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ORIGINAL ARTICLE

Reappraisal of sinonasal undifferentiated carcinoma: SMARCB1 (INI1)-deficient sinonasal carcinoma: a single-institution experience

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Abstract Sinonasal carcinomas are rare and of diverse histology, often involve critical anatomic structures, and are associated with an aggressive clinical course and poor prognosis. Differentiating these tumor types may have clinical impact as advances in entity-specific therapeutic intervention could increase survival and quality of life and occasionally result in a cure. Recently, a unique subset of sinonasal carcinomas characterized by basaloid/rhabdoid tumor morphology and loss of expression of SMARCB1 (INI1) was identified. We tested a total of 256 tumors including head and neck (n=241) and thoracic (n=15) tumors with basaloid/rhabdoid morphology for loss of expression of SMARCB1 (INI1) using full tissue sections and tissue microarrays. Among these, four tumors of the sinonasal tract were found to be SMARCB1 (INI1) deficient and were reclassified as SMARCB1 (INI1)-deficient sinonasal carcinomas. These tumors appear to be restricted to the sinonasal tract, and their unique clinical, morphological, and immunohistochemical features seem to warrant inclusion as a separate new entity among the existing high-grade sinonasal neoplasms. Separation from the other types of sinonasal malignancies is important as the identification of SMARCB1 (INI1) deficiency may provide a new target for novel treatment approaches and may ultimately lead to improved patient survival.

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Introduction

The sinonasal tract is the location of a wide variety of histogenetically and clinically and biologically distinctive benign and malignant neoplasms. Malignant sinonasal tract tumors account for less than 1 % of all neoplasms and about 3 % of neoplasms of the upper aerodigestive tract. Sinonasal tract malignancies most commonly affect the maxillary sinuses (60 %), followed by the nasal cavity (22 %), ethmoid sinuses (15 %), and frontal and sphenoid sinuses (less than 3 %) [1]. Sinonasal tract tumors are histologically diverse, with the majority being squamous cell carcinomas or variants thereof (55 %); others include non-epithelial neoplasms (20 %), glandular tumors (15 %), undifferentiated carcinomas (7 %), and miscellaneous tumors (3 %). Survival rates of paranasal sinus malignancies have improved from 20 % in the 1950s to 60–80 % as reported in the current literature [1]. In recent years, several studies have identified novel diagnostic markers for sinonasal carcinomas, and increasing evidence shows the importance of immunophenotyping and genotyping for differentiating among these not rare overlapping neoplasms [1].

High-grade poorly differentiated and undifferentiated sinonasal carcinomas include nasopharyngeal-type undifferentiated carcinomas (lymphoepithelial carcinomas), sinonasal undifferentiated carcinomas (SNUCs), small cell neuroendocrine carcinomas, teratocarcinosarcomas, and poorly differentiated keratinizing and non-keratinizing variants of squamous cell carcinoma. In the last decade, another entity has been added—nuclear protein of testis (NUT)-midline carcinomas (NMCs). Very recently, two independent groups introduced a new entity characterized by loss of SMARCB1 (INI1), for

which the term SMARCB1 (INI1)-deficient sinonasal carcinoma has been proposed.

Herein, we report 4 new cases of SMARCB1-deficient sinonasal carcinomas, along with a review of other high-grade sinonasal carcinomas and challenges in the differential diagnosis of these tumors.

Materials and methods

This retrospective single-institution study was approved by the institutional review board. Four cases of SMARCB1-deficient sinonasal carcinomas were identified from the surgical pathology files at the Department of Pathology of the MD Anderson Cancer Center. Based on prior two reported series [2, 3], the files of head and neck and thoracic subspecialties were searched for tumors with basaloid and/or rhabdoid features within the last years. Ninety cases were retrieved and subjected to immunohistochemistry using anti-INI1 antibody. These cases (summarized in Table 1) included the following tumor types: 11 high-grade olfactory neuroblastomas (Hyams grades 3–4), 13 low-grade olfactory neuroblastomas (Hyams grades 1–2), 6 SNUCs (3 of these with scattered rhabdoid cells), 13 paranasal and lacrimal duct non-keratinizing squamous cell carcinoma (SCC) arising/associated with Schneiderian papillomas (5+ high-risk HPV), 7 sinonasal adenoid cystic carcinomas (solid variant), 1 low-grade sinonasal seromucinous adenocarcinoma, 1 nasal high-grade dedifferentiated basal cell adenocarcinoma, 1 parotid high-grade dedifferentiated acinic cell carcinoma, 4 sinonasal high-grade/small cell-type neuroendocrine carcinomas, 2 submandibular high-grade large cell-type neuroendocrine carcinomas, 2 recurrent intermediate-grade neuroendocrine carcinomas of middle ear (with rhabdoid cells), 3 sinonasal Ewing sarcoma (EWS)/primitive neuroectodermal tumor (PNET), 1 nasal alveolar-type rhabdomyosarcoma (RMS), 2 maxillary ameloblastomas, 2 NMCs, 1 atypical teratoid (AT)/rhabdoid tumor (RT) of the central nervous system, 4 paragangliomas (succinate dehydrogenase subunit B (SDHB) mutated), 2 sinonasal teratocarcinosarcomas, 1 sinonasal high-grade mixed germ cell tumor, 1 gastric basaloid adenocarcinoma, and 15 thoracic basaloid squamous carcinomas (12 pulmonary and 3 thymic carcinomas).

Table 1 MDACC head and neck and thoracic tumors studied for SMARCB1 expression

Tumor type	Number of cases
Sinonasal undifferentiated carcinoma (SNUC)	14
Olfactory neuroblastoma	40
Squamous carcinoma (sinonasal primary)	94
Neuroendocrine carcinoma (sinonasal primary)	20
Melanoma (sinonasal primary)	12
EWS/PNET (sinonasal primary)	12
Rhabdomyosarcoma (sinonasal primary)	15
Basal adenocarcinoma and basal cell carcinoma	6
Adenoid cystic carcinoma (solid pattern)	9
Teratocarcinosarcoma (sinonasal)	2
Mixed germ cell tumor (sinonasal)	1
Sinonasal non-intestinal type/seromucinous adenocarcinoma	1
Atypical teratoid/rhabdoid tumor (AT/RT)	1
NUT-midline carcinoma	2
Neuroendocrine carcinoma, major salivary	2
Neuroendocrine carcinoma, middle ear	2
High-grade/dedifferentiated acinic cell carcinoma, major salivary gland	1
Ameloblastoma	2
Basaloid adenocarcinoma, gastric	1
Paraganglioma, SDHB deficient	4
Basaloid squamous carcinoma, thoracic	15 (12 pulmonary and 3 thymic)

In parallel, four tissue microarrays (TMAs) previously constructed from cases from the head and neck files and not including the above cases were screened with anti-INI1 antibody. These TMAs included keratinizing and non-keratinizing primary SCC of paranasal sinuses (70 cases) and a variety of sinonasal small round cell tumors (8 SNUCs, 16 neuroendocrine carcinomas, 14 rhabdomyosarcomas, 16 olfactory neuroblastomas, 9 EWSs/PNETs, 12 mucosal melanomas, 2 solid adenoid cystic carcinomas, 5 basal/basaloid carcinomas, 11 squamous cell carcinomas/Schneiderian carcinomas). Among these, 2 additional cases of potential SMARCB1-deficient sinonasal tumors were identified. For these 2 cases which demonstrated loss of SMARCB1 immunoexpression on TMA, additional immunostaining was performed on full tissue sections.

Immunohistochemistry

All tumors were evaluated by immunohistochemistry for SMARCB1 (INI1) (BAF4, 1:50; BD Biosciences, San Diego, CA, USA) on 4-μm-thick sections from paraffin blocks using a Ventana automatic stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA). Only complete, clean nonstaining tumor cell nuclei clearly contrasting with clear-cut expression in normal cells in the background were considered to show *loss of expression* or *deficient status*. Normal mucosal glands, stromal fibroblasts, endothelial cells, and inflammatory cells served as internal controls in all cases.

Results

Clinical features

The clinical characteristics of patients with SMARCB1-deficient sinonasal carcinomas are summarized in Table 2.

Table 2 Clinical presentation of SMARCB1 (INI1)-deficient sinonasal carcinomas

Case	Age	Sex	Presentation	Site	Stage	Primary treatment	Clinical course	Follow-up
1	64	F	Diplopia	Frontal sinus	T_4N_0	Surgery CRT	NED	NED (3 months)
2	75	M	Headache, epistaxis, swelling of nasal bridge	Nasal cavity Large mass obstructing the left nasal passage	T_4N_0	Induction CRT Surgery+RT	Locoregional recurrence	AWD (12 months)
3	51	F	Sinusitis	Left temporal fossa/sphenoid	T_4N_0	Surgery CRT	Local recurrence, distant metastases	DOD (24 months)
4	33	F	Headache, epistaxis	Anterior skull base eroding through the cribriform plate	T_4N_0	Surgery CRT	Local recurrence	AWD (33 months)

CRT chemoradiotherapy, *RT* radiotherapy, *NED* no evidence of disease, *AWD* alive with disease

Histological findings

The SMARCB1-deficient carcinomas grew as epithelioid nests in the sinonasal submucosa architecturally mimicking the more common squamous cell carcinomas and occasionally presented a papillary growth reminiscent of Schneiderian carcinomas. High-grade features such as tumor necrosis and elevated mitotic activity along with infiltrative growth and bone invasion were present. Rhabdoid-like and/or plasmacytoid-like cells were recognized in each of these SMARCB1-deficient sinonasal carcinomas to a variable extent. Similar to SNUC, prominent nucleoli were present but no significant nuclear pleomorphism along with lack of frank squamous or glandular differentiation with morphological features was seen in these cases (Figs. 1, 2, 3, and 4).

Immunohistochemical features

Four of the 90 cases (4.4 %) with basaloid and/or rhabdoid features demonstrated complete loss of SMARCB1 immunohistochemical expression (Figs. 1, 2, and 3). Two other cases were detected from the primary sinonasal tumors studied on the TMA. For these 2 cases, additional immunohistochemistry was performed on full tissue sections and these did not demonstrate complete loss of SMARCB1, therefore failing to be classified as SMARCB1-deficient sinonasal carcinomas (Fig. 4).

Thus, of the total of 256 studied cases (of which 230 were sinonasal tumors), 4 cases of SMARCB1-deficient sinonasal carcinomas were identified. All these cases showed strong nuclear SMARCB1 staining in surrounding non-neoplastic tissues. Another SMARCB1-deficient tumor was the AT/RT control.

Ancillary studies and initial tumor classification

The immunohistochemical findings and original diagnoses are compiled in Table 3. Immunophenotypically, all 4 SMARCB1-deficient cases expressed pancytokeratins while showing complete lack of expression of SMARCB1. The neuroendocrine markers (synaptophysin and chromogranin) were expressed in 1 case; p63 was detected in all cases (focally positive in 3 cases while diffuse in 1 case); p16 was strongly expressed in case 1 (>75 %, nuclear and cytoplasmic patterns) (Fig. 1) with focal expression (45 % of tumor cells) in case 2 (Fig. 2); of note, both these cases were negative for high-risk HPV (by in situ hybridization); phosphatase and tensin homolog (PTEN) expression was also lost in case 1 (Fig. 1). Case 4 showed diffuse expression of Spalt-like transcription factor 4 (SALL4) and equivocal AFP staining, while other germ cell markers were negative (CD30, CD117) (Fig. 3). The 2 cases initially scored on TMAs as SMARCB1 deficient were diagnosed as SNUC and neuroendocrine carcinoma, respectively; subsequent full tissue sections from these samples did not

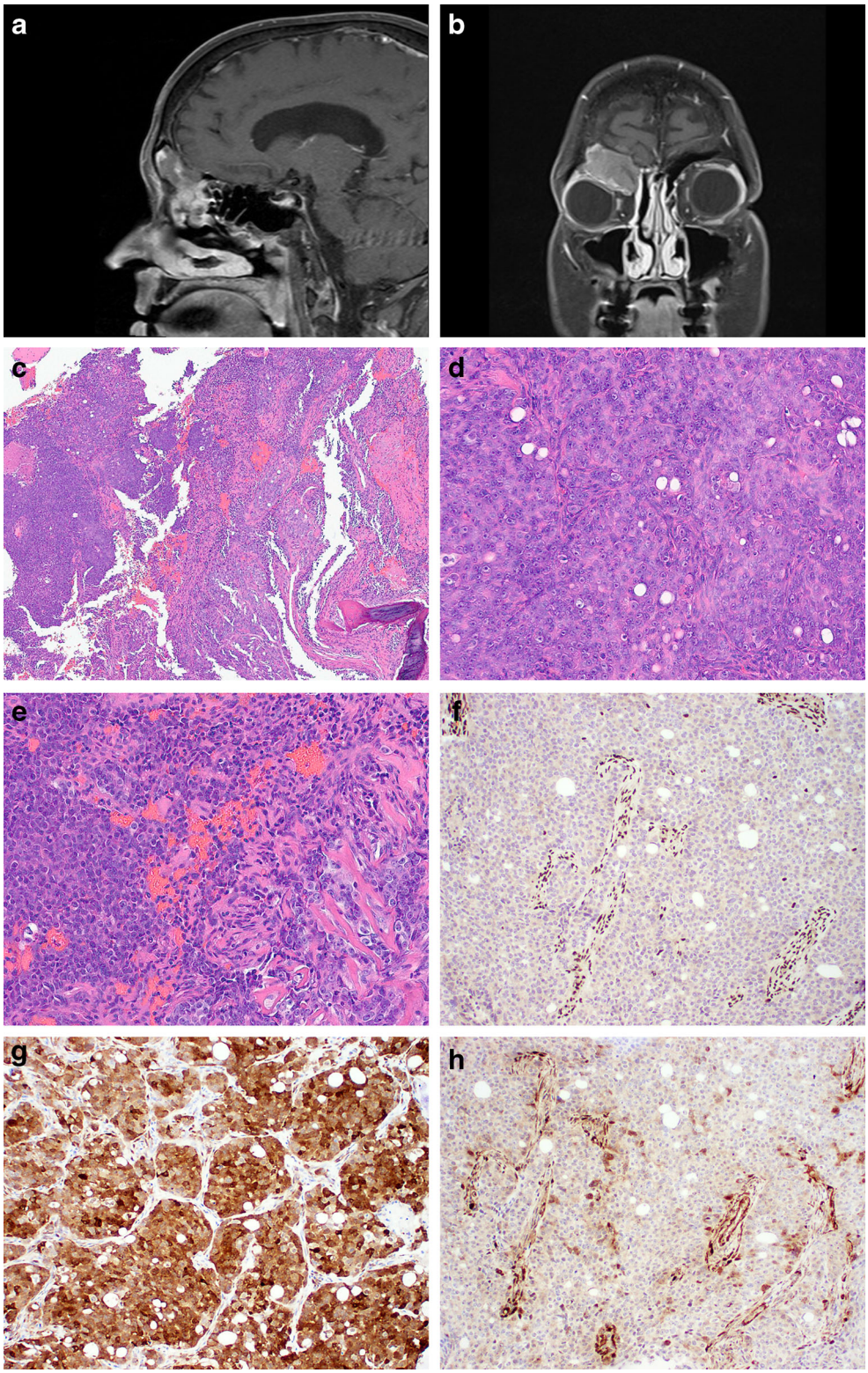

Fig. 1 MRI of the orbits, face, and skull base of case 1. An enhancing tumor is present which is filling the supraorbital portion of the frontal sinus, destroying the orbital cavity and extending into ethmoid sinus (**a**, **b**). Hematoxylin and eosin-stained sections (low- and high-power magnifications) with carcinoma growing as epithelioid nests in the sinonasal submucosa, architecturally mimicking the more common squamous cell carcinoma (**c**). **d**, **e** Complete loss of SMARCB1 (INI1) immunohistochemical expression (**f**). Diffuse p16 immunoreactivity (**g**) and PTEN loss (**h**)

confirm loss of SMARCB1 and were therefore not classified as SMARCB1 deficient. This is an important illustration for tumor heterogeneity and the drawbacks associated with utilization of tissue microarrays (Fig. 4) [4].

The first case among our 4 SMARCB1 (INI1)-deficient cases had a recurrence, with similar phenotype, including total loss of SMARCB1. At the request of treating oncologist, case 4 was tested for 50 hotspot mutations (CMS 50) and whole

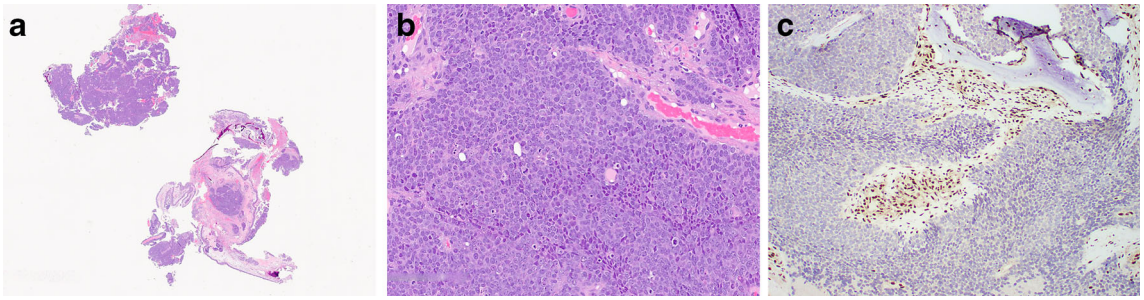

Fig. 2 Low-power (**a**) and high-power (**b**) magnification of the tumor of case 2 showing basaloid features. Complete loss of SMARCB1 expression (**c**)

exome sequencing (CMS 409), with no point mutations detected.

Discussion

SMARCB1 (INI1) is a tumor suppressor gene located on chromosome 22q11.2 [2]. Its gene product SMARCB1 (INI1) is ubiquitously expressed in nuclei of all normal tissues. *SMARCB1* gene inactivation has been implicated in the pathogenesis of a diverse group of malignant neoplasms that tend to share rhabdoid cytomorphology [5]. This group of SMARCB1-deficient tumors includes AT/RT, malignant rhabdoid tumors of the kidney and soft tissue, epithelioid sarcoma, renal medullary carcinoma, and subsets of myoepithelial carcinoma of the soft tissue, epithelioid malignant peripheral nerve sheath tumor, and extraskeletal myxoid chondrosarcoma. Recently, two independent groups introduced a new member of the SMARCB1-deficient tumor family—SMARCB1 (INI1)-deficient sinonasal carcinoma [2, 3].

To date, 16 SMARCB1-deficient sinonasal carcinomas have been described in details in the medical literature (including our 4 cases); 3 cases were identified in a cohort of 112 sinonasal carcinomas at the University Hospital of Erlangen, 9 cases were derived from the files of the Johns Hopkins Medical Institutions, Baltimore (142 sinonasal tumors screened on TMAs), and 4 cases were identified at MD Anderson Cancer Center among 230 primary sinonasal tumors (and a total of 254 screened tumors with basaloid and/or rhabdoid features). These represent 3.3 % out of a combined series of 484 sinonasal primary tumors. From these three studies, it appears that morphologically, these carcinomas are characterized by rounded or anastomosing nests of tumor cells set in a fibrous stroma. In some cases, a prominent exophytic component with papillary fronds can be noted but generally, the tumors show a cohesive pattern of growth. Peripheral palisading and radial growth around blood vessels imparting a pseudorosette-like

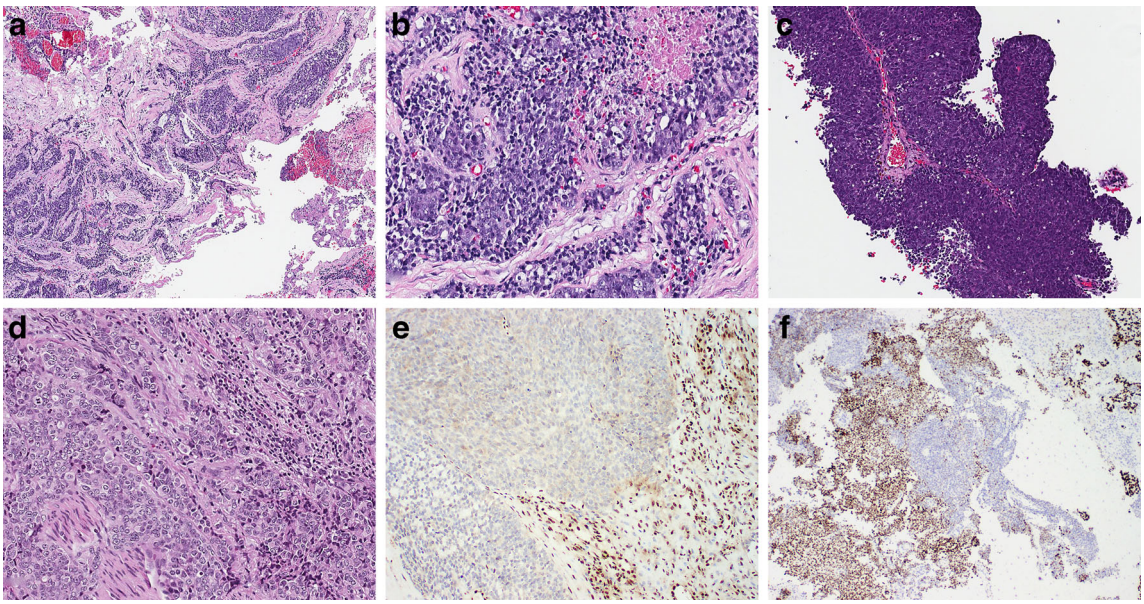

Fig. 3 Case 4 was initially diagnosed as a mixed germ cell tumor. The tumor has microreticulated (**a**, **b**) and basaloid papillary (**c**) patterns. Perineural invasion and rhabdoid cells are seen in some areas (**d**). Phenotypically, the tumor is SMARCB1 (INI1) deficient in both basaloid and microreticular areas (**e**), while SALL4 is positive within the microreticular areas (**f**)

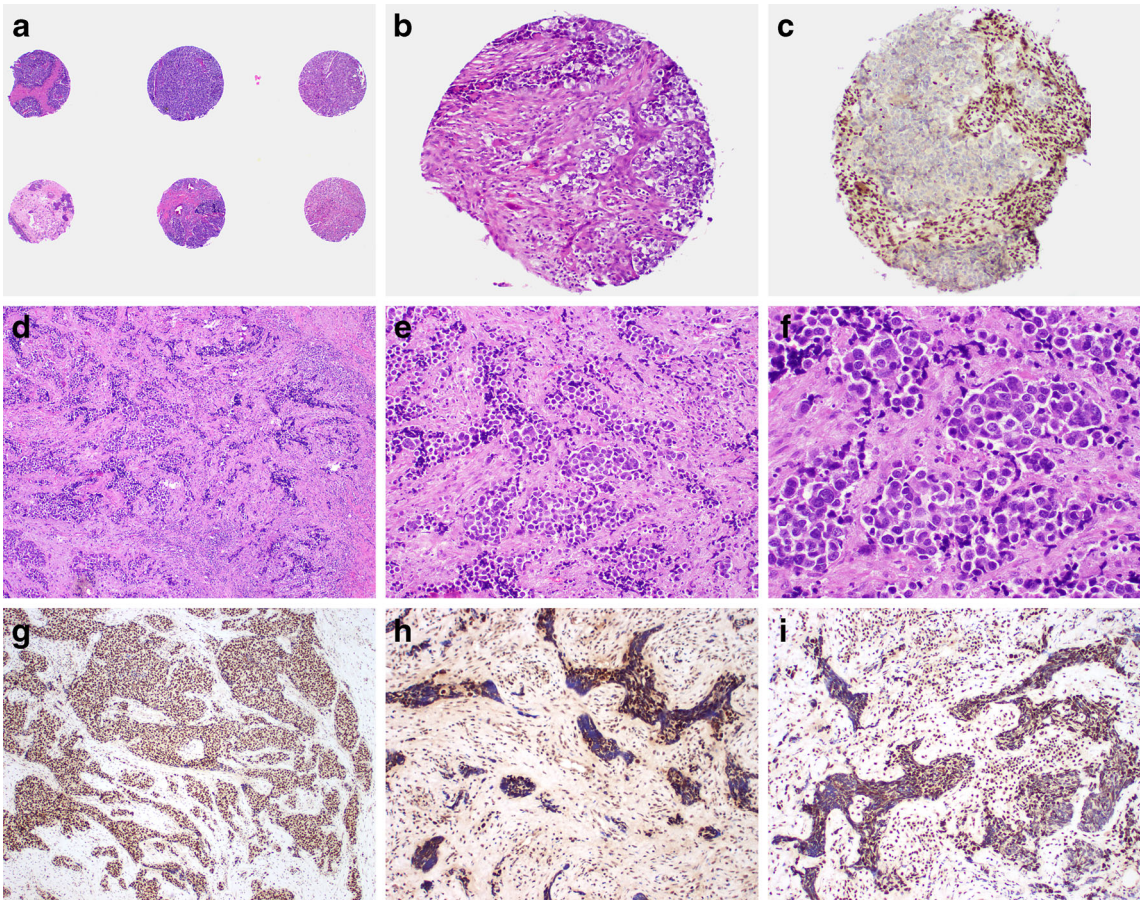

Fig. 4 TMA constructed from sinonasal tumors (**a**, **b**). SMARCB1 (INI1) immunoperoxidase study (**c**). High-grade sinonasal carcinoma with focal rhabdoid elements (**d**–**f**). The tumor retains SMARCB1 (INI1) expression (**g**–**i**), although scattered foci were SMARCB1 (INI1) deficient (**h**, **i**) as an illustration of tumoral heterogeneity

pattern are further characteristics. The tumors are highly infiltrative and often show invasion of the underlying bone. Cytologically, the cells have large round nuclei and prominent nucleoli. The cytoplasm can vary and ranges from scant (basaloid) to more abundant with prominent eccentric eosinophilic cytoplasm (rhabdoid). Necrosis and a high mitotic rate are common findings. Isolated cases contained scattered ductlike spaces, but squamous or glandular differentiation is not a feature of reported SMARCB1-deficient sinonasal carcinomas. However, more recently, a series of 8 new cases presented in abstract form suggested a wider histological spectrum as initially recognized with rare cases showing squamoid, oncocytoid, nodular epithelioid sarcoma-like and small cell carcinoma-like pattern [8]. However, frank squamous differentiation or keratinization has never been observed in this entity.

Immunophenotypically, SMARCB1-deficient cases express pancytokeratins while showing complete lack of expression of SMARCB1. In addition, variable staining can be seen with CK5, p63, p40, p16, E-cadherin, and synaptophysin. Original tumor diagnosis often includes poorly differentiated non-keratinizing (basaloid) squamous cell carcinoma, SNUC,

Table 3 Phenotype for SMARCB1 (INI1)-deficient sinonasal carcinomas

Case	Original diagnosis	Pan-CK	CK5/6	Synaptophysin chromogranin	p16	p63	HPV	EBER (EBV)	PTEN
1	Sinonasal undifferentiated carcinoma (SNUC)	+	Focally +	−	+	Focally +	−	−	−
2	Basaloid squamous carcinoma	Focally +	+	−	Focal (45 %)	+	−	−	+
3	SNUC	+	Focally +	Focally +	n/a	Focally +	−	−	n/a
4	High-grade mixed germ cell tumor	+	n/a	−	n/a	Focally +	n/a	n/a	n/a

CK cytokeratin, *n/a* not available

or carcinoma, not otherwise specified. In our series, the original diagnosis was SNUC (2 cases), basaloid squamous carcinoma (1 case), and high-grade mixed germ cell tumor (1 case). Case 4 was tested for 50 hotspot mutations (CMS 50) and whole exome sequencing of SMARCB1 (CMS 409), with no mutations detected. Possible explanations for this discrepant loss of SMARCB1 protein expression and lack of *SMARCB1 (INI1)* mutations are that mutations exist in other exons that are not covered by the CMS 50, possible deletion that is not detected by the CMS 50 [CMS 50 covers three amplicons for this gene: 2(35–72), 4–5(144–206), and 9(373–386)], methylation of the promoter *SMARCB1 (INI1)* gene, or posttranslational/epigenetic silencing mechanisms.

As observed in a recent study [4], assessment of SMARCB1 expression on tissue microarrays should be interpreted with caution. Tumor areas with suboptimal tissue preservation and areas closely associated with tumor cell necrosis may show variable reduction in the SMARCB1 reactivity. In line with these observations, we identified 2 cases as possible SMARCB1-deficient tumors on TMAs but reassessment on conventional slides showed intact expression.

SMARCB1 (INI1) represents the first member of an ATPase chromatin remodeling complex (the SWI/SNF complex) to be implicated in the genesis of cancer. Subsequent studies investigating its mechanism of tumor suppression showed that *SMARCB1 (INI1)* loss causes cell cycle progression in part via downregulation of $p16^{INK4a}$ and upregulating *E2Fs* and *cyclin D1*. However, the aberrant proliferative stimulus caused by *SMARCB1 (INI1)* loss also triggers cell cycle checkpoints causing arrest and apoptosis. Thus, despite causing the upregulation of proliferation-associated gene pathways, *SMARCB1 (INI1)* loss is lethal to most primary cells [6, 7]. Disruption of checkpoints via inactivation of *TP53* in vivo results in dramatic oncogenic synergy with *SMARCB1 (INI1)* loss, leading to cancer formation at a median of 3 weeks, although loss of *TP53* itself is insufficient to circumvent arrest in cultured SMARCB1-deficient cells [6, 7].

Several targets have been identified that may play a role in oncogenic transformation following *SMARCB1* loss [7]. Cyclin D1 is expressed at high levels in rhabdoid tumors, and this effect appears specific as other cyclins are not similarly overexpressed compared to other cancers. SMARCB1 binds to *cyclin D1* promoter and regulates its expression. Ablation of *cyclin D1* has been shown to prevent rhabdoid tumor growth, and pharmacologic inhibition has shown promise in mouse models [6, 7]. *C-MYC* is highly expressed in rhabdoid tumors and SMARCB1, and the SWI/SNF complex has been reported to modulate *c-MYC* activity. The SWI/SNF complex binds to the *C-MYC* promoter and has been reported to repress the expression of c-MYC. However, SMARCB1 protein has also been shown to directly interact with c-MYC and is required for its transactivation potential. Thus, it is currently unclear whether the high levels of c-MYC in rhabdoid tumors contribute to oncogenesis or whether this is secondary due to loss of c-MYC transactivation function [7]. Loss of *SMARCB1 (INI1)* has also been shown to interfere with activation of interferon target genes, and treatment with either interferon or *PLK1* inhibitors may hold therapeutic promises [7].

The cases screened in this study included a large range of basaloid/rhabdoid sinonasal and non-sinonasal neoplasms of the head and neck and thoracic region. From these, only 4 cases emerged that matched the previous description of SMARCB1-deficient carcinomas. Interestingly, these tumors were all located in the sinonasal tract which implies that these tumors may be site restricted, further emphasizing the notion that these tumors may represent a specific clinicopathological entity. Despite some recent improvements in therapy, rhabdoid tumors remain highly lethal cancers. Better understanding of the mechanisms by which loss of *SMARCB1* drives oncogenesis has the potential to identify novel therapeutic approaches for these aggressive tumors and related cancers.

To conclude, we have identified 4 more cases of SMARCB1 (INI1)-deficient sinonasal carcinomas among a large series of 256 cases of head and neck and thoracic neoplasms. The clinical, morphological, and immunohistochemical features of these tumors seem to be unique, warranting inclusion as a new entity among high-grade sinonasal malignancies. Unraveling of the underlying molecular aberrations may provide future targeted therapies for these unusual tumors.

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Conflict of interest The authors declare that they have no competing interests.

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