SMARCA4 mutations and LOH; TP53 mutations	None	Poor; median survival 7 months	Heavy smoking
Undifferentiated endometrial carcinoma [14, 20]	Mean 59 years (range 40–69 years)	Endometrium	KRAS, PTEN, CTNNB1, MMR proteins, ARID1A SMARCA4, SMARCB1	None	Stage dependent; survival of months to years	Unknown
SMARCA4-deficient uterine sarcoma	Mean 36 years (range 25–58 years)	Uterus	SMARCA4	Unknown	Poor; average survival ~6 months	Unknown, possibly radiation therapy

Table 4 Possible cases of SMARCA4-deficient uterine sarcoma previously reported in the literature

Case and references	Age (years)	Outcome	Original diagnosis	Immunohistochemistry	Likelihood of SMARCA4-deficient uterine sarcoma
1 [14]	26	DOD at 5 months	Undifferentiated endometrial carcinoma	SMARCA4-deficient, MMR intact	High
2 [41]	39	DOD at 17 months	Malignant rhabdoid tumor	Positive: vimentin Negative: keratins and desmin	Possible
3 [28]	33	Unknown, but tumor involved bladder	Undifferentiated sarcoma with rhabdoid cells	Positive: vimentin, focally for keratins, EMA, CD10, INI-1 (intact) Negative: SMA, desmin, myogenin, S100, and CD99 negative	Possible
4 [29]	46	Unknown	Malignant rhabdoid tumor	Positive: vimentin, keratins, EMA Negative: MSA, desmin, myoglobin	Possible
5 [30]	37	DOD at 4 months	Malignant rhabdoid tumor of uterine corpus	Positive: vimentin Negative: keratins, EMA	Possible
6 [42]	36	ANED at 38 months	Malignant rhabdoid tumor of uterine cervix	Positive: vimentin Negative: keratin, desmin, myoglobin, CD34, EMA, S100, HMB-45	Possible
7 [43]	71	Died 11 days post-op	Uterine sarcoma with features of malignant rhabdoid tumor	Positive: keratin, EMA, vimentin	Possible
8 [44]	56	ANED at 7 months	Uterine rhabdoid tumor	Positive: keratins, vimentin	Possible
9–13 [45–47]	51–72	ANED at 14 months, dead from other causes at 6 months, DOD in 2.5 and 3 months, no follow-up in one case	Carcinosarcoma with rhabdoid tumor component/features	Positive: keratins in carcinomatous component	Low; likely carcinosarcoma with rhabdomyoblastic differentiation
14 [48]	49	DOD at 4 months	Collision of uterine rhabdoid tumor and endometrioid adenocarcinoma	Positive: keratins in carcinoma, vimentin in rhabdoid component Negative: keratins in rhabdoid component, vimentin in carcinoma	Low; possible dedifferentiated carcinoma

DOD dead of disease, *ANED* alive with no evidence of disease

adenosarcoma to date has shown loss of SMARCA4 by immunohistochemistry (which would indicate biallelic inactivation). Single *SMARCA4* mutations are uncommonly seen in adenosarcomas, but they are always in the setting of other pathogenic alterations [25]. Also, many tumors (such as carcinosarcoma and benign endometrial polyps) may mimic adenosarcoma by showing focal phyllodiform growth, and this pattern is not diagnostic of adenosarcoma [26]. In our case, it was only focal, and no spindled growth was present. Epithelioid areas of adenosarcoma are rarely seen [27]. However, these epithelioid areas in adenosarcoma have only been reported in tumors with smooth muscle differentiation, and only as a focal finding. The tumor cells in case 1 were negative for SMA, desmin, and caldesmon, and the epithelioid morphology was seen throughout the tumor, ruling out this possibility.

While small cell carcinoma of the ovary, hypercalcemic type and malignant rhabdoid tumor are associated with germline mutations in 8–50% and 15–30% of cases, respectively, SMARCA4-deficient thoracic sarcoma—which occurs in a much older age group—is not. Because the age distribution of SMARCA4-deficient uterine sarcoma more closely mirrors that of small cell carcinoma of the ovary, hypercalcemic type than SMARCA4-deficient thoracic sarcoma, it is conceivable that a subset of our cases is associated with a germline mutation in *SMARCA4*. Association with germline mutations in *SMARCA4* should be investigated, as it may have implications for family members who may be carriers (e.g., consideration of hysterectomy and bilateral oophorectomy). Of note, patients with rhabdoid tumor predisposition syndrome 2 (OMIM: 613325, caused by heterozygous germline mutations in *SMARCA4*), have not been reported to have uterine tumors.

Others have previously postulated that uterine tumors with rhabdoid cells may be genetically related to malignant rhabdoid tumors [28–30]. While we feel the unqualified term "malignant rhabdoid tumor" is not completely accurate (SMARCA4-deficient uterine sarcoma does not occur in infants, is not currently associated with *SMARCB1* mutations, and has been used historically to describe a variety of tumors of many anatomic sites), their idea of linking rhabdoid tumors in the uterus with other SWI/SNF-deficient tumors ultimately proved correct, and was enabled by the availability of immunohistochemical and genomic assays to study *SMARCA4* in tumor biology.

In other SWI/SNF-deficient tumors, there is usually not total exclusivity between tumors caused by SMARCA4 and SMARCB1 deficiencies. For example, malignant rhabdoid tumors are usually associated with mutations in *SMARCB1*, but may rarely be caused by *SMARCA4* loss. Although the cases presented here have SMARCA4 loss-of-function, other cases of SMARCA4-deficient uterine sarcoma may be shown to have SMARCB1 loss.

Unlike SMARCA4-deficient thoracic sarcoma, which is usually associated with heavy smoking [7], we have identified no known risk factors for SMARCA4-deficient uterine sarcoma, similar to small cell carcinoma of the ovary, hypercalcemic type in its lack of known non-genetic risk factors. Although two of our cases had endometriosis, the extra-uterine location of endometriosis would make it unlikely to have a role in the pathogenesis of this disease. Notably, the exposure to radiation therapy in case 4 may have had a role in tumorigenesis.

SMARCA4-deficient uterine sarcomas have not been previously reported as a distinct clinicopathologic entity. However, there have been reports of uterine tumors, diagnosed as undifferentiated carcinomas, sarcomas, or malignant rhabdoid tumors, which may be examples of SMARCA4-deficient uterine sarcoma (Table 4; cases 1–8). Although immunohistochemistry for SMARCA4 and claudin-4 and molecular studies would be needed to establish a diagnosis of SMARCA4-deficient uterine sarcoma, the constellation of clinical, morphologic and immunophenotypic features of the above cases are suggestive of this entity. There are also reports of other uterine rhabdoid tumors (Table 4; cases 9–14) which likely represent other tumors or have insufficient morphologic or immunohistochemical work-up to assess as candidate cases. Some of these may be better classified as another tumor type (e.g., carcinosarcoma or dedifferentiated carcinoma) which happen to display rhabdoid morphologic features.

In summary, recognizing and differentiating SMARCA4-deficient uterine sarcoma from its histologic mimics is important, as it has a dismal prognosis. This tumor type may be a rational candidate for targeted therapy (such as EZH2 inhibitors and anti-PD-1 therapy), and the possible association with germline mutations in *SMARCA4* should be elucidated in future studies.

Compliance with ethical standards

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