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ARTICLE

Primary cutaneous SMARCA4-deficient undifferentiated malignant neoplasm: first two cases with clinicopathologic and molecular comparison to eight visceral counterparts

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SMARCA4-deficient undifferentiated malignant neoplasms (SD-UMN) comprise a group of aggressive tumors with epithelioid morphology that are characterized by loss of function of SMARCA4, a component of the SWI/SNF chromatin remodeling complex. SD-UMN was first recognized in the thoracic cavity but is now appreciated to occur at multiple anatomic sites. A notable exception has been skin. Here we report the first two cases of primary cutaneous SD-UMN and compare their features to a cohort of eight visceral cases arising in lung, gastrointestinal tract, and gallbladder. Evidence for a bona fide cutaneous origin included extensive clinical, radiologic, and serologic analyses that failed to identify a metastatic source as well as the molecular identification of a UV-associated mutational pattern. The cutaneous cases showed strikingly similar morphologic, immunohistochemical, and molecular features to the visceral cases, strongly suggesting that they belong to this family of tumors. In addition to biallelic inactivation of *SMARCA4*, both cutaneous tumors also showed biallelic inactivation of *TP53* and *CDKN2A*, findings which also appear common in visceral cases. One patient died of disease at 18 months after diagnosis, consistent with the aggressive nature of this tumor. Our results expand the anatomic spectrum of SD-UMN, adding this entity to an already challenging differential diagnosis that includes melanoma, squamous cell carcinoma, Merkel cell carcinoma, epithelioid sarcoma, and others. Given the potentially aggressive nature of SD-UMN, the timely and accurate diagnosis of this entity may have implications for prognosis and therapy.

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INTRODUCTION

SMARCA4-deficient undifferentiated malignant neoplasms (SD-UMN) encompass a family of malignant epithelioid tumors often associated with poor clinical outcomes. The defining feature is the presence of inactivating *SMARCA4* mutations, with the resultant loss of protein expression being detectable by immunohistochemistry[1,2]. SMARCA4 (also known as BRG1) is an ATPase which comprises one of the two catalytic subunits of the SWItch/Sucrose Nonfermentable (SWI/SNF) chromatin remodeling complex (the other being SMARCA2; reviewed in Mittal et al.[3]). The SWI/SNF complex is involved in transcriptional regulation and cellular proliferation, and loss of function of SMARCA4 is a well-established oncogenic driver both in experimental models and in various cancer types[4,5].

Malignancies characterized by SMARCA4 deficiency have a wide anatomic distribution and include tumors of the lung, gastrointestinal tract, sinonasal tract, and gynecologic tract[6–12]. Regardless of the site of origin, SD-UMN are characterized by common histopathologic characteristics, including an undifferentiated epithelioid large cell or rhabdoid cytomorphology. Atypical mitoses, apoptotic debris, and tumor necrosis are also common findings. Their relatively undifferentiated appearance can make the diagnosis challenging, and raises consideration of high-grade epithelial, mesenchymal, endothelial, and melanocytic neoplasms in the differential diagnosis.

One notable exception within the recognized anatomic spectrum of SD-UMN is skin. Here we describe the first cases of primary cutaneous SD-UMN and report their genomic characterization by next generation DNA sequencing. We compare the clinicopathologic, immunohistochemical, and molecular features of these primary cutaneous tumors to SD-UMN arising at visceral sites. Our findings have important implications for the diagnosis of undifferentiated epithelioid malignancies in the skin.

MATERIALS AND METHODS

Case selection

Approval was obtained for this study from our Institutional Review Board. Two cases of primary cutaneous SD-UMN were identified at our institution between 2019 and 2021. In an effort to identify more cases, our institutional files were searched for cutaneous cases with terms including "epithelioid", "rhabdoid", "poorly differentiated carcinoma/neoplasm", and "undifferentiated carcinoma/neoplasm"; however, no additional cutaneous cases have been identified to date. Additional representative cases of SD-UMN at visceral sites were retrospectively selected from our institutional files. These eight visceral cases were selected based upon the availability of slides for review and the completion of relevant molecular studies. No cases were subsequently excluded after initial selection.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 4 µm-thick formalin fixed, paraffin-embedded tissue sections. SMARCA4 IHC was performed following citrate buffer pressure cooker epitope retrieval (Target Retrieval Solution,

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Table 1. Clinical Features of Cutaneous and Visceral SMARCA4-Deficient Undifferentiated Malignant Neoplasms.

Site	Age	Sex	Smoking status	Treatment	Follow-up interval	Disease course	Outcome	Histologic characteristics
Cutaneous primary (case 1)	84	Male	Former smoker	Excision and radiation	18 months	Local progression	Deceased	Poorly differentiated epithelioid neoplasm
Cutaneous primary (case 2)	70	Male	Never smoker	Excision	9 months	No recurrence	No recurrence	Poorly differentiated epithelioid neoplasm with rhabdoid features
Lung primary (n = 4)	66–71 (median 68.5)	2 Male 2 Female	Former smoker ($n = 2$) Current smoker ($n = 2$)	Chemotherapy and checkpoint blockade ($n = 2$) Resection ($n = 1$) Resection and chemotherapy ($n = 1$)	14 months - 5 years	No recurrence ($n = 2$) Progressive visceral disease ($n = 2$)	No recurrence (after 5 years, and at 15 months) Deceased (at 1 year, and at 14 months)	Poorly differentiated epithelioid neoplasm
Esophageal primary (n = 2)	68 (both)	2 Male	Current smoker ($n = 1$) Never smoker ($n = 1$)	Chemotherapy, radiation, and checkpoint blockade ($n = 1$) Unknown ($n = 1$)	2 months and 19 months	Progressive visceral disease Unknown	Deceased 19 months post-diagnosis Unknown	Undifferentiated carcinoma
Rectal primary ($n = 1$)	58	Female	Former smoker	Chemotherapy	2 months	Progressive visceral disease	Deceased 2 months post-diagnosis	Undifferentiated carcinoma
Gallbladder primary ($n = 1$)	42	Female	Never smoker	Chemotherapy	9 months	Progressive metastatic disease to liver	Deceased 9 months post-diagnosis	Undifferentiated carcinoma

pH 6.1; Dako, Carpinteria, CA) using a rabbit anti-SMARCA4 (BRG1) monoclonal antibody (1:50 dilution, 40 min incubation, clone EPR3912; Abcam, Cambridge, MA). Sox-2 IHC was performed on 4 µm-thick formalin-fixed paraffin-embedded tissue sections following citrate buffer pressure cooker epitope retrieval (Target Retrieval Solution, pH 6.1; Dako) using a rabbit anti-Sox-2 monoclonal antibody (1:75 dilution, 40 min incubation; CLONE D6D9; Cell Signaling Techonology, Danvers, MA). Pan-keratin IHC was performed on 4 µm-thick formalin-fixed paraffin-embedded tissue sections following protease enzyme digestion epitope retrieval using a mouse anti-pan-keratin monoclonal antibody (clone MNF116, 1:300 dilution, 40 min incubation; Agilent/Dako, Santa Clara, CA). EnVision plus detection system (Dako Link 48) was used for all antibodies.

Molecular profiling

Two cases of primary cutaneous SD-UMN were analyzed using a next-generation DNA sequencing platform (Oncopanel), as previously described[13,14]. Cases met adequacy requirements of at least 20% tumor cells in specimens measuring at least 3 mm in greatest linear dimension. Eight additional cases of primary visceral SD-UMN were analyzed in parallel using this method. The Oncopanel assay surveys exonic DNA sequences of 447 cancer genes and can identify alterations including insertions, deletions and substitutions. In addition, 191 regions across 60 genes are interrogated for the detection of chromosomal rearrangements. A complete list of genes included can be found in supplemental table S1. Regions harboring single nucleotide polymorphisms (SNPs), spaced approximately every 4 MB across the genome are also captured and sequenced to facilitate assessment of copy number variations. Notably the Oncopanel assay includes *SMARCA4* (*BRG1*), the related gene *SMARCB1* (*INI1*), and diverse driver genes involved in the pathogenesis of entities in the histopathologic differential diagnosis such as melanoma, squamous cell carcinoma, and cutaneous metastases (such as from lung and gastrointestinal primaries). Additionally, for tumors with 16 or more mutations, mutational signature analysis is performed based upon the pattern of nucleotide substitutions, allowing recognition of signatures associated with DNA damage due to ultraviolet light (UVA) exposure, tobacco smoke exposure, prior treatment with alkylating agents (including temozolomide), impaired POLE DNA polymerase function, and APOBEC (Apolipoprotein B mRNA Editing Catalytic Polypeptide-like) enzyme dysregulation. The Oncopanel mutational signature detection tool is based upon previously published signatures derived from whole exome sequencing data[15] and was subsequently refined by training on targeted exome sequencing data[16]. The reported mutational patterns reflect those observed in vitro following exposure to relevant mutagens. The presence of these signatures was further validated against the clinicopathologic features in 740 Oncopanel samples including origin at a sun-exposed site, smoking history, prior treatment with temozolomide, concurrent POLE hotspot mutation, or MMR deficiency, as detected by Oncopanel.

RESULTS

Clinical features of cutaneous and visceral SMARCA4-deficient undifferentiated malignant neoplasms

The clinical features of all ten cases are summarized in Table 1. Both cases of primary cutaneous SD-UMN were received in consultation at our institution with a referring diagnosis of Merkel cell carcinoma. Both patients were men (84 and 70 years old). Patient 1 presented with a single papule on the left neck, clinically concerning for a basal cell carcinoma. Clinical and radiologic examination, including a full body PET scan, did not reveal evidence of visceral or other metastatic disease. Serologic testing for Merkel cell carcinoma-associated antibodies was negative. A wide excision was performed but tumor recurred locally within one year. The patient underwent radiation therapy, but within 4 months new lesions arose in the radiation field. Despite yet another excision, the disease progressed with continued local recurrences and the patient was referred to hospice care where he died approximately 18 months following initial diagnosis. Patient 2 developed a rapidly growing keratotic papule on the right cheek. The patient had a history of melanoma, also on the right cheek, 10 years previously and for which he underwent wide local excision and negative sentinel lymph node biopsy. The excision of his SD-UMN did not show a scar, confirming that the two tumors were at distinct sites on the cheek. Extensive clinical and radiologic evaluation, including computed tomography (CT) of the thorax, abdomen, and pelvis, as well as full body PET scan,

failed to reveal a metastatic source or other foci of disease. Serologic testing for Merkel cell carcinoma-associated antibodies was again negative. The patient underwent complete excision of the biopsy site with minimal residual tumor. One year following diagnosis the patient remained recurrence-free. Repeat CT scans of the head, neck, and have been negative to date, and the patient remains under close surveillance with repeat imaging every six months.

Patients with primary visceral SMARCA4-deficient undifferentiated malignant neoplasms ($n = 8$: female = 4, male = 4) ranged in age from 42–71 with a median age of 68. Primary tumor sites included lung ($n = 4$), esophagus ($n = 2$), rectum ($n = 1$) and gallbladder ($n = 1$). Five patients had progressive disease despite treatment and died between 2–19 months post-diagnosis. Two patients remain disease-free at 15 months and 5 years post-diagnosis, respectively. Follow-up data was not available for the remaining case. Further details including treatment and follow-up interval information are summarized in Table 1.

Morphologic and immunohistochemical features of cutaneous and visceral SMARCA4-deficient undifferentiated malignant neoplasms

The morphologic features of the primary cutaneous SD-UMN were highly similar to those of visceral origin. All of the tumors were composed of medium to large epithelioid cells with prominent nucleoli (sometimes multiple), a fine chromatin pattern, and thickened and irregular nuclear membranes (Fig. 1). The cells contained moderate amounts of amphophilic cytoplasm and some displayed areas of rhabdoid cytomorphology. Mitotic activity and apoptotic debris were frequent findings. For the cutaneous tumors, there was no intraepidermal component. Rather the tumors were primarily located in the dermis (Fig. 1) and showed sheet-like growth with some areas showing a more nested pattern. The tumor border was relatively well-circumscribed for case 1; this feature was difficult to assess in case 2 due to the small size of the sample. Subsequent wide excision of the tumors revealed focal extension of tumor into the subcutis in case 1 and only focal residual dermal involvement in case 2. Perineural invasion was identified in case 1.

Complete loss of SMARCA4 immunohistochemical expression was demonstrated in all cutaneous and visceral cases (Figs. 2 and 3). Nuclear expression was retained in neighboring non-neoplastic cells. The primary cutaneous tumors additionally expressed pan-keratin and Sox2 (Fig. 2). Given the broad differential diagnosis that included poorly differentiated squamous cell carcinoma, melanoma, Merkel cell carcinoma, NUT midline carcinoma, and other epithelioid malignancies, a wide array of additional immunohistochemical studies were performed but all were negative including p63, INSM1, cytokeratin 7 (CK7), CK20, ERG, CD34, MCPyV (Merkel cell polyoma virus large T antigen), NUT (nuclear protein in testis), Sox-10, S100, Melan-A, and HMB45. INI1 expression was retained, arguing against an epithelioid sarcoma.

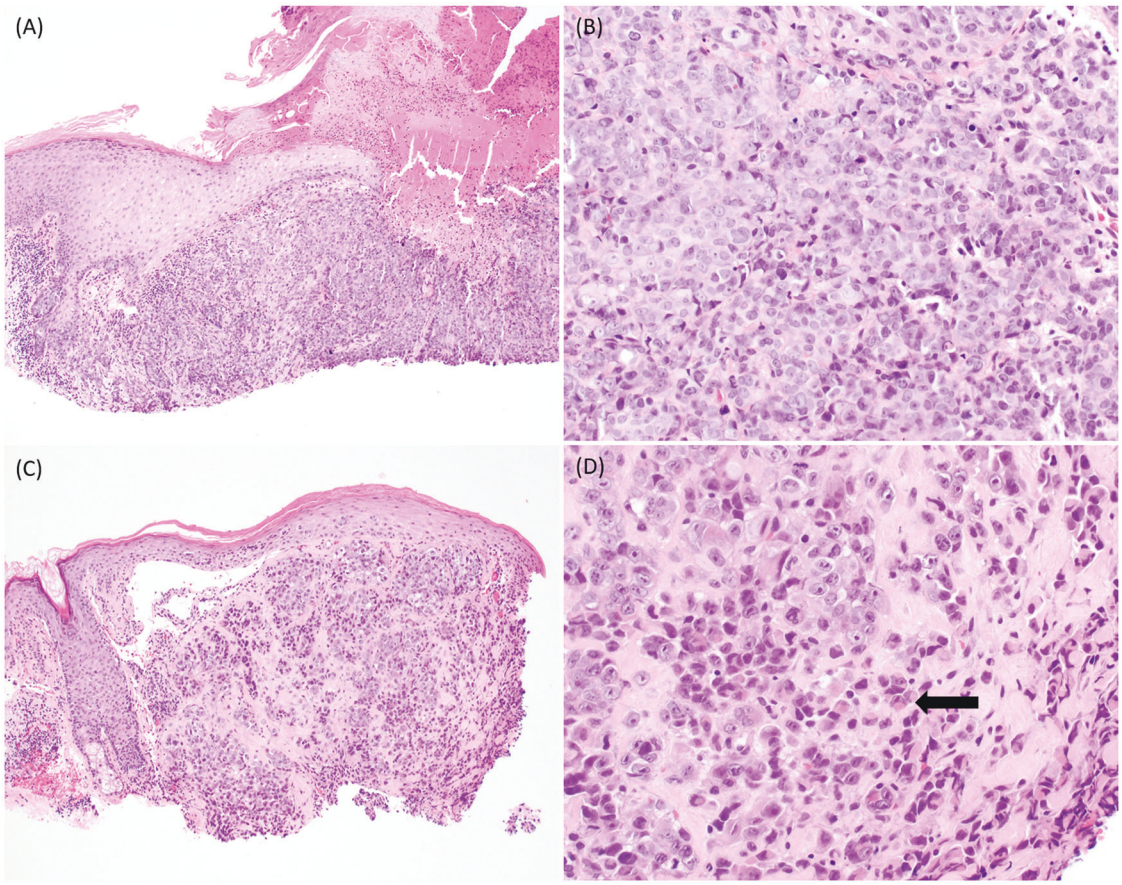

Fig. 1 Primary cutaneous SMARCA4-deficient undifferentiated malignant neoplasm. Case 1 **A**, **B** shows a poorly differentiated malignant neoplasm with sheet-like growth occupying the dermis after component:...without an in-situ component (A). Higher magnification shows that the tumor is composed of pleomorphic epithelioid cells with amphophilic cytoplasm and variably prominent nucleoli. Mitoses are easily identified, and apoptotic cells are present (**B**). Case 2 **C**, **D** shows a remarkably similar morphology. A low power image shows a dermally-based tumor lacking an in-situ component with a more nested architecture (**C**). Higher magnification shows that the tumor is composed of pleomorphic epithelioid cells with variably prominent nucleoli and focal rhabdoid morphology (arrow, **D**). Mitoses are frequent. (All images show H&E-stained slides. Panels A and C, 100 x magnification. Panels B and D, 400 x magnification).

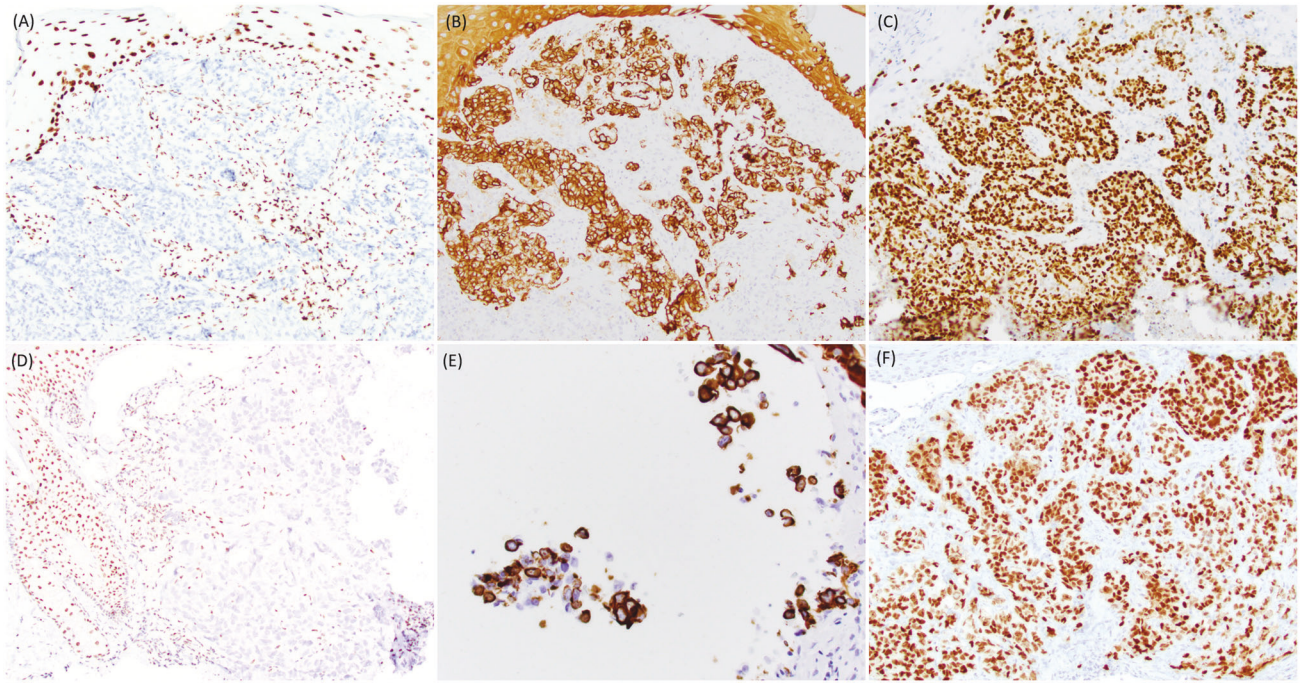

Fig. 2 Primary cutaneous SMARCA4-deficient undifferentiated malignant neoplasm immunohistochemical profile. Case 1 is depicted in the top row **A**–**C**, and case 2 is depicted in the bottom row **D**–**F**. Both cases showed complete loss of staining of SMARCA4 (**A**, **D**; note retained nuclear expression in non-neoplastic cells). Primary cutaneous SMARCA4-deficient malignant neoplasms also express pan-keratin (**B**, **E**) and Sox-2 (**C**, **F**). **E** shows small volume of tumor due to loss of tissue on deeper levels. All images depict 200x magnification.

Tumors arising in the lung showed variable keratin and Sox2 expression and were negative for TTF-1, napsin-A, p40, INSM1, and NUT. Tumors arising in the esophagus showed variable keratin expression and retention of INI1; they were negative for CDX-2, p63, INSM1, and S100 expression. The rectal primary showed keratin expression, focal SATB2 expression and was negative for CDX-2. The gallbladder primary was negative for Sox10 and INSM1 expression.

Molecular characteristics of cutaneous and visceral SMARCA4-deficient undifferentiated malignant neoplasms

Next-generation DNA sequencing was performed on all ten cases, with results summarized in Table 2. Cutaneous SD-UMN case 1 harbored two nonsense variants in *SMARCA4*, c.535 C > T and c.4531 A > T, which result in premature termination at Q179 and K1511, respectively. Neither mutation has been previously reported in COSMIC (Catalogue of Somatic Mutations in Cancer)[17]. While the sequencing data cannot distinguish between the two mutations occurring in the same allele or in different alleles, the complete loss of SMARCA4 expression (Fig. 2) supports biallelic inactivation in this patient. The second cutaneous SD-UMN patient also showed two *SMARCA4* mutations, again consistent with biallelic inactivation. The first was a known pathogenic variant (c.1492 C > T) which results in premature termination at Q498 while the second was a splice site variant predicted to lead to a frameshift alteration (c.1943_1943 + 1delinsAA). This patient additionally showed single copy deletion of the related gene *INI-1* (also known as *SMARCB1*). This is interesting because *INI-1* is also a member of the SWI/SNF complex. However, INI-1 expression was retained in this patient's tumor, and so the pathologic significance of this finding remains uncertain. Importantly, both cutaneous tumors harbored an ultraviolet radiation (UV)-associated mutational signature, which supports their cutaneous origin and which was absent in all visceral cases.

Two other mutational events were shared between the two skin tumors. The first was apparent biallelic inactivation of *CDKN2A* (p16). In case 1, this occurred through two-copy deletion of the locus (Fig. 4), while in case 2 this occurred through loss of one allele combined with the acquisition of a known pathogenic mutation in the other allele (c.341_342delinsTT; p.P114L). The second shared mutational event was apparent biallelic loss of *TP53*. Case 1 showed two known pathogenic mutations (c.772 G > A; p.E258K and c.833 C > T; p.P278L) while case 2 showed loss of one allele at chromosome 17p13.1 coupled with three known pathogenic mutations in the remaining allele (c.783-1 G > A, c.796_797delinsAA;p.G266K, and c.836_837delinsAA; p.G279E).

All 8 visceral SD-UMN cases also showed loss of function of *SMARCA4* aberrations. Interestingly, this occurred through a variety of mechanisms including mutation, two copy loss of the locus, and mutation combined with single copy loss of the locus (Table 2). Nearly all cases from visceral sites harbored *CDKN2A* loss-of-function aberrations (7/8 cases, 88%), suggesting that this may be a characteristic feature of this tumor family. *TP53* loss-of-function aberrations were also prominent (4/8 cases, 50%), although less uniformly so than *CDKN2A*.

There were some additional findings of interest. One of the lung tumors (case 2) showed known pathogenic mutations in *KRAS*, *STK11*, and *KEAP1*, which are recognized mutations in lung carcinomas. A different lung tumor (case 3) showed single copy loss of *INI-1* (*SMARCB1*), similar to one of the cutaneous cases. Interestingly, none of the visceral cases, including the four lung cases, showed a tobacco-associated mutational profile.

A variety of other mutations and copy number changes were present within all 10 cases, but we did not identify other clear patterns of shared or recurrent aberrations.

DISCUSSION

Here we report the first cases of primary cutaneous SD-UMN. Our assignment of these cases as bona fide primary tumors is based on extensive clinical, radiologic, and serologic analyses which failed to reveal any metastatic source, coupled with the molecular

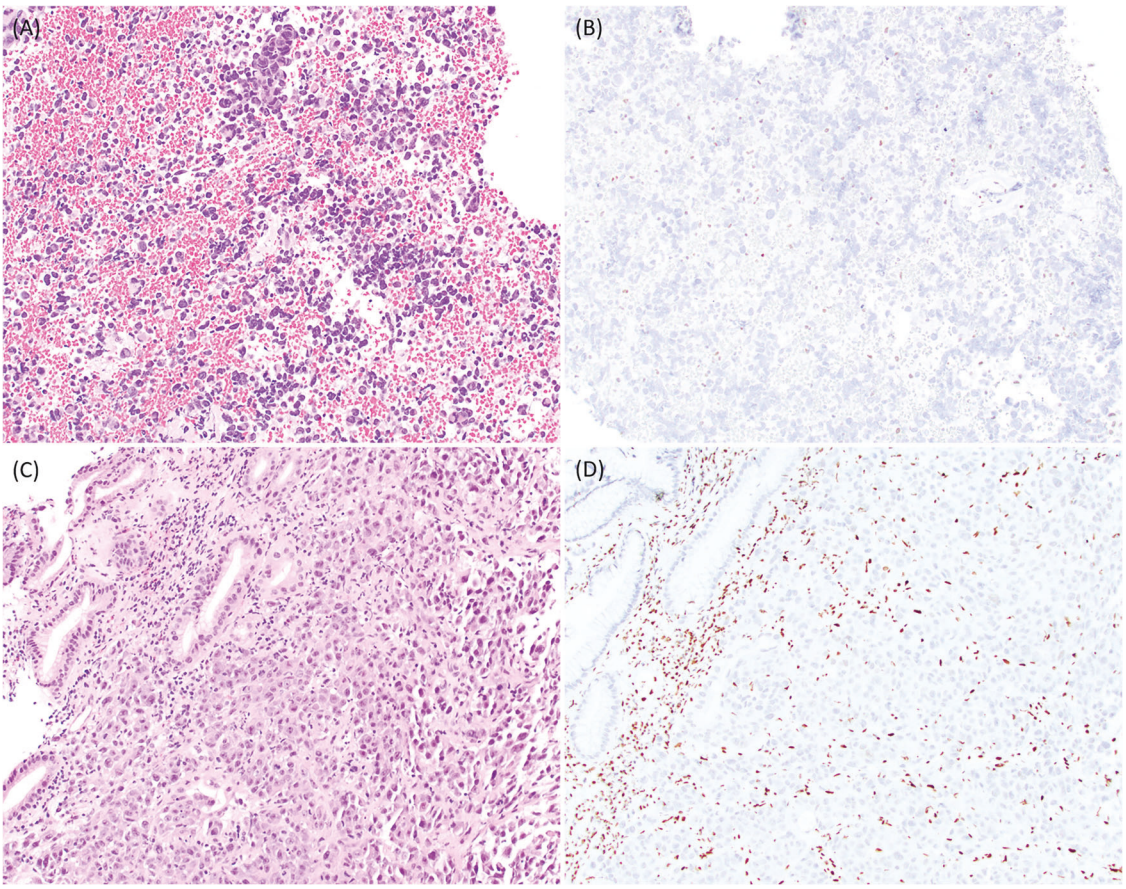

Fig. 3 Visceral SMARCA4-deficient undifferentiated malignant neoplasms are histologically highly similar to cutaneous counterparts. **A** shows a primary lung SMARCA4-deficient undifferentiated malignant neoplasm composed of pleomorphic epithelioid cells, frequent mitoses, and apoptotic debris. This tumor shows complete loss of SMARCA4 expression (with retention of expression in non-noeplastic cells) (B). A primary rectal SMARCA4-deficient undifferentiated malignant neoplasm shows classic epithelioid morphology with variably prominent nucleoli (**C**). This tumor shows complete loss of SMARCA4 expression (with retention of expression in non-neoplastic cells). Interestingly, the rectal epithelium also shows loss of SMARCA4 expression, which is of uncertain significance but suggests that it may represent neoplastic or pre-neoplastic epithelium (**D**). All images depict 200x magnification.

identification of a UV-associated mutational profile. This latter finding strongly supports a cutaneous origin and was not seen in any of the visceral tumors. We considered the possibility of metastasis from a regressed visceral primary. This sort of spontaneous regression is not well described for visceral SD-UMN and, in any case, the presence of a UV signature in these tumors argues against this model. The morphologic, immunohistochemical, and molecular features of cutaneous SD-UMN are strikingly similar to its visceral counterparts, arguing that they belong in the overall family of SD-UMN. Thus, our work expands the anatomic spectrum of this potentially aggressive tumor with important implications for diagnosis, prognosis, and therapy.

The differential diagnosis for poorly differentiated epithelioid malignancies in the skin is broad and challenging. The main considerations included poorly differentiated squamous cell carcinoma, melanoma, Merkel cell carcinoma, NUT midline carcinoma, epithelioid sarcoma, and others[18,19]. Our work highlights the importance of a broad immunohistochemical work-up which, in principle, should be able to distinguish between these entities through the identification of SMARCA4 loss coupled with exclusion of the other entities (e.g. lack of melanocytic or squamous markers, lack of CK20 and MCPyV, retention of INI-1, absence of NUT, and so on). Accordingly, when dealing with poorly differentiated epithelioid or rhabdoid dermal neoplasms which lack lineage-specific markers, evaluation of SMARCA4 expression may be helpful before rendering a diagnosis of poorly differentiated carcinoma/malignant neoplasm. Molecular analysis may be useful in further confirming the diagnosis and in excluding the known driver mutations of other entities, although most diagnoses should be possible by morphology and immunohistochemistry, coupled with strong clinico-pathologic correlation. Care should also be taken, primarily through clinical correlation, to exclude metastatic SD-UMN to the skin from an underlying visceral source, as has been recently documented[19]. Even within the differential diagnosis discussed above, SD-UMN stands out as being particularly aggressive. As more primary cutaneous SD-UMN cases are identified, it will be important to determine whether they show comparable rates of aggressiveness to their visceral counterparts[6,9,20]. Some of the entities in the differential diagnosis also have individualized therapeutic approaches, including sentinel node sampling, immunotherapy, and others[21,22]. Thus, the timely and accurate recognition of SD-UMN will be important to ensure selection of the proper therapeutic approach.

Our data also provide detailed molecular analysis of a relatively large series of SD-UMN from diverse anatomic sites, with a number of interesting findings. First, while loss of SMARCA4 expression is a hallmark of this entity (Figs. 2 and 3), the data reveal multiple molecular pathways to this end, including mutation, copy number loss, and a combination of the two. Indeed, this emphasizes the utility of additionally measuring copy number changes, and not just mutations, in understanding the full genomic profile. There may be other pathways to *SMARCA4*

Table 2. Molecular characteristics of cutaneous and visceral SMARCA4-deficient undifferentiated malignant neoplasms.

Site	SMARCA4 alterations	UV mutation signature	Other alterations of interest
Cutaneous Case 1	SMARCA4 c.4531 A > T (p.K1511*)[a] SMARCA4 c.535 C > T (p.Q179*) [a]	Present	9p21.3 two copy deletion of CDKN2A TP53 c.772 G > A (p.E258K) TP53 c.833 C > T (p.P278L)
Cutaneous Case 2	SMARCA4 c.1492 C > T (p.Q498*)[b] SMARCA4 c.1943_1943 + 1delinsAA ()[b]	Present	Single copy deletion of CDKN2A at 9p21.3 CDKN2A c.341_342delinsTT (p.P114L), exon 2 TP53 c.783-1 G > A () TP53 c.796_797delinsAA (p.G266K) TP53 c.836_837delinsAA (p.G279E) Single copy deletion of TP53 at 17p13.1 SMARCB1 single copy deletion at 22q11.23
Lung ($n = 4$)			
Case 1	SMARCA4 c.3169-2 A > T[c]	Absent	TP53 c.455_456insT (p.P153Afs*28)
Case 2	SMARCA4 c.942_943insC (p.A317Cfs*70)[c] Chromosome 19p arm level loss (including SMARCA4)[c]	Absent	9p21.3 two copy deletion of CDKN2A KRAS c.34 G > T (p.G12C) KEAP1 c.880 G > C (P.D294H) STK11 c.597 + 2 T > A ()
Case 3	SMARCA4 19p13.2 two copy deletion (exons 2-7)[c]	Absent	Chromosome 9 two copy loss of CDKN2A TP53 c.725 G > C (p.C242S) Chromosome 17p arm level loss (including TP53) Chromosome 22q arm level loss (including SMARCB1)
Case 4	SMARCA4 19p13.2 two copy deletion (exons 4-11)[c]	Absent	Chromosome 9p loss of CDKN2A CDKN2A c.251 A > G (p.D84G)
Esophagus ($n = 2$)			
Case 1	SMARCA4 c.3013 C > T (p.R1005*)[d] SMARCA4 exon 7 (chr19:11098548):: SMARCA4 exon 7 (chr19:11098573)	Absent	9p21.3-p24.3 single copy deletion of CDKN2A TP53 c.734 G > A (p.G245D) Chromosome 17p arm level loss (including TP53)
Case 2	SMARCA4 c.4741 G > A (p.G1581S)[d] SMARCA4 c.526 C > T (p.Q788R) SMARCA4 c.2363 A > G (p.Q176*)	Absent	9p21.3 single copy deletion of CDKN2A
Rectum			
($n = 1$)	SMARCA4 c.1603G > T (p.E535*)[e] SMARCA4 C.4318 C > T (p.Q1440*)	Absent	9p21.3 two copy deletion of CDKN2A
Gallbladder ($n = 1$)	SMARCA4 c.3383-3_3411del[f] CAGGAACCACGAAGGCGGAGGACCGGGGCATG SMARCA4 intron 25 (chr19:11141402):: SMARCA4 exon 26 (chr19:11141434) deletion	Absent	9p21.3-p24.3 single copy deletion of CDKNA2 TP53 c.637 C > T (p.R213*)

*Denotes the introduction of a STOP codon.

[a]Cutaneous case 1: These two SMARCA4 nonsense variants are predicted to confer biallelic inactivation.

[b]Cutaneous case 2: The nonsense variant and frameshift mutation leading to a splice site variant in SMARCA4 likely confer biallelic inactivation.

[c]Lung case 1: SMARCA4 splice site mutation predicted to lead to loss of function. Lung case 2: SMARCA4 frameshift variant predicted to lead to loss of function and chromosome 19p arm level loss including the SMARCA4 locus. Lung case 3: Two copy deletion of SMARCA4 in exons 2-7 causing loss of function. Lung case 4: Two copy deletion of SMARCA4 in exons 4-11 causing loss of function.

[d]Esophagus case 1: Biallelic inactivation of SMARCA4 resulting from a nonsense alteration and a 25 base pair deletion frameshift alteration (SMARCA4 p.Gln356Argfs*47). Esophagus case 2: One nonsense mutation (p.Q176) and two missense mutations likely lead to SMARCA4 loss of function.

[e]Rectal case: These two nonsense variants likely lead to biallelic loss of function of SMARCA4.

[f]Gallbladder case: SMARCA4 splice site deletion and SMARCA4 intron 25 (chr19:11141402):: SMARCA4 exon 26(chr19:11141434) deletion imply biallelic SMARCA4 inactivation.

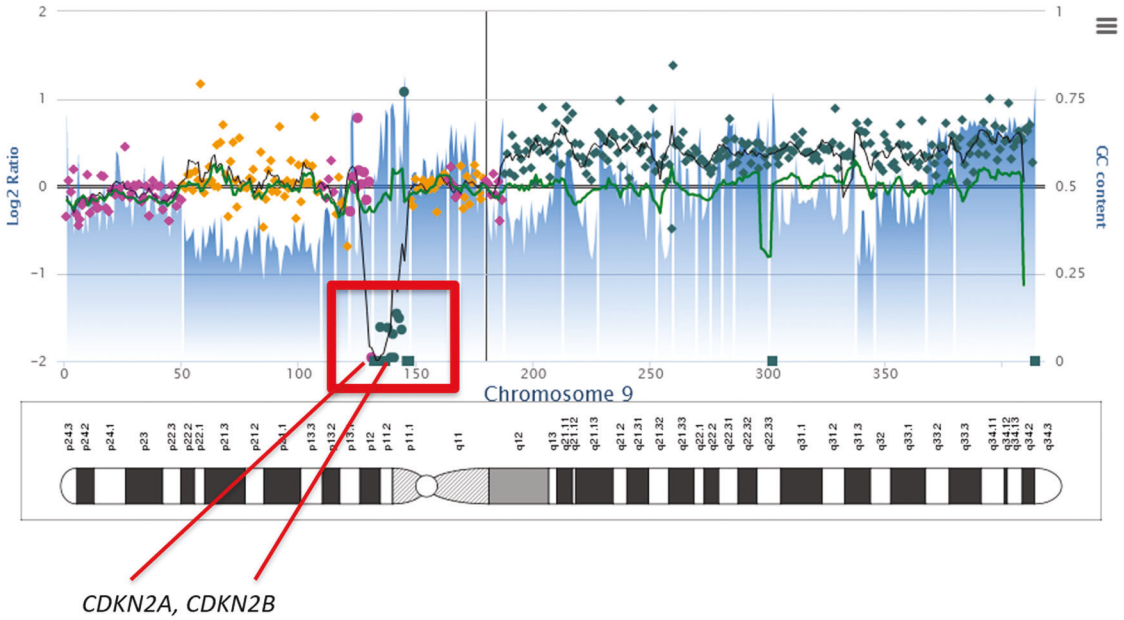

Fig. 4 Cutaneous case 1 copy number plot of chromosome 9 showing focal, two-copy loss of *CDKN2A* and *CDKN2B*. Magenta and gold dots indicate the log2 ratio of sample copy number relative to a pooled normal at the level of individual exons and selected introns for each of the targeted genes. Green dots indicate a copy number other than neutral as called by automated algorithms. Pale blue tracing shows the percent guanine and cytosine (GC) in the targeted region. The green line shows the median log2 ratio for all samples on a plate; the black line shows the ratio for the specific case.

inactivation that remain to be discovered. Targeted protein degradation and epigenetic regulation are interesting candidates. A second interesting feature was the presence of recurrent loss-of-function alterations in *CDKN2A* (9/10 cases, illustrated in Fig. 4) and *TP53* (6/10 cases). While these two genes are among the most commonly mutated in cancer, their prominence in this series of SD-UMN suggests that they, along with *SMARCA4*, may represent a core mutational module for this entity. Indeed, prior studies have found *TP53* (5/5 cases) and *CDKN2A* (2/5 cases) alterations in SMARCA4-deficient thoracic sarcoma[23]. Interestingly, in contrast, genomic profiling of SMARCA4-deficient uterine sarcoma and small cell carcinoma of the of the ovary, hypercalcemic type (which is defined by biallelic loss of function of *SMARCA4*), failed to reveal recurrent *TP53* or *CDKN2A* alterations, suggesting that *TP53* and *CDKN2A* alterations may play a more important role in tumors arising outside of the gynecologic tract[12,24,25].

As for nomenclature, there has been much discussion about whether this entity represents a carcinoma or a sarcoma[6,7]. Our understanding of SD-UMN is evolving, as highlighted by the improved characterization of the SMARCA4-deficient group of thoracic neoplasms. Initially described as SMARCA4-deficient thoracic sarcomas[6,26,27], recent work now proposes that these thoracic tumors largely represent smoking-related undifferentiated carcinomas rather than sarcomas[7]. Conversely, the lack of claudin-4 expression and the presence of Sox2 expression in some SMARCA4-deficient neoplasms with epithelioid morphology has been proposed by some authors to support a mesenchymal origin for these tumors[28]. We do not know the cell type of origin for the cutaneous SD-UMN and have therefore used the neutral term "malignant neoplasm" for now.

In summary, we report here the first two cases of primary cutaneous SD-UMN and show that their morphologic, immunohistochemical, and molecular features are highly similar to their visceral counterparts. Increased awareness of this entity should improve both diagnosis and clinical care. We expect that the identification of additional cases will clarify important aspects of this tumor including its true incidence, natural history, and pathophysiologic mechanisms.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Concept and design: E.R.G. and J.H. Case contribution: E.R.G. and J.H. Pathology review: E.R.G. and J.H. Molecular analysis and interpretation: L.M., E.R.G. and J.H. Original manuscript draft preparation: E.R.G. and J.H. Review and editing of manuscript: All authors.

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The authors declare no competing interests.

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