SMARCA4-deficient Thoracic Sarcomas
Clinicopathologic Study of 30 Cases With an Emphasis on Their Nosology and Differential Diagnoses

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Abstract: SMARCA4-deficient thoracic sarcoma (SMARCA4-DTS) is a recently described entity with an aggressive clinical course and specific genetic alterations of the BAF chromatin remodeling complex. In the present study, we reviewed the clinicopathologic features of 30 cases of SMARCA4-DTS, discussed its main differential diagnoses and the challenging diagnostic scenarios that the average pathologist may face. In addition, we tested the specificity of the "SMARCA4-DTS immunohistochemical signature" (co-loss of SMARCA4 and SMARCA2 with overexpression of SOX2) in a large cohort of intrathoracic malignancies. Patients ranged from 28 to 90 years of age (median: 48 y), with a marked male predominance (male:female = 9:1) and they were usually smokers. Tumors were generally large compressive masses located in the mediastinum (n = 13), pleura (n = 5), lung (n = 2) or in 2 or more of these topographies (n = 10). Treatment strategies were varied, including 1 case treated with EZH2 inhibitors. Median overall survival was 6 months. Histologically, tumors were poorly differentiated frequently showing rhabdoid features. A subset of cases showed a focal myxoid stroma (7%, n = 2/30) and rare cases displayed a previously unreported pattern simulating desmoplastic small round cell tumors (7%, n = 2/30). Making a diagnosis was challenging when dealing with biopsy material from massively necrotic tumors and in setting the expression of SOX2, CD34, and SALL4 proved useful. All tested cases displayed concomitant loss of SMARCA4 and SMARCA2 and most tumors expressed epithelial markers (Pan-keratin or EMA) (n = 29/30), SOX2 (n = 26/27), and CD34 (n = 17/27). SMARCB1 expression was retained in all cases (23/23). SALL4 and Claudin-4 were expressed in a subset of cases (n = 7/21 and 2/19, respectively). TTF-1 and P63 were focally expressed in 1 case each. P40 and NUT were not expressed (0/23 and 0/20, respectively) The SMARCA4-DTS immunohistochemical signature was both sensitive and specific, with only a subset of small cell carcinoma of the ovary hypercalcemic type showing overlapping phenotypes. Our study confirms and expands the specific features of SMARCA4-DTS, emphasizing the fact that they can be straightforwardly identified by pathologists.

Key Words: BAF complex, SMARCA4, SMARCA2, SOX2, SMARCA4-deficient thoracic sarcomas, immunohistochemical signature, BRG1, BRM, rhabdoid, small cell carcinoma of the ovary hypercalcemic type


SMARCA4-deficient thoracic sarcoma (SMARCA4-DTS) is a new tumor type associated with particular clinicopathologic and molecular features. Affected patients are predominantly males with a previous history of smoking presenting with rapidly progressive intrathoracic tumors often complicated by surrounding tissues. Because of a particularly aggressive clinical course and the absence of effective therapies, in the vast majority of cases the disease
leads rapidly to patients’ death (median overall survival of 7 months1).

Prototypical cases of SMARCA4-DTS display features of poorly differentiated neoplasms with epithelioid/rhabdoid cells organized in a solid pattern. From a molecular viewpoint, these tumors show alterations in the switch/sucrose nonfermenting complex, also known in humans as BRG1-associated factors (BAF). This key complex is responsible for chromatin remodeling through changes in the nucleosome conformation, hence, regulates DNA transcription, replication, and repair.2-4 Recent studies have emphasized the importance of the tumor suppressive properties of the BAF complex as alterations of its subunits are being increasingly detected in human malignancies.5

Notably, SMARCA4-DTS is defined by recurrent inactivating mutations of SMARCA4 which encodes one of the ATPase subunits of the BAF complex and displays a “rhabdoid-like” transcriptomic signature, clustering with SMARCB1 and SMARCA4 mutated malignant rhabdoid tumors and small cell carcinoma of the ovary hypercalcemic type (SCCOHT). Moreover, their transcriptomic profiles are enriched in stem cell transcripts such as SRY-box 2 (SOX2) and neurodevelopmental genes1 and exhibit a dramatically decreased expression of the SMARCA2 subunit. Altogether, these molecular findings have led us to suggest an immunohistochemical signature as a major criterium for the diagnosis of SMARCA4-DTS, characterized by the co-loss of expression of SMARCA4 and SMARCA2 and over-expression of SOX2.

Since the original description of SMARCA4-DTS, 2 series of 12 cases each and an isolated case report have been published6-8 confirming our initial findings and describing frequent overexpression of SALL4.6 In the current study we have prospectively analyzed the cases of SMARCA4-DTS referred to our institution and the French national sarcoma network. Their clinicopathologic features were reviewed, detailed immunohistochemical characterization was performed and the main differential diagnoses and diagnostically challenging scenarios were discussed. In addition, we assessed the specificity of the SMARCA4-DTS immunohistochemical signature on a large group of intrathoracic malignancies.

MATERIALS AND METHODS

Main Cohort Case Selection

The files of our institutions were combed through for confirmed cases of SMARCA4-DTS between 2016 and 2018. In addition, a group of 16 cases from the original series1 were included in the study cohort. The cases were originally diagnosed based on the criteria used routinely in our institutions (initially proposed by one of the authors, F.L.L.), which include: rhabdoid and/or poorly differentiated phenotype (no specific line of differentiation); complete loss of expression of SMARCA4 and SMARCA2; focal or diffuse expression of at least 2 of 3 of the following markers: SOX2, CD34 or SALL4. In the isolated cases where material was unavailable for assessment of SMARCA2 expression (n = 6), the remaining criteria were considered sufficient for the inclusion in the final cohort if the rest of the clinicopathologic features were typical of SMARCA4-DTS. Of note, in 2 of the 6 previously published cases1 RNA sequencing analysis showed a reduction of SMARCA2 expression.

The neoplasms were reviewed and extensively characterized morphologically and immunohistochemically by 2 soft tissue pathologists (R.P. and F.L.L.). Data regarding patients’ clinical information, demographics and medical imaging were retrieved from the medical records of our institutions or from the referring centers.

Immunostaining

Immunohistochemistry was carried out on 4µm paraffin sections as per standard technique on a Ventana Benchmark ULTRA automat (Ventana, Roche Diagnostics) using antibodies against the following proteins: SMARCA4 (BRG1) (clone EPNCIR111A, 1:200 dilution; Abcam), SMARCA2 (BRM) (clone D9E8B, 1:100 dilution; Cell Signaling Technology), SOX2 (clone D6D9, 1:200 dilution; Cell Signaling Technology), SMARCB1 (clone 25 [ini-1] mouse monoclonal, 1:30 dilution; BD Biosciences), Claudin-4 (clone EPRR17575, 1:500 dilution; Abcam/Epitomics), CD34 (clone QBEnd10, ready to use; Roche Diagnostics [790-2927]), SALL4 (clone 6E3, ready to use; Roche Diagnostics [760-4864]), TTF-1 (clone 8G7G3/1, ready to use; Roche Diagnostics [790-4398]), P63 (clone 4A4, ready to use; Roche Diagnostics [790-4509]), P40 (clone BC28, ready to use; ZYTOMED [BMS050]), pan-keratin (clone AE1/AE3/PCK26, ready to use; Roche Diagnostics [760-2135]), NUT (clone C52B1, 1:100 dilution; Cell Signaling).

The following criteria were used for assessing the positivity of tumor cells: negative (0% to 9% stained cells); +/− (10% to 75% of cells); + (> 75% of cells). In addition, for the antibodies targeted against the subunits of the BAF complex, complete absence of expression was also stated when appropriate. The staining was determined in a semiquantitative manner by 2 pathologists (R.P. and F.L.L.). Immunostainings performed initially by the referring pathologists were also assessed when available. Internal and/or external controls were used in all tested cases as appropriate.

Immunohistochemical Screening Group

A total of 431 cases were included in the screening group for which expression of SMARCA4 was assessed. In addition, due to limited material, SMARCA2 and SOX2 staining were determined in a portion of these cases (220 and 280 cases, respectively). Selected neoplasms consisted of various carcinomas and sarcomas arising primarily in the lung, pleura, and mediastinum which do not typically present BAF complex alterations and a miscellaneous group of BAF-altered sarcomas/carcinomas, which can develop either primarily or secondarily in the thoracic cavity.
Statistical Analysis

Statistical analysis was performed using Graphpad Prism 6.01 (GraphPad Software, La Jolla, CA). The sensitivity and specificity of the SMARCA4-DTS immunohistochemical signature were analyzed using the Fisher exact test. A value of $P<0.001$ was considered statistically significant.

RESULTS

Clinical Findings

A total of 30 cases met the inclusion criteria, of whom 27 were males and 3 females (male:female ratio of 9:1). Affected patients were predominantly adults, with a median age of 48 years and a wide age range, from 28 to 90 years (the main clinical findings are described in Table 1). Most of the patients were either current or ex-smokers (79% and 8%, respectively) with a median pack-year of 18.5 (range: 5 to 60).

The lesions appeared as large infiltrative masses usually complicated with compression of adjacent structures (respiratory tract and/or blood vessels of the mediastinum) (Fig. 1), and were frequently suspected clinically and radiographically to represent lymphomas, NUT midline carcinomas or germinal tumors. At the time of diagnosis, the neoplasms were mainly located in the mediastinum followed by pleura and lung. However, many cases were multifocal and due to size and locoregional extension affected more than one of the previously mentioned topographies. Metastatic disease was very frequent (77% of cases) with commonly affected sites being, in decreasing order of frequency: lymph nodes, bones (mainly from the axis), adrenal glands, liver, digestive tract, central nervous system, and kidneys.

Treatment strategies were diverse and comprised: (1) chemotherapy, with the most frequent regimens being: CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone/prednisone), MAID (mesna, doxorubicin, ifosfamide and dacarbazine), and VIDE (vincristine, ifosfamide, doxorubicin and etoposide); (2) surgical intervention either alone or in combination with chemotherapy; (3) adjuvant radiotherapy in a minority of cases. One case (patient 26) was included in a clinical trial and received an inhibitor of the Enhancer of zeste homolog 2 (EZH2) as a second line treatment but the response could not be assessed due to complications and death soon after the onset of treatment (iatrogenic). Overall, whatever the chosen treatment scheme, therapeutic response was very poor with progressive disease developing in all patients and a median overall survival of 6 months (follow-up data available for 27 cases). Virtually all patients died of local complications.

Pathologic Findings

Histologically, the neoplasms presented a solid architecture consisting of variably cohesive epithelioid cells arranged in irregular sheets or nests, typically infiltrating the surrounding tissues (Figs. 2A, B). Necrosis was ubiquitously present, frequently had a geographic distribution and in some cases was so extensive that viable cells were difficult to find.

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<th>Sex/Age (y)</th>
<th>Topography (At Presentation)</th>
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The cellular elements consisted of ovoid or polygonal cells with moderate to abundant amounts of eosinophilic to clear cytoplasm (Figs. 2D, E), sometimes presenting intracytoplasmic inclusions (rhabdoid morphology) (Fig. 2F). Nuclei were round shaped, mildly pleomorphic, usually large and irregular with vesicular chromatin and prominent nucleoli. Mitotic activity was brisk in all studied cases. Although tumors showed extensive necrotic and apoptotic debris, inflammatory host response was usually mild. In a subset of cases (7%, n = 2/30), tumor cells were embedded in a prominent desmoplastic stroma showing variable amounts of spindled fibroblasts which conferred an aspect reminiscent to desmoplastic small round cell tumor (Figs. 3A, B). In addition, focal stromal myxoid/edematous changes were noted in rare cases (7%, n = 2/30), which when present, induced cells to organize individually, in cords or in reticular patterns (Figs. 3C, D). When mentioned, the following diagnoses were proposed in the referred cases: SCCOHT (n = 1); undifferentiated malignant neoplasm (n = 1),...
FIGURE 2. Main histologic features of SMARCA4-DTS. A, Typical solid architecture with large nests and sheets of neoplastic cells. B, Most tumors show massive infiltration of surrounding tissues, in this case adipocytes are individually dissected by tumor cells. C, Large areas of tumor necrosis are typical, note the perivascular cuffs of viable cells. D, Small-sized to medium-sized discohesive tumor cells with marked nuclear atypia, prominent nucleoli and mild pleomorphism. Abundant apoptotic debris is also a frequent finding (upper right corner). E, In a subgroup of cases, neoplastic cells are cohesive and have an epithelioid morphology, note the abundant pale cytoplasm in this example. F, Rhabdoid cells showing abundant eosinophilic cytoplasm and large vesicular nuclei with prominent nucleoli.
FIGURE 3. Rare morphologic patterns and challenging scenarios of SMARCA4-deficient sarcomas. A and B, Desmoplastic round cell tumor-like pattern showing sheets and nests of tumors cells surrounded by a prominent desmoplastic stroma with a variably prominent fusocellular (fibroblastic) component. C and D, Myxoid stromal change was focal in rare tumors. In these areas, tumors cells were arranged in a cord-like manner (C) or floating individually in mucin pools (D). E and F, Biopsy showing a massively necrotic biopsy of a SMARCA4-DTS. In these settings, the presence of rhabdoid cells in viable areas (F) as well the expression of positive markers like CD34, SOX2, and/or SALL4 are useful (inset shows SALL4 positive cells from the same case).
epithelioid sarcoma (n = 1), granulocytic sarcoma (n = 1) undifferentiated carcinoma (n = 2), and SMARCA4-DTS (n = 3).

The immunohistochemical profile of the tested cases is summarized in Table 2. Most of the tumors (97%, n = 29/30) expressed at least one epithelial marker (either pan-cytokeratin AE1-AE3 or EMA), usually focally (Fig. 4A). CD34 positivity was seen in a majority of cases (63%, n = 17/27) and it was frequently expressed in the majority of neoplastic cells (Fig. 4B). In all tested cases, the expression of SMARCA4 and SMARCA2 was absent (Figs. 4C, D). In addition, SOX2 was overexpressed in most cases (96%, n = 26/27), commonly in a marked and diffuse manner (Fig. 4E). SALL4 expression was present in a subset of cases (33%, n = 7/21) and it was usually focal. SMARCB1 expression was ubiquitously preserved. TTF-1 and p63 were expressed in 1 case each. All tested cases were negative for p40 and NUT. Finally, claudin-4 was diffusely lost in most cases with focal expression seen in 2 cases (Fig. 4F).

In a majority of the included cases wide immunohistochemical panels were initially performed and were available for reassessment. At least focal expression was seen for: pan-cytokeratin KL1 (35%, n = 6/17), CK7 (5%, n = 1/20), calretinin (7%, n = 1/14), CD99 (40%, n = 6/15), and synaptophysin (18%, n = 3/17). In addition, tumors lacked expression of: CD5 (n = 0/16), CK5/6 (n = 0/17), CD45 (n = 0/18), chromogranin A (n = 0/17), PLAP (n = 0/10), CD117 (n = 0/16), WT1 (n = 0/6), Desmin (n = 0/23), Myogenin (n = 0/23), and CD56 (n = 0/16).

**Immunohistochemical Screening Group**

The results are summarized in Supplementary Material 1 (Supplemental Digital Content 1, http://links.lww.com/PAS/A710). Of all tested cases, only a subset of SCCOHT shared the SMARCA4-DTS signature. As shown, complete lack of expression of SMARCA4 was rarely seen in all tumor categories and when present it was usually in a subset of tested cases, including in the lung subgroup: large cell carcinoma (21%, n = 11/53) adenocarcinoma (12%, n = 6/50) and sarcomatoid carcinoma (33%, n = 5/15); in the mediastinum and pleura group: adenocarcinoma NOS (25%, n = 1/4) and epithelioid mesothelioma (13%, n = 4/31) and in the soft tissue sarcoma group: SMARCA4-lost malignant rhabdoid tumor (100%, n = 2/2) and SCCOHT (3/3).

Similarly, complete loss of expression of SMARCA2 was limited, including in the lung neoplasms: large cell carcinoma (7%, n = 3/46) and adenocarcinoma (3%, n = 1/30) and among soft tissue sarcomas: SMARCB1-lost MRT (33%, n = 3/9), and SCCOHT (100%, n = 3/3). Of note, among all the cases that showed complete lack of expression of SMARCA2, only the cases of SCCOHT had concomitant loss of SMARCA4.

Finally, diffuse overexpression of SOX2 was restrained to a subset of lung squamous cell carcinoma cases (37.5%, n = 18/48) and one case of extrathoracic undifferentiated pleomorphic sarcoma with rhabdoid phenotype.

Statistical analysis showed that the specificity and sensitivity of the SMARCA4-DTS immunohistochemical signature were 99.5% (95% confidence interval, 97.4%-99.9%) and 87.5% (95% confidence interval, 67.6%-97.3%), respectively.

**DISCUSSION**

The present study is the largest series to date of SMARCA4-DTS, further expanding their clinical and histologic spectrum. In addition, it confirms the defining clinical and pathologic features displayed by SMARCA4-DTS (Table 3). As mentioned, these tumors seem to occur mainly in patients with a history of smoking and tend to affect the male population predominantly. In our first study, patients were frequently young adults with a median age of 41 years old, the same onset as in the Japanese series. However, the present results suggest that SMARCA4-DTS may develop in a wider age range as the median age of presentation (48 y old) is located between the one reported in the Japanese (39 y old)6 and American cohorts (59 y old).7 As reflected in previous studies, SMARCA4-DTS are highly aggressive neoplasms which are frequently disseminated at presentation and tend to progress rapidly despite multimodal treatment strategies. The current median overall survival of 6 months was slightly worse than the 7 months reported in our previous series.

The morphologic features of SMARCA4-DTS are not entirely specific but distinctive enough to infer the diagnosis in the correct clinical setting. As highlighted, typical cases are characterized by the presence of round to epithelioid cells arranged in a solid pattern showing variably prominent rhabdoid differentiation. Cytologic atypia is frequently prominent and nuclear pleomorphism tends to be mild, partly reflecting the complex genomic profiles seen in this group of tumors. Moreover, immunohistochemistry is useful and indispensable when dealing with small specimens as it typically shows dual loss of SMARCA4 and SMARCA2 and frequent diffuse expression of SOX2. In addition, as in MRTs, at least focal expression of epithelial markers (either CK AE1-AE3 or EMA), CD34, and/or SALL4 are seen in most cases. In our experience, such “positive” markers are particularly useful in the setting of small challenging biopsies consisting of extensively necrotic tissue (Figs. 3E, F). Finally, other markers like P40 and NUT are always negative which is also a requirement for confirming a diagnosis of SMARCA4-DTS.

An interesting finding was the presence of a new architectural pattern simulating “desmoplastic round cell tumor”; in such cases the presence of high-grade nuclear

**TABLE 2. Immunohistochemical Features of SMARCA4 Deficient Sarcomas**

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<th></th>
<th>CK AE1-AE3</th>
<th>EMA</th>
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<th>SMARCA2</th>
<th>SMARCA4</th>
<th>SOX2</th>
<th>SALL4</th>
<th>Claudin-4</th>
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atypia and the immunophenotype (absence of expression of desmin and WT1) are generally enough for a correct classification. In addition and in line with previous studies, we found a subset of cases showing stromal myxoid changes. Although in our cohort this feature was displayed in focal areas of resection specimens, keeping in

FIGURE 4. Immunohistochemical profile of SMARCA4-deficient sarcomas. A, Focal epithelial membrane antigen (EMA) staining (at least focal expression of epithelial markers was seen in the vast majority of cases). B, Diffuse expression of CD34 (present in 50% of cases). C and D, SMARCA4 (C) and SMARCA2 (D) are diffusely lost in tumor cells. Note the endothelial and inflammatory cells with retained staining. E, Diffuse expression of SOX2 in tumor cells. F, Claudin-4 loss of expression.
mind this aspect may be of prime importance in small biopsy material as primary pulmonary myoid sarcoma or myoepithelioma may share similar histologic aspects.

In some particular circumstances, reaching an accurate diagnosis of SMARCA4-DTS may be challenging as there are various entities to consider that share histologic features. In this matter, although our screening cohort indicates that the SMARCA4-DTS immunohistochemical signature is both sensitive and specific, some points should be discussed. As previously mentioned, neoplasms showing rhabdoid differentiation like MRTs and SCCOHT frequently harbor BAF complex alterations.9–11 In such cases, adequate clinicopathologic correlation is critical as MRTs usually affect a younger age group (infants and young children) and typical cases present biallelic inactivation of SMARCB1.9,14 In addition, as highlighted in the screening cohort, all MRTs tested showed lack of expression of SOX2. Lastly, it is important to note that some rare MRTs may present with diffuse loss of SMARCA2 or SMARCA4 but unlike SMARCA4-DTS dual loss of these proteins and p53 overexpression have never been reported. In a similar way to MRTs, the clinical context is sufficient for ruling out SCCOHT in most circumstances. These tumors are restricted to the female population and typically present as primary intra-abdominal masses sometimes associated with hypercalcemia. However, simultaneous intra-abdominal and intrathoracic presentation of a SMARCA4-deficient neoplasm in a female may render the differential diagnosis impossible. Particularly, if molecular techniques for evidencing germline mutations of SMARCA4 and the simple genomic profiles of SCCOHT10,16 are unavailable. In this setting, assessing SOX2 expression may be somewhat useful as it was not diffusely expressed in the tested cases of SCCOHT. Nevertheless, the importance of this marker for discriminating between these 2 tumors needs to be further studied as our cohort included only 3 cases of this rare ovarian tumor.

The BAF complex is in charge of the modification of chromatin architecture, a highly regulated process among species which permits the access or restriction of the transcription machinery to specific zones of the genome. Through the power of ATP hydrolysis, it facilitates DNA accessibility by “loosening” the chromatin structure; on the other hand, the Polycomb group complexes (PcG) increases chromatin compaction and hence, induces transcriptional repression.15 Understanding the highly regulated nature of this process is critical in order to comprehend the pathophysiology of BAF deficient neoplasms and the therapeutic strategies being presently developed. Indeed, BAF related aberrations are seen in as much as 19% of human cancers16,17 which can be grouped in 6 main categories: (1) “differentiated” carcinomas of various origins, in which these anomalies may confer a worse prognosis18–21; (2) undifferentiated/dedifferentiated carcinomas22–24; (3) hematological malignancies25–26; (4) gliomas27; (5) melanomas28; and (6) an evolving group of sarcomas, including: MRTs, epithelioid sarcomas, SMARCA4-DTS and the recently described SMARCA4-deficient undifferentiated uterine sarcoma.29 Because of the frequency of these anomalies, the usefulness of targeted therapy aiming at key components of the chromatin regulation machinery is actively being studied.17 Indeed, preclinical models have shown promising results in MRT28 and SCCOHT29 and presently, a phase 1 clinical trial using an inhibitor of a subunit of the PRC2, the Enhancer of zeste homolog 2 (EZH2), has shown encouraging results in MRTs.30 These inhibitors impede the accumulation of the H3K27me3 chromatin repressive epigenetic signature (caused by the PRC), ultimately leading to tumor cell apoptosis and hampering tumor cell proliferation.31 Furthermore, biological data support the potential benefits of specific therapies combining topoisomerase II inhibitors with EZH2 inhibitors to treat SMARCA4-deficient malignancies.32 Altogether, these prognostic and potentially therapeutic implications denote the importance of correctly identifying the new members of the “BAF altered family of tumors.”

An important question that has been raised is whether SMARCA4-DTS may represent an undifferentiated form of lung carcinoma.6,7 Some of the main arguments used supporting this statement are the following: (1) SMARCA4-DTS develop frequently in smokers and can primarily affect the lung; (2) molecular similarities with a subset of lung adenocarcinomas. (3) As mentioned above, BAF complex alterations are seen in a subset of undifferentiated/dedifferentiated carcinomas, particularly of endometrial origin that may show dual loss of SMARCA4/SMARCA2, focal keratin/CD34 positivity, and similar sheet-like pattern of monotonous cells. On the contrary, present and past results arguing that SMARCA4-DTS constitutes a separate entity include:...
(1) primary location in the lung is rare and many cases arise in young adults; (2) preneoplastic lesions or tumors showing heterogenous features with specific lines of differentiation have not been reported; (3) genomic expression profile is similar to rhabdoid tumors at least in a subset of cases. An additional argument is that SMARCA4-DTS do not consistently express claudin-4, a protein which may discriminate rhabdoid tumors from rhabdoid carcinomas.33 Nevertheless, the usefulness of this marker remains to be defined as a recent cohort of dedifferentiated and undifferentiated endometrial carcinomas with BAF aberrations consistently lacked its expression as well.34 Overall, we believe that establishing whether SMARCA4-DTS are indeed dedifferentiated neoplasms difficult to answer. As per definition, to consider a diagnosis of SMARCA4-DTS no histological evidence of specific lines of differentiation should be seen. In our routine practice, we reserve the diagnosis of SMARCA4-deficient carcinoma to cases expressing epithelial markers but not fulfilling the 3 main criteria proposed in the methods section. Although these aspects are based on our personal experience and the present series lacked autopsies specimens, so far we have not come across a single case morphologically supporting the process of dedifferentiation as the origin of these neoplasms. In addition, it is worth noting that SMARCA4-DTS immunophenotype is reminiscent of the vicinity of their transcriptomes with those of bona fide malignant rhabdoid tumors. The loss of SMARCA2 together with SMARCA4 represent a particular biological feature of the cluster of BAF deficient rhabdoid tumors to which SMARCA4-DTS may belong.1 This loss of expression, unexplained at the genomic level may be related to a common cell of origin shared by MRT, SCCOHT, and SMARCA4-DTS. In spite of the uncertainty, due to the potential therapeutic implications, the correct identification of these neoplasms remains of utmost importance.

In conclusion, we have herein described the clinicopathologic aspects of the largest group to date of SMARCA4-DTS. Our findings and the clinicopathologic series of independent groups confirm the reproducibility of the diagnostic criteria and the usefulness of the immunohistochemical signature for identifying this new group of neoplasms. Even though the immunophenotype of SMARCA4-DTS is quite specific, appropriate clinicopathologic correlation should be performed in all cases in order to avoid misclassification, particularly with other rhabdoid tumors. Furthermore, correct identification of these group of neoplasms is essential as present evidence suggests important prognostic and potentially therapeutic implications. It will be interesting to see in future studies whether SMARCA4-deficient carcinomas and sarcomas show different sensitivity profiles to these novel treatment strategies.

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REFERENCES
17. Perret et al Am J Surg Pathol • Volume 43, Number 4, April 2019


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