SMARCA4/BRG1-Deficient Sinonasal Carcinoma
Morphologic Spectrum of an Evolving Entity

Aanchal Kakkar, MD; Subiyathul Farah Ashraf, MD; Amber Rathor, MSc; Amit Kumar Adhya, MD; Suresh Mani, MS; Kapil Sikka, MS; Deepali Jain, MD, DNB, FIAC

**Context.**—Molecular analysis of poorly differentiated/undifferentiated sinonasal neoplasms has resulted in identification of a growing number of genetically defined tumors. SMARCA4-deficient sinonasal carcinoma is one such recently described entity that emerged from within sinonasal undifferentiated carcinoma (SNUC), neuroendocrine carcinoma (NEC), and teratocarcinosarcoma (TCS).

**Objective.**—To identify SMARCA4-deficient sinonasal carcinomas from a large institutional cohort of poorly differentiated/undifferentiated carcinomas and evaluate their clinicopathologic features.

**Design.**—SMARCA4/BRG1 immunohistochemistry was performed on all tumors diagnosed as SNUC, poorly differentiated carcinoma, NEC, and TCS during a 12-year period. SMARCA2/BRM and INSM1 immunostaining was performed in SMARCA4-deficient cases.

**Results.**—Twelve SMARCA4-deficient sinonasal carcinomas were identified among 299 cases. Morphologically, 5 cases were large cell NEC, 2 cases were small cell NEC, and 5 were TCS. SMARCA4 loss was diffuse and complete in 10 cases, while 2 cases showed focal retention. Most cases showed diffuse cytoplasmic staining accompanied by weak, usually focal staining for chromogranin and synaptophysin. INSM-1 showed negativity in most cases. All cases showed retained SMARCA2 expression. IDH1/2 mutation was absent in all cases analyzed. Four of 7 patients died of disease, and aggressive multimodality treatment had better outcome.

**Conclusions.**—SMARCA4-deficient sinonasal carcinomas are morphologically akin to sinonasal poorly differentiated NECs and TCS, display cytokeratin positivity and only focal staining for neuroendocrine markers, and have aggressive biological behavior. Inclusion of SMARCA4 in the immunohistochemical panel for diagnostic workup of all sinonasal NEC and TCS phenotypes will facilitate their early recognition. Comprehensive germline and somatic mutational analyses of these tumors are necessary for further insights into their molecular pathogenesis.

(Arch Pathol Lab Med. doi: 10.5858/arpa.2021-0001-OA)

The sinonasal tract harbors a diverse spectrum of high-grade neoplasms, many of which have considerably overlapping morphologic features owing to their poorly differentiated or undifferentiated appearance. Some of these tumors have now been characterized as distinct entities on the basis of their specific etiology, for example, human papillomavirus–related multiphenotypic carcinoma, or specific molecular alterations, namely NUT carcinoma and isocitrate dehydrogenase 2 (IDH2)–mutant sinonasal undifferentiated carcinoma (SNUC). Precise classification of these distinct neoplasms within the poorly/undifferentiated sinonasal carcinoma subgroup has significant prognostic and therapeutic implications.

The mammalian switch/sucrose nonfermenting (mSWI/SNF) complex is a family of ATP-dependent chromatin remodeling proteins that is a master regulator of gene transcription. It consists of 12 to 15 subunits encoded by 29 genes, including 2 mutually exclusive catalytic ATPase subunits, namely SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2 (SMARCA2)/Brahma (BRM) and SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4 (SMARCA4)/Brahma-related gene 1 (BRG1), and several core subunits one of which is SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily B, member 1 (SMARCB1). Mutations in the genes encoding these subunits occur in more than 20% of all human cancers, with biallelic inactivation of SMARCB1 in malignant rhabdoid tumor. Not surprisingly, SMARCB1 was also the first gene of this family to be associated with sinonasal neoplasms, with SMARCB1-deficient sinonasal carcinoma characterized by loss of INI1 (integrator interactor 1) immunexpression. Subsequently, loss of expression of SMARCA4 was documented in a few cases of poorly/undifferentiated sinonasal carcinomas. SMARCA4 mutations have also been

Accepted for publication August 9, 2021.

From the Departments of Pathology (Kakkar, Ashraf, Rathor, Jain) and Otorhinolaryngology and Head and Neck Surgery (Mani, Sikka), All India Institute of Medical Sciences, New Delhi, India; and the Department of Pathology and Laboratory Medicine, All India Institute of Medical Sciences, Bhubaneswar, India (Adhya).

This research received funds from Collaborative intramural grant, Research Section, All India Institute of Medical Sciences, New Delhi (AC-005).

The authors have no relevant financial interest in the products or companies described in this article.

Corresponding author: Deepali Jain, MD, DNB, FIAC, Department of Pathology, All India Institute of Medical Sciences, New Delhi - 110029, India (email: deepalijain76@gmail.com).
described in small cell carcinoma of ovary, hypercalcemic type (SCCOHT),16,17 in lung carcinomas,9,18 and in a subset of aggressive thoracic and uterine sarcomas.19–21 Additionally, Rooper et al22 recently reported SMARCA4 loss in a large proportion of sinonasal teratocarcinomas (TCSs), indicating that the morphologic spectrum of SMARCA4-deficient sinonasal tumors is wider than shown previously.22 Eleven cases of SMARCA4-deficient sinonasal carcinoma have been described to date, and much remains to be learned about this novel entity, including its morphologic spectrum, immunohistochemical profile, and biological behavior.7,16,18 We therefore conducted this study to identify cases of SMARCA4-deficient carcinomas from a retrospective cohort of sinonasal poorly differentiated/undifferentiated carcinomas and TCSs, and document their clinicopathologic and immunohistochemical features for insights into improving diagnosis and classification, as well as providing directions for development of targeted therapies.

METHODS

Approval was obtained from the Institute Ethics Committee to conduct this study on archival patient tumor samples. All tumors diagnosed as sinonasal undifferentiated carcinoma (SNUC), poorly differentiated carcinoma, neuroendocrine carcinoma (NEC), and TCS between 2009 and 2020 were retrieved from our departmental records. Histopathologic features and immunohistochemical staining performed at the time of reporting were reviewed. NECs from head and neck sites other than sinonasal were retrieved and included for comparison.

Immunohistochemistry (IHC) was performed manually on formalin-fixed, paraffin-embedded (FFPE) tissue microarray for screening in 230 cases followed by confirmation in whole sections; in the remaining 69 cases, IHC was performed on whole sections, using primary antibodies against SMARCA4/BRG1 (1:800; clone E8V5B, Cell Signaling Technology), Sections from normal tonsil were used as positive controls; endothelial cell nuclei served as internal positive controls. SMARCA4 immunostaining was interpreted in viable tumor nuclei clear of necrotic foci. Cases with loss of SMARCA4 nuclear staining with intact staining in endothelial cells were considered as SMARCA4-deficient sinonasal carcinomas. In all SMARCA4-deficient cases, IHC was performed with SMARCA2/BRM (1:800; clone D9E8B, Cell Signaling Technology), insulinoma-associated protein 1 (INSM1) (1:1000; clone A8, Aldrich), catenin (1:100; clone 14/Beta-Catenin, BD Transduction Laboratories), CHN1 (1:500; clone 5E8, Cell Signaling Technology), and MET (1:100; clone D5E2, Cell Signaling Technology). Sections from normal tonsil were used as internal positive controls. Genomic DNA was extracted from FFPE tissue samples by using Reliaprep FFPE gDNA Miniprep system DNA extraction kit (catalog No. A2351, Promega) following slight modifications in the manufacturer’s instructions. IHDI/2 mutation analysis was done by using TRUPCR IHDI/2 detection kit (catalog No. 3B1329, 3B BlackBio Biotech India Ltd) designed to detect various IHDI/2 somatic mutations (IDH1: R132H, R132C, R132Q, R132x; IDH2: R172K, R172x) and p53 (1:200; clone DO-1, Santa Cruz). Rb (1:1000; clone SP100, Cell Signaling Technology), p53 (1:200; clone DO-1, Santa Cruz), and Rb (1:1000; clone G3-245, Zeta Corporation, on Ventana GX autostainer with OptiView DAB IHC Detection Kit). Clinical, treatment, and follow-up data of the cases with SMARCA4 loss were retrieved from patient records and by telephonic interview.

Genomic DNA was extracted from FFPE tissue samples by using Reliaprep FFPE gDNA Miniprep system DNA extraction kit (catalog No. A2351, Promega) following slight modifications in the manufacturer’s instructions. IHDI/2 mutation analysis was done by using TRUPCR IHDI/2 detection kit (catalog No. 3B1329, 3B BlackBio Biotech India Ltd) designed to detect various IHDI/2 somatic mutations (IDH1: R132H, R132C, R132Q, R132x; IDH2: R172K, R140x, R172x). The qPCR assay was performed with 50 to 75 ng genomic DNA on QIAGEN real-time PCR cycler, Rotor-gene Q.

RESULTS

A total of 299 sinonasal carcinomas were evaluated by SMARCA4 IHC, including 244 poorly differentiated/undifferentiated carcinomas, 38 NECs, and 17 sinonasal TCSs. Fourteen tumor specimens from 12 patients (4%) showed SMARCA4 loss, of which have been published previously (cases 3 to 5).16 The clinical details of the patients are summarized in Table 1 and representative imaging for case 1 is shown in Figure 1, A and B. Mean age of the patients was 38.5 years (median, 35.5 years; range, 22–70 years). They included 10 males and 2 females. Cases 1 and 9 had 2 specimens each, from a biopsy and an endoscopic excision. Five cases had previously been diagnosed as poorly differentiated NEC, 5 as TCS, 1 as poorly differentiated carcinoma with focal neuroendocrine differentiation, and 1 as poorly differentiated malignant tumor. Among the 5 poorly differentiated NECs, 2 had earlier been classified as small cell NEC (SCNEC), 1 as SCNEC with overlying squamous dysplasia, 1 as large cell NEC (LCNEC), and 1 had not been categorized as small cell or large cell type. Thus, 13.2% of all NECs evaluated, 29.4% of all TCSs, and 0.8% of all poorly differentiated/undifferentiated carcinomas were identified as SMARCA4-deficient sinonasal carcinoma. All 6 NECs from other head and neck sites did not show loss of SMARCA4 expression.

On review of the microscopic features (Figure 2, A through F; Figure 3, A through F; Figure 4, A through F), 5 cases had an appearance akin to LCNEC (Figure 2, A; Figure 3, A), with tumor cells having scant to moderate amount of cytoplasm, ovoid nuclei with stippled chromatin, some with enlarged nuclei having prominent nucleoli. Tumor cells were arranged in nests and sheets, and showed whorls and palisading in 1 case. Two cases showed features resembling SCNEC (Figure 2, B; Figure 4, A), with organoid architecture and rosettes. One of these also showed dysplasia in the overlying squamous epithelium (Figure 4, A). Abrupt anisonucleosis, nuclear molding, brisk mitoses, and crushing of tumor cells were frequent in both LCNEC- and SCNEC-like tumors.

The 5 cases previously diagnosed as TCS (Figure 2, C; Figure 4, D) showed a primitive neuroectodermal component accompanied by a malignant epithelial component in the form of infiltrating glands (5 cases) or squamous islands (1 case), and a sarcomatous component with rhabdomyoblastic differentiation evidenced by desmin and/or myogenin immunopositivity in 3 cases (cases 8, 9, 12) and undifferentiated sarcoma in 2 cases (cases 10, 11). The primitive neuroectodermal component in 4 TCS cases had LCNEC-like morphology, with nested architecture and palisading of tumor cells, which had oval to carrot-shaped nuclei with stippled chromatin and prominent nucleoli, resembling the SMARCA4-deficient LCNECs. In the remaining TCS case, LCNEC-like areas as well as olfactory neuroblastoma-like foci were seen. The latter showed uniform cells with scant cytoplasm and round to ovoid nuclei having stippled chromatin embedded in a neurofibrillary matrix; rosettes were also present. Corresponding images for SMARCA4 staining in LCNEC, SCNEC, and TCS phenotypes are shown in Figure 2, D through F.

On immunohistochemistry, 10 SMARCA4-deficient cases showed diffuse cytokeratin staining (Figure 3, B) accompanied by weak staining for chromogranin (Figure 3, C), synaptophysin (Figure 3, D), and CD56, which was frequently focal. Interestingly, INSM1 displayed negativity in most cases, being focally positive with weak intensity (Figure 3, E) in 2 cases (cases 1, 6) and moderate intensity in only 1 case (case 12). SMARCA4 loss was diffuse and complete in 10 cases, while 2 cases (cases 6, 7) showed severe reduction with focal retention of expression in a small proportion of the tumor area (Figure 2, E), both of which showed SCNEC phenotype. Notably, SMARCA4 expression
Table 1. Clinical Features, Initial Diagnosis, and Clinical Course of Patients With SMARCA4-Deficient Sinonasal Carcinoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, y/ Sex</th>
<th>Location</th>
<th>Clinical Presentation</th>
<th>Procedure</th>
<th>Initial Diagnosis</th>
<th>Clinical Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48/M</td>
<td>Bilateral nasal cavities, sphenoid, ethmoid mass with intracranial extension</td>
<td>Nasal obstruction, epistaxis, epiphora × 4 mo, polyoid mass</td>
<td>Biopsy; excision</td>
<td>Poorly differentiated NEC, small cell type</td>
<td>3 cycles of cisplatin, etoposide NACT with partial response; endoscopic excision of residual tumor in superior meatus after 7 mo; received definitive CT RT (60 Gy in 30 fractions over 6 wk); concurrent cisplatin 65 mg × 6 cycles; alive without disease at 35 mo</td>
</tr>
<tr>
<td>2</td>
<td>70/M</td>
<td>Right nasal cavity</td>
<td>Necrotic mass in right nasal cavity × 6 mo</td>
<td>Biopsy</td>
<td>Poorly differentiated NEC, large cell type</td>
<td>Received CT; progressive disease on treatment; died 9 mo from disease onset</td>
</tr>
<tr>
<td>3</td>
<td>30/M</td>
<td>Nasal cavity</td>
<td>NA</td>
<td>Biopsy</td>
<td>NEC</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>41/M</td>
<td>Nasal cavity</td>
<td>NA</td>
<td>Biopsy</td>
<td>Poorly differentiated malignant tumor</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>51/F</td>
<td>Sinonasal mass, unspecified</td>
<td>NA</td>
<td>Excision</td>
<td>Small cell NEC</td>
<td>Died at 3 mo, before starting radiation therapy</td>
</tr>
<tr>
<td>6</td>
<td>30/M</td>
<td>Left nasal cavity; intracranial extension</td>
<td>Nasal cavity mass</td>
<td>Biopsy</td>
<td>High-grade poorly differentiated carcinoma with focal NE differentiation</td>
<td>Palliative CT; alive with residual disease at 34 mo from symptom onset</td>
</tr>
<tr>
<td>7</td>
<td>43/M</td>
<td>Sinonasal mass, unspecified, with intracranial extension</td>
<td>Case of sinonasal cancer, post CT RT; recurrent nasal cavity growth after 18 mo</td>
<td>Excision</td>
<td>Poorly differentiated NEC, small cell type with overlying squamous dysplasia</td>
<td>Progressive disease with intracranial extension; no adjuvant treatment; died 34 mo from disease onset</td>
</tr>
<tr>
<td>8</td>
<td>30/M</td>
<td>Nasal cavity</td>
<td>NA</td>
<td>Excision</td>
<td>Teratocarcinosarcoma</td>
<td>Received 3 cycles of CT; progressive disease; died at 14 mo from disease onset</td>
</tr>
<tr>
<td>9</td>
<td>25/M</td>
<td>Left nasal cavity</td>
<td>Nasal obstruction, epistaxis × 2 mo</td>
<td>Biopsy; excision</td>
<td>Teratocarcinosarcoma</td>
<td>Examination 3 mo after biopsy showed tumor filling the nasal cavity, endoscopic excision was done; lost to follow-up</td>
</tr>
<tr>
<td>10</td>
<td>45/F</td>
<td>Right nasal mass</td>
<td>Nasal obstruction, epistaxis × 3 mo</td>
<td>Excision</td>
<td>Teratocarcinosarcoma</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>28/M</td>
<td>Left nasal cavity mass, intracranial extension</td>
<td>Nasal cavity mass × 2 mo</td>
<td>Biopsy</td>
<td>Teratocarcinosarcoma</td>
<td>Surgical debridement of tumor at another hospital; residual intracranial disease at 7 mo, awaiting RT</td>
</tr>
<tr>
<td>12</td>
<td>22/M</td>
<td>Left sinonasal mass with intracranial extension</td>
<td>Nasal cavity mass</td>
<td>Biopsy</td>
<td>Teratocarcinosarcoma</td>
<td>Recent case; awaiting treatment</td>
</tr>
</tbody>
</table>

Abbreviations: CT, chemotherapy; NA, not available; NACT, neoadjuvant chemotherapy; NE, neuroendocrine; NEC, neuroendocrine carcinoma; RT, radiotherapy.

Figure 1. Contrast-enhanced magnetic resonance imaging coronal section (A) shows a large enhancing mass filling the left nasal cavity and ethmoid sinus, with destruction of the cribriform plate and nasal septum and intracranial extradural extension. Axial image (B) shows an enhancing mass involving bilateral ethmoid sinuses.
was lost in all 3 components of the TCS cases (Figure 2, F). None of the cases showed loss of SMARCA2 expression (Figure 3, F). Two cases showed loss of Rb immunopositivity (Figure 4, B), both of which were of SCNEC phenotype. p53 positivity was present in 6 cases (Figure 4, C). IDH1/2 displayed immunopositivity in 9 of 11 cases (Figure 4, E); however, real-time PCR analysis did not reveal any IDH1/2 mutations. Two cases showed patchy nuclear immunopositivity for $\beta$-catenin (Figure 4, F). Histopathologic, immunohistochemical, and molecular features of the cases are detailed in Table 2.

Treatment and follow-up details are included in Table 1. Most patients presented with advanced disease, with intracranial extension, that is, clinical stage T4 in 5 of 8 patients (62.5%). Of the 7 patients with available follow-up, 4 died of disease (median duration to death: 11.5 months), 2 are alive with disease, and 1 is alive without disease. Notably, the sole patient who is alive without disease at 35 months (case 1) had received neoadjuvant chemotherapy followed by surgery and combined chemoradiotherapy.

**DISCUSSION**

Molecular analysis of poorly/undifferentiated sinonasal tumors has resulted in the identification of a growing number of genetically defined neoplasms. While most were previously diagnosed as SNUCs, sinonasal NECs, and poorly differentiated carcinomas, SMARCA4-deficient sinonasal carcinomas have also been found to harbor a subset of genetically distinct tumors known as SMARCA4-deficient sinonasal carcinomas. As more data emerge on newly described tumor entities, their histologic spectrum is expanding, providing clues to diagnosis. We therefore assessed a large cohort of sinonasal poorly/undifferentiated carcinomas for SMARCA4 and SMARCA2 expression to gain further recognition of the clinical, morphologic, and immunohistochemical features of this novel entity.

The first case of SMARCA4-deficient sinonasal carcinoma reported by Agaimy and Weichert in 2017 was composed of sheets of small to medium-sized “blue-appearing” cells with hyperchromatic nuclei and focal plasmacytoid features imparted by a thin eccentric rim of eosinophilic cytoplasm. The tumor was diffusely positive for cytokeratin and focally for neuroendocrine markers, and had been diagnosed as a poorly differentiated neuroendocrine carcinoma on an initial biopsy. The next case was encountered shortly thereafter by Jo et al while evaluating a cohort of SNUCs by large-panel targeted next-generation sequencing. Although they did not describe the morphologic features, a figure shows that this tumor bearing a SMARCA4 splice site variant had sheets of cells with scant cytoplasm and round to ovoid nuclei with stippled chromatin and prominent nucleoli, that is, LCNEC-like and brisk mitoses, and showed loss of SMARCA4 immunoexpression; the clinical presentation and course were not included. Agaimy et al subsequently described the detailed features of 10 cases of SMARCA4-deficient sinonasal carcinoma (3 included in the present study, cases 3 to 5), 5 of which had been diagnosed initially as NEC, 1 as NEC versus olfactory neuroblastoma, 1 as SNUC, and the rest as poorly differentiated carcinoma/malignancy. Most tumors showed interconnected nests and sheets of large cells with vesicular nuclei and prominent nucleoli, with extensive necrosis, that is, LCNEC-like appearance. Tumor cells stained diffusely with cytokeratin, and focally and often weakly with neuroendocrine markers. Rhabdoid cells were seen in 3 cases. One of 8 cases also showed loss of SMARCA2 expression. Notably, 4 of 6 patients with follow-up died of disease, and the remaining...
Figure 3. Large cell neuroendocrine carcinoma showing tumor cells with scant to moderate amount of cytoplasm, large round to ovoid nuclei with stippled chromatin, some with prominent nucleoli, brisk mitoses, and nuclear molding (A). Tumor cells show diffuse cytokeratin staining (B), and patchy chromogranin (C) and synaptophysin (D) positivity. Focal INSM1 (E) and retained SMARCA2 (F) (hematoxylin-eosin, original magnification ×200 [A]; original magnifications ×200 [B, C, D, and F] and ×100 [E]).
were receiving palliative therapy, indicating the poor outcome of SMARCA4-deficient sinonasal carcinoma.\textsuperscript{16} Sinonasal NECs are rare neoplasms characterized by morphologic evidence of neuroendocrine differentiation, such as nested, organoid, or trabecular growth, rosette formation, and stippled chromatin along with diffuse immunopositivity for epithelial marker (ie, cytokeratin) and for neuroendocrine markers (ie, chromogranin, synaptophysin, and CD56).\textsuperscript{24} INSM1 is a nuclear transcription factor that has been recently established as a marker of neuroendocrine differentiation in head and neck cancers, apart from other sites, with higher sensitivity and specificity than traditional neuroendocrine markers, and appears to have high utility in diagnosis of sinonasal NEC.\textsuperscript{25} Like other head and neck NECs, sinonasal NECs are categorized as well, moderately, and poorly differentiated, with the latter being composed of SCNEC and LCNEC, which morphologically resemble their pulmonary counterparts.\textsuperscript{24} The distinction between SCNEC and LCNEC is based on cell size; nuclear to cytoplasmic ratio, which is high in SCNEC and lower in LCNEC; and nuclear features, that is, hyperchromatic nuclei with stippled chromatin and nuclear molding in SCNEC, and large hyperchromatic to vesicular nuclei with prominent nucleoli in LCNEC.\textsuperscript{24,26} Sinonasal poorly differentiated NECs are locally aggressive tumors that present at advanced stage, often with intraorbital and intracranial extension as well as distant metastases.\textsuperscript{27,28} They have a dismal prognosis with reported 5-year overall and disease-free survival rates of 42.6% and 27.8%, respectively.\textsuperscript{28} There is sparse prior data on molecular genetics of NECs; thus, it is uncertain whether these are all truly NECs or represent SMARCA4-deficient sinonasal carcinomas. Although the latter show morphologic neuroendocrine phenotype, they can be differentiated from bona fide NECs by presence of only focal immunoreactivity for neuroendocrine markers. Owing to the rarity of sinonasal NEC, treatment approaches remain uncertain; however, multimodality treatment and induction chemotherapy have been found to be associated with improved outcomes.\textsuperscript{24,28}

TCS is a rare sinonasal malignancy with aggressive clinical behavior, whose oncogenic mechanisms remain poorly understood. Histologically, TCS is composed of immature ectodermal, mesodermal, and endodermal (ie, teratoid) elements, accompanied by carcinoma and sarcoma. Primitive neuroectodermal tissue, fetal-type squamous epithelium, and glandular structures are most commonly encountered.\textsuperscript{29} Its morphologic diversity often leads to misdiagnosis, particularly on small biopsy specimens when only some of the components are identified. Until recently, not much was known about the molecular basis of this enigmatic tumor, with only 1 report of CTNNB1 mutation in a single case of TCS giving insights into its pathogenesis.\textsuperscript{30} However, Rooper et al\textsuperscript{22} evaluated SMARCA4 staining in 22 TCS cases, and reported loss of expression in 82% of cases. Further, they identified biallelic inactivating SMARCA4 mutations by next-generation sequencing in 3 representative cases, providing much needed data on the genetics of this tumor. Interestingly, none of the SNUCs, SCNECs, and LCNECs included in their study showed loss of SMARCA4 expression. Dogan et al\textsuperscript{31} in their study on methylation profiling of sinonasal carcinomas, documented somatic mutations in SMARCA4 in 2 cases, one SCNEC–squamous cell carcinoma and one poorly differentiated carcinoma with neuroendocrine and glandular differentiation. However, the clinicopathologic features of these cases were not detailed.

Figure 4. Small cell neuroendocrine carcinoma with dysplasia in overlying squamous epithelium (A) showing loss of RB1 protein (B) and p53 positivity (C). Teratocarcinosarcoma showing neuroectodermal component resembling large cell neuroendocrine carcinoma, malignant glands, and stroma (D), with positive IDH (E) and β-catenin (F) staining (hematoxylin-eosin, original magnifications ×100 [A] and ×400 [D]; original magnifications ×200 [B, C, and F] and ×100 [E]).
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Salient Histologic Features</th>
<th>SMARCA4</th>
<th>SMARCA2</th>
<th>CK</th>
<th>CK5/6</th>
<th>CK7</th>
<th>CG</th>
<th>SYN</th>
<th>INSM1</th>
<th>CD56</th>
<th>p16</th>
<th>p63</th>
<th>p40</th>
<th>NUT</th>
<th>INI1</th>
<th>β-Catenin</th>
<th>p53</th>
<th>Rb</th>
<th>IDH</th>
<th>IDH Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LCNEC-like</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>LCNEC-like, hyperchromatic nuclei, prominent nuclei, crushing</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>LCNEC-like, crushing</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>LCNEC-like, crushing</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>+</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>LCNEC like, whorls, palisades</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>+</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>SCNEC-like, organoid architecture, rosettes, crushing, abrupt anisonucleosis, brisk mitoses</td>
<td>Loss*</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Retained</td>
<td>+</td>
<td>Loss</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>SCNEC-like, squamous dysplasia</td>
<td>Loss*</td>
<td>Retained</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>Loss</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>TCS: primitive neuroectodermal component with nests, palisades; oval/carrot-shaped nuclei, stippled chromatin, prominent nuclei; glands; rhabdomyosarcoma</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>TCS: primitive blastemal appearance with spindling, rosettes, palisades, hyperchromatic nuclei, prominent nuclei; glands; rhabdomyosarcoma</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>F</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>in squamous areas</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>TCS: primitive neuroectodermal component with nests, palisades, rosettes; oval/carrot-shaped nuclei, stippled chromatin, prominent nuclei; primitive glands; undifferentiated sarcoma</td>
<td>Loss</td>
<td>Retained</td>
<td>Focal</td>
<td>ND</td>
<td>–</td>
<td>F</td>
<td>–</td>
<td>F</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>ND*</td>
</tr>
<tr>
<td>11</td>
<td>TCS: primitive neuroectodermal component with cells in clusters, nests, short fascicles; occasional gland; undifferentiated sarcoma</td>
<td>Loss</td>
<td>Retained</td>
<td>+</td>
<td>in glands only</td>
<td>ND</td>
<td>–</td>
<td>F</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
<td>Retained</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>TCS: LCNEC-like foci; ONB-like foci; glands; squamous islands; rhabdomyosarcoma</td>
<td>Loss</td>
<td>ND</td>
<td>+</td>
<td>in glands only</td>
<td>+</td>
<td>in squamous foci only</td>
<td>+</td>
<td>in glands only</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>in squamous areas</td>
<td>Retained</td>
<td>ND*</td>
<td>ND*</td>
</tr>
</tbody>
</table>

Abbreviations: CG, chromogranin; CK, cytokeratin; F, focal; IHC, immunohistochemistry; LCNEC, large cell neuroendocrine carcinoma; ND, not done; ONB, olfactory neuroblastoma; SCNEC, small cell neuroendocrine carcinoma; SYN, synaptophysin; TCS, teratocarcinosarcoma; +, positive; –, negative.

* Severe reduction with partial retained expression.

* Consultation case, tissue not available for further testing.
In the present study, we identified 12 cases of SMARCA4-deficient sinonasal carcinoma, which did not have any distinguishing demographic or clinical features. Seven of these had histomorphologic features resembling NEC, with LCNEC-like phenotype being more frequent than SCNEC, while 5 were TCS on histology. The primitive neuroectodermal component in all 5 TCS cases morphologically resembled the SMARCA4-deficient LCNECs, with nested architecture and palisading of tumor cells that had oval to carrot-shaped nuclei with stippled chromatin and prominent nucleoli. Thus, LCNEC-like morphology, irrespective of presence of other morphologic components, with focal weak staining of traditional neuroendocrine markers, and INSM1 negativity/staining in occasional cells serve as clues to diagnosis of SMARCA4-deficient sinonasal carcinoma, and aid in differentiating sinonasal NEC from TCS. In most of our cases, SMARCA4 loss was complete and global; however, 2 cases with SCNEC phenotype showed partial retained expression. This phenomenon has also been described by Rooper et al in 3 TCS cases, and in thoracic SMARCA4-deficient undifferentiated tumors as well. Interestingly, both these cases also showed loss of Rb immunoexpression, a surrogate marker for Rb1 biallelic loss, accompanied by p53 positivity, which is well documented in small cell lung carcinoma (SCLC). Thus, it is possible that these sinonasal SMARCA4-deficient SCNECs are molecularly akin to SCLC, but during their clonal evolution have also developed SMARCA4 loss. Our finding is supported by that of Gandhi et al, who recently reported SMARCA4 loss in cases of TFF–1-negative SCLC and LCNEC of the lung.

Unlike composite tumors in uterus, kidney, and gastrointestinal tract, where loss of SWI/SNF proteins is limited to the dedifferentiated component, loss of SMARCA4 expression in all components of TCS supports the hypothesis that this tumor originates from a multipotential somatic stem cell with the ability to undergo divergent differentiation. Further, the identification of SMARCA4 loss in TCS not only gives insights into its molecular mechanisms, but also provides a robust immunohistochemical marker that can aid in rapid diagnosis of this challenging and often baffling neoplasm.

Until recently, it was noted that most SWI/SNF-driven neoplasms have certain common phenotypic features such as monotonous small blue cell or anaplastic morphology, and presence of rhabdoid cells. However, it now appears that while unified by loss of SMARCA4 immunoreexpression, SMARCA4-deficient tumors at different sites show varied clinical, histomorphologic, immunohistochemical, and genetic features and should therefore be defined by their site of origin. SCCOHT occurs in females and, contrary to its name, may display large cells with abundant cytoplasm, rhabdoid inclusions, lower nuclear-cytoplasmic ratio, and prominent nucleoli, along with the classical small cell phenotype; they frequently harbor germline or somatic SMARCA4 mutations. Undifferentiated thoracic sarcomas with SMARCA4 loss are composed of large epithelioid cells with abundant cytoplasm, vesicular nuclei, and prominent nucleoli. These tumors usually demonstrate a complex genomic profile. SMARCA4-deficient non–small cell lung carcinomas may have phenotypic features of adenocarcinoma, particularly with clear cells, or large cell carcinoma, rarely show rhabdoid cells, and have somatic SMARCA4 mutations. Our data and those from previous reports indicate that SMARCA4-deficient sinonasal carcinomas are seen over a wide age range, are more common in males, and morphologically and immunohistochemically most closely resemble SCCOHT among the gamut of SMARCA4-deficient neoplasms. However, mutation analysis for germline and somatic SMARCA4 mutations is necessary to establish genotypic similarity. We performed immunohistochemical and molecular testing for β-catenin (previously documented in TCS), p53 (known to occur in NEC), and IDH1/2 (seen in sinonasal undifferentiated carcinomas) and found that IDH1/2 mutations were absent, and β-catenin and p53 positivity was seen across the morphologic spectrum of SCNEC, LCNEC, and TCS, which further supports the theory of common molecular pathogenesis despite morphologic heterogeneity among SMARCA4-deficient sinonasal carcinomas. Although IDH1/2 mutation–specific antibody is documented in literature to be reasonably specific for IDH mutations, we did not find IDH mutations in any of the IDH immunopositive cases, suggesting that systematic analysis of specificity of IDH1/2 mutation–specific antibody in different types of genetically characterized tumors is warranted.

Lastly, while follow-up data are limited, sinonasal SMARCA4-deficient sinonasal carcinomas, like other SMARCA4-deficient neoplasms, appear to be associated with poor overall survival, with more than half the patients succumbing to the disease. Although we did not have follow-up for all our patients, it is evident that most patients presented with advanced-stage disease, had an aggressive clinical course, and poor survival outcome with median time to death of 11.5 months, as compared to a baseline population of sinonasal cancers at our institute that had 5-year overall survival and disease-free survival rates of 72% and 44%, respectively.

SMARCA4/BRG1 and SMARCA2/BRM are core ATPases of the SWI/SNF complex, of which multiple configurations exist. SMARCA4 and SMARCA2 act as paralogs of one another, and are present in all SWI/SNF complexes, that is, cBAF, PBAF, and ncBAF in a mutually exclusive manner. Both these proteins interact with key regulatory proteins such as RB, p53, and β-catenin, and thus modulate cellular processes. In thoracic SMARCA4-deficient undifferentiated tumors, loss of SMARCA4 expression is frequently accompanied by deficient SMARCA2 expression, which has been attributed to epigenetic silencing of SMARCA2, and correlates with reduced survival. This suggests that therapeutic approaches for thoracic SMARCA4-deficient undifferentiated tumors and SCCOHT may not necessarily apply to SMARCA4-deficient sinonasal carcinoma, and novel perspectives may be required on this front.

CONCLUSIONS

Our findings add to the literature on SMARCA4-deficient sinonasal carcinomas and reinforce their aggressive clinical
behavior, necessitating accurate diagnosis and appropriate management. These neoplasms are morphologically akin to sinonasal NECs and TCS, emphasizing the need for a designation that unifies them as a distinct entity. It is recommended that SMARCA4 immunohistochemistry be applied along with neuroendocrine markers in the initial panel for the diagnostic workup of all sinonasal tumors with a NEC phenotype, to facilitate their early recognition. In poorly/undifferentiated carcinomas, immunopositivity for cytokeratin accompanied by negativity for INSM1 and focal positivity for other neuroendocrine markers may help identify likely candidates for SMARCA4 loss. Lastly, there is a need for comprehensive germline and somatic mutational analyses of these tumors for further insights into their molecular pathogenesis and consequent novel therapeutic approaches.

References

SMARCA4-Deficient Sinonasal Carcinoma—Kakkar et al 9