SMARCA4-deficient thoracic sarcoma: a distinctive clinicopathological entity with undifferentiated rhabdoid morphology and aggressive behavior

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A distinct subset of thoracic sarcomas with undifferentiated rhabdoid morphology and *SMARCA4* inactivation has recently been described, and potential targeted therapy for *SMARC*-deficient tumors is emerging. We sought to validate the clinicopathological features of *SMARCA4*-deficient thoracic sarcomas. Clinicopathological information was gathered for 40 undifferentiated thoracic tumors with rhabdoid morphology (mediastinum ($n=18$), lung ($n=14$), pleura ($n=8$)). Thymic carcinomas ($n=11$) were used as a comparison group. Immunohistochemistry included BRG1 (*SMARCA4*), BRM (*SMARCA2*), INI-1 (*SMARCB1*), pan-cytokeratin, desmin, NUT, S-100 protein, TTF1, CD34, and SOX2. BRG1 loss was present in 12 of 40 rhabdoid thoracic tumors (30%): 7 of 18 in mediastinum (39%), 2 of 8 in pleura (25%), and 3 of 14 in lung (21%). All BRG1-deficient tumors tested for BRM ($n=8$) showed concomitant loss. All thymic carcinomas showed retained BRG1 and INI-1. Morphologically, tumors with BRG1 loss showed sheets of monotonous ovoid cells with indistinct cell borders, abundant eosinophilic cytoplasm, and prominent nucleoli. Scattered areas with rhabdoid morphology (ie, eccentric nuclei, dense eosinophilic cytoplasm, discohesion) were present in all the cases. *SMARCA4*/BRG1-deficient sarcomas showed rare cells positive for cytokeratin in 10 cases (83%). One showed rare TTF1-positive cells. All were negative for desmin, NUT, and S-100 protein. CD34 was positive in three of five (60%) BRG1-deficient tumors tested. SOX2 was positive in all four BRG1-deficient tumors tested, and negative in all seven tested cases with retained BRG1. *SMARCA4*/BRG1-deficient sarcomas occurred at median age of 59 years (range 44–76) with male predominance (9:3) and had worse 2-year survival compared with BRG1-retained tumors (12.5% *vs* 64.4%, $P=0.02$). *SMARCA4*-deficient thoracic sarcomas can be identified based on their distinctive high-grade rhabdoid morphology, and the diagnosis can be confirmed by immunohistochemistry. Identification of these tumors is clinically relevant due to their aggressive behavior, poor prognosis, and potential targeted therapy.
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The *SMARCA4* gene is located on chromosome 19p and encodes the BRG1 protein, one of the two mutually exclusive catalytic subunits of the switch/sucrose-nonfermenting (SWI/SNF) chromatin-remodeling complex. The other catalytic subunit is BRM, encoded by the *SMARCA2* gene. The SWI/SNF complex is formed by multiple proteins, of which INI-1 (encoded by *SMARCB1* gene) is the best characterized. The complex acts as a tumor suppressor by regulating transcription and promoting cell differentiation.[1–8] Inactivation of *SMARCA4* has been reported in several aggressive tumors with high-grade undifferentiated rhabdoid morphology, including small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), and a subset of atypical teratoid–rhabdoid tumors of the central nervous system.[9–15] Carcinomas from various sites have also been shown to have *SMARCA4* inactivation including those arising in the endometrium, gastrointestinal tract, and lung.[16–26] Morphologically, carcinomas with *SMARCA4* inactivation include

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both differentiated carcinomas (adenocarcinomas and squamous cell carcinomas), and undifferentiated carcinomas with variably rhabdoid morphology.

SCCOHT is the prototypic rhabdoid-appearing tumor with *SMARCA4* inactivation, and has a histologic phenotype morphologically similar to the better characterized *SMARCB1*/INI-1-deficient tumors. This expanding group has been referred to as '*SMARCB1*-deficient neoplasia',[27] and includes atypical teratoid–rhabdoid tumors,[28] rhabdoid meningiomas,[29] malignant rhabdoid tumors (MRT) of the kidney and soft tissue,[30–32] rhabdoid carcinomas of the gastrointestinal tract,[33] pancreatic undifferentiated rhabdoid carcinomas,[34] and epithelioid sarcomas.[35,36] These tumors share overlapping morphologic features including rhabdoid histology, loss of INI-1 by immunohistochemistry, focal cytokeratin/EMA immunoexpression, and occasional positive immunostaining for desmin and CEA.[27] Inactivation of alternative SWI/SNF complex members, such as *SMARCA4* and *SMARCA2*, may explain the molecular pathway underlying tumors histologically similar to *SMARCB1*/INI-1-deficient tumors, but with retained INI-1 immunoexpression.

In a recent genetic study, Le Loarer *et al*[23] identified a group of undifferentiated thoracic malignancies with *SMARCA4* inactivation presenting as compressive tumors often involving the mediastinum, with or without lung involvement, occurring in young patients and displaying aggressive behavior. They found these tumors to be genetically distinct from lung carcinomas with *SMARCA4* deficiency, and to display a closer molecular relationship to SCCOHT and MRT. In contrast to lung carcinomas with isolated *SMARCA4* inactivation, these *SMARCA4*-deficient thoracic malignancies also showed co-deficiency of *SMARCA2*. Because of these clinicopathological and molecular differences, Le Loarer and colleagues proposed the term '*SMARCA4*-deficient thoracic sarcomas' for this distinctive entity.[23] A subsequent study further confirmed the unique molecular and clinicopathological features of *SMARCA4*-deficient thoracic sarcomas in a Japanese cohort.[22]

Currently, there is a clinical trial available for patients with *SMARC*-deficient tumors with an inhibitor to the histone-lysine *N*-methyltransferase Enhancer of Zeste Homolog 2 or EZH2,[37] and alternative targeted therapies are under development for tumors deficient in *SMARCA4* expression.[38–43] Given the potential therapeutic implications, it is important to identify *SMARCA4*-deficient thoracic sarcomas, including diagnosis from small biopsies. In this study, our main objectives were to determine the frequency of *SMARCA4* inactivation in thoracic sarcomas with undifferentiated rhabdoid morphology, and to further validate the clinicopathological features of these tumors in a Western population.

Materials and methods

Case Selection

Following institutional review board approval, two thoracic pathologists (JLS and JMB) retrospectively reviewed hematoxylin and eosin-stained slides from mediastinum, pleura and lung tumors from institutional surgical and consultation archives, identified using search terms that included 'rhabdoid', 'undifferentiated', or 'poorly differentiated malignant neoplasm'. Undifferentiated rhabdoid tumors encountered in our consultation practice were also prospectively included. Possible or known metastatic tumors from extra-thoracic primary sites were excluded, as were cases with an alternative definitive diagnosis (eg, NUT carcinoma, differentiated carcinoma, rhabdomyosarcoma, melanoma, etc) established by clinical or study immunohistochemical work-up. Study cases ($n=40$) with undifferentiated rhabdoid morphology were selected. A group of surgically resected thymic carcinomas ($n=11$) confirmed by morphology and immunohistochemistry were included as a comparison group given their mediastinal location, including nine squamous cell carcinomas, one adenocarcinoma, and one undifferentiated carcinoma.

Clinical information was obtained from the electronic medical record and from referring physicians, if available. Selected clinical parameters were recorded, including demographics, clinical presentation, location and size of tumor at presentation, type of surgical procedure, treatment, and outcome.

Immunohistochemistry

Immunohistochemistry for BRG1 was performed on whole paraffin-embedded tissue sections from the 40 rhabdoid thoracic tumors and 11 thymic carcinomas. Following EDTA antigen retrieval, monoclonal BRG1 antibody (clone EPNCIR111A, Abcam, Cambridge, MA, USA) was used at 1:200 dilution (15 min incubation) with polymer refine detection (Leica DS9800). Negative and positive controls were performed with each run. Immunostaining was also performed on all undifferentiated rhabdoid tumors, using antibodies directed against INI-1 (clone 25/BAF47, BD Transduction Laboratories, San Jose, CA, USA), desmin (clone DE-R-11, Novocastra, New Castle, UK), NUT (clone C52B1, Cell Signaling Technology, Danvers, MA, USA), S-100 protein (Dako, Glostrup, Denmark), and TTF1 (clone SPT24, Novocastra). Pan-keratin clones included OSCAR keratin (Biolegend, San Diego, CA, USA) and/or keratin AE1/AE3 (Dako). Immunostains for CD34 (Novocastra), BRM, and SOX2 (clone EPR3131, Abcam) were performed on subsets of the rhabdoid tumors with tissue available for additional immunohistochemistry. Following citrate antigen retrieval, a polyclonal BRM antibody (Sigma-Aldrich, St. Louis,

Table 1 Clinicopathologic features of BRG1-deficient/retained thoracic tumors and thymic carcinomas

Features	*BRG1-deficient tumors* (n = *12*)	*BRG1-retained tumors* (n = *28*)	*Thymic carcinomas* (n = *11*)
Median age (yrs), (range)	59 (44–76)	54.5 (19–84)	51 (32–79)
Male	9 (75%)	22 (79%)	6 (55%)
Site			
Mediastinum	7 (58%)	11 (39%)	11 (100%)
Lung	3 (25%)	11 (39%)	—
Pleura	2 (17%)	6 (22%)	—
Metastatic disease	7/9 (78%)	10/22 (45%)	6/11 (55%)
Unresectable tumor	6/6 (100%)	6/16 (38%)	6/11 (55%)
Treatment, *n*	6	16	11
Surgery only	4 (67%)	3 (19%)	0
Surgery+chemo	2/6 (33%)	3 (19%)	0
Surgery+XRT		1 (6%)	3 (27.3%)
Surgery+chemo+XRT		3 (19%)	2 (18.2%)
Chemo only		2 (12%)	1 (9%)
XRT only		1 (6%)	2 (18.2%)
Chemo and XRT		3 (19%)	3 (27.3%)
Available follow-up, *n*	8	18	11
Median (range) follow-up (mos)	4.0 (0.7–108.5)	17.4 (0.6–183.6)	34.3 (2.3–162.8)
Outcome, *n*	8 DOD	9 DOD	8 DOD

Abbreviations: Chemo, chemotherapy; DOD, dead of disease; mos, months; XRT, radiation therapy; yrs, years.

MO, USA) was used at 1:400 dilution on Leica Bond III stainer.

Loss of BRG1, INI-1, and BRM immunoexpression was defined as complete unequivocal loss of expression in tumor nuclei, in the presence of positive internal control (eg, background inflammatory cells, stromal fibroblasts, endothelial cells, or benign epithelial cells). Heterogeneous patterns of retained BRG1, INI-1, or BRM immunoexpression with cell-to-cell variability in expression were scored as 'retained'.

Statistical Analysis

The median survival and 2-year overall survival were estimated with the Kaplan–Meier method. Survival was compared with likelihood ratio tests.

Results

Clinicopathological Characteristics of Study Population

Forty primary thoracic tumors with undifferentiated rhabdoid morphology were included: 18 from the mediastinum, 14 from the lung, and 8 from the pleura (Table 1). Specimens included 21 surgical excisions/biopsies and 19 core or transbronchial biopsies. Eleven surgically resected thymic carcinomas were also included as a comparison group. Altogether, the study cohort included 37 men and 14 women, with a median age of 56 years (range 19–84). Thirteen patients had a history of smoking, 1 patient was a never smoker, and the smoking status of the remaining patients was not known.

Immunohistochemistry for SWI/SNF Complex Proteins

Analysis of *SMARCA4* expression based on BRG1 immunohistochemistry was performed in all the cases. Of the 40 undifferentiated/rhabdoid thoracic tumors, loss of BRG1 immunoexpression was present in 12 (30%): 7 of 18 in the mediastinum (39%), 2 of 8 in the pleura (25%), and 3 of 14 in the lung (21%; Table 1). Eight of the tumors with BRG1 loss had sufficient tissue available to evaluate for BRM (*SMARCA2*) co-deficiency, which was present in all the cases tested (Table 2; Figures 1a and b). Isolated *SMARCA2* inactivation was also seen in one additional mediastinal tumor with undifferentiated rhabdoid morphology that showed retained BRG1 and INI-1 immunoexpression (Table 2). INI-1 staining was performed in all the cases, and loss was seen in one pleural tumor with retained BRG1 immunoexpression, while all the others demonstrated retained INI-1 (Table 2; Figure 1c). All thymic carcinomas showed retained BRG1 and INI-1. Several cases ($n = 7$; three mediastinal tumors, two pleural tumors, one lung tumor and one thymic carcinoma) showed heterogeneous patterns of nuclear positivity for BRG1, characterized by cell-to-cell variation in BRG1 expression within the tumor. Since the significance of 'mosaic' expression is unknown, the immunoexpression in these cases was interpreted as retained.

Table 2 Clinical data for patients with thoracic tumors showing loss of BRG1, BRM, or INI-1 immunoexpression

Patient	*Age (yrs)*	*Sex*	*Site (size)*	*BRG1*	*BRM*	*INI-1*	*Presenting symptoms*	*Imaging features*	*Biopsy type*	*Treatment*	*Outcome*	*Time to death (mos)*
1	69	F	Mediastinum (NA)	L	L	R	Pain from iliac bone mets	Superior anterior mediastinum mass, mediastinal LAD, pelvis and iliac bone mets	Surgical	Unresectable, chemo +XRT	DOD	4
2	66	M	Mediastinum (9.6 cm)	L	R	R	Dyspnea	Anterior mediastinal/hilar mass, echogenic area in pericardium	Needle	Unresectable, chemo; progression on 2 regimens	DOD	4
3	44	M	Mediastinum (NA)	L	R	R	NA	Mediastinal mass, mediastinal LAD, axilla and lung mets	Surgical	Unresectable, chemo	DOD	1
4	56	M	Mediastinum (10 cm)	L	L	R	Wt loss, fatigue, reflux	Mediastinal/hilar mass	Needle	Unresectable, chemo; no response on 2 regimens	DOD	4
5	50	M	Mediastinum (16 cm)	L	L	R	Dyspnea, chest pain	Left mediastinal mass encasing major vessels, severe midline shift, left jugular vein thrombosis	Surgical	Unresectable, chemo	DOD	2
6	48	M	Pleura (NA)	L	L	R	Pleural effusion	NA	Surgical	NA	DOD	108
7	57	M	Mediastinum (NA)	L	L	R	Dyspnea	Mediastinal mass	Surgical	Unresectable, chemo; no response, subsequent brain and axillary mets	DOD	4
8	61	M	Lung (1.3 cm)	L	NA	R	NA	PET avid (SUV max 3.44) RLL lung mass, mediastinal LAD, left adrenal mets	Surgical	NA	NA	
9	65	M	Pleura (NA)	L	L	R	NA	NA	Surgical	NA	NA	
10	48	F	Mediastinum (10 cm)	L	NA	R	NA	PET avid (SUV max 12) anterior mediastinal mass encasing and narrowing trachea and R main stem bronchus, supraclavicular LAD	Needle	NA	NA	
11	76	F	Lung (NA)	L	L	R	NA	Solitary RUL lung mass	Needle	NA	DOD	4
12	67	M	Lung (1.4 cm)	L	L	R	Seizure	1.4 cm RUL nodule, brain and right adrenal mets	Surgical	NA	NA	
13	36	M	Mediastinum (NA)	R	L	R	NA	NA	Surgical	NA	NA	
14	60	M	Pleura (NA)	R	NA	L	Pleural effusion, LLL lung consolidation	NA	Surgical	NA	NA	

Abbreviations: Chemo, chemotherapy; DOD, dead of disease; dx, diagnosis; F, female; L, lost; LAD, lymphadenopathy; LLL, left lower lobe; M, male; mets, metastases; mos, months; NA, not available; PET, positron emission tomography; R, retained; RLL, right lower lobe; RUL, right upper lobe; SUV max, maximum standard uptake value; wt, weight; XRT; radiation therapy; yrs, years.

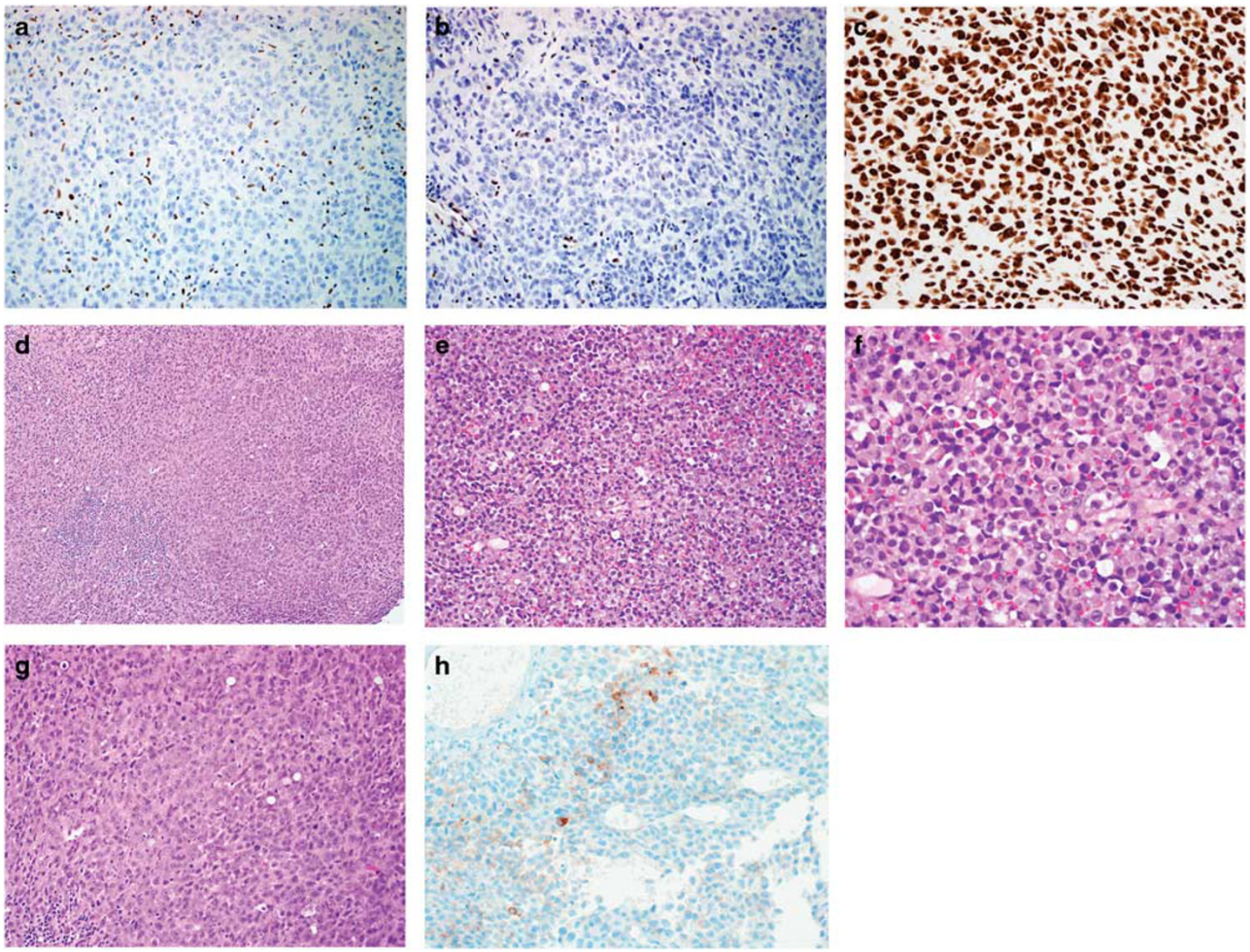

Figure 1 *SMARCA4*-deficient thoracic sarcoma demonstrating loss of BRG1 (*SMARCA4*) immunostaining in all tumor nuclei, with internal control demonstrated by staining of inflammatory and stromal cell nuclei (**a**; × 200). BRM (*SMARCA2*) immunoexpression is also lost, with positive internal control (**b**; × 200). INI-1 (*SMARCB1*) immunoexpression is retained (**c**; × 400). Solid architecture is seen in *SMARCA4*-deficient thoracic sarcomas (**d**; × 100). A region with more classic rhabdoid morphology, characterized by discohesive cells with eccentric displacement of nuclei by condensation of eosinophilic cytoplasm (**e**; × 200 and **f**; × 400). Ovoid tumor cells with abundant eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli (**g**; × 200). An example of positive cytokeratin (OSCAR) immunoexpression in only rare tumor cells (**h**; × 200).

Clinicopathological and Immunohistochemical Features of SMARCA4-Deficient Thoracic Sarcomas

SMARCA4/BRG1-deficient thoracic sarcomas ($n = 12$) occurred at median age of 59 years (range, 44–76), with male predominance (9:3). The epicenter of disease was mediastinum in 7 patients, lung in 3, and pleura in 2. The smoking status was known for six patients, five of whom were either current or former smokers. Presenting symptoms included dyspnea, chest pain, pleural effusion, reflux, fatigue, and weight loss. One patient presented with right lower extremity pain and weakness due to metastases in the iliac bone, and one presented with seizures due to brain metastases (Table 2).

Of six patients with detailed presentation data, all presented with unresectable tumors. The tumor size was available in four mediastinal tumors, and ranged from 9.6 to 16 cm (Table 2). PET data was available for two tumors, both of which were PET avid (SUV max 3.44 and 12). The lung tumors presented at smaller sizes (1.3 and 1.4 cm in the two patients with available size), but both had metastatic disease at the time of the presentation (adrenal (both patients), brain and mediastinal lymph nodes). In total, six patients with BRG1-deficient tumors had known evidence of metastases at presentation, and one developed progressive metastases 1 month after diagnosis. The sites of metastatic disease included lymph nodes (mediastinal, axillary, and supraclavicular), adrenal, pericardium, lung, bone, and brain.

The morphology of the tumors was similar among all the cases and had a characteristic appearance. A striking feature observed in all the cases was a relatively monotonous appearance of the tumor cells, especially when considering their otherwise high-grade histological characteristics. Secondary or complex architectural structures were not present;

Table 3 Overall survival among patients with follow-up

	N	*Deaths*	*Median survival (mo)*	*2-Year survival % (95% CI)*	*Hazard ratio (95% CI)*
BRG1-deficient	8	8	4.0	12.5% (0.0%, 35.4%)	2.86 (1.02, 8.04)
BRG1-retained	18	9	39.9[a]	64.4% (38.7%, 90.1%)	0.87 (0.33, 2.33)
Thymic carcinoma	11	8	36.3[b]	63.6% (35.2%, 92.1%)	Reference

[a]Survival of BRG1-deficient *vs* BRG1-retained: $P = 0.02$.
[b]Survival of BRG1-deficient versus thymic carcinoma: $P = 0.06$.

rather, the tumor cells formed solid sheets or vaguely anastomosing islands or nodules of tumor cells (Figure 1d and g). The tumor cells in many cases displayed a syncytial-like growth pattern, with indistinct cell borders. They generally had vesicular chromatin with prominent moderately sized nucleoli. Careful examination typically revealed variably represented areas of more classic rhabdoid morphology, including discohesive ovoid tumor cells with abundant eosinophilic cytoplasm, eccentric nuclei, and vague perinuclear hyaline inclusions (Figures 1e–f). Brisk mitotic activity (Figure 1f) and necrosis were universal.

BRG1-deficient cases showed rare tumor cells with cytokeratin expression in 10 cases (83%; Figure 1h). However, diffuse positivity for cytokeratin was not seen in any of the BRG1-deficient cases. One BRG1-deficient case showed weak TTF1 immunoexpression, and cytokeratin was negative in this case. All BRG1-deficient cases were negative for desmin, NUT, and S-100 protein. CD34 immunostaining was positive in three of five BRG1-deficient tumors tested (60%). SOX2 immunohistochemistry was performed in four cases with BRG1 loss, all of which showed strong and diffuse SOX2 expression; SOX2 was negative in seven tested cases with retained BRG1 expression.

Clinical Outcome

In total, follow-up information was available for 37 patients (median, 12 months; range, 0.5–184), including 8 with BRG1 loss, 18 with retained BRG1, and 11 thymic carcinomas (Table 3). Known metastases occurred in 7 patients with *SMARCA4*-deficient thoracic sarcomas, 10 with BRG1-retained tumors, and 6 thymic carcinomas (Tables 1 and 2). All patients ($n = 6$) with *SMARCA4*/BRG1-deficient thoracic sarcomas and available data had unresectable tumors, 4 of whom were treated with chemotherapy only, and 2 received both chemotherapy and radiation therapy (Tables 1 and 2). Six of 16 patients with BRG1-retained tumors and 6 of 11 patients with thymic carcinoma presented with unresectable tumors, and were treated with a variety of modalities (Table 1).

All patients with *SMARCA4*/BRG1-deficient sarcomas and available follow-up ($n = 8$) were dead of disease at a median of 4 months (range, 1–108), compared with 9 of 18 in the BRG-retained group (median 39.9 months; range 0.5–96), and 8 of 11 in the thymic carcinoma group (median 36.3 months; range 2–54; Tables 1–3). Patients with *SMARCA4*/BRG1-deficient thoracic sarcomas had worse 2-year survival compared with those with BRG1-retained tumors and those with thymic carcinomas (12.5% *vs* 64.4% and 63.3%, respectively; Table 3). The overall survival of patients with *SMARCA4*/BRG1-retained tumors and thymic carcinomas was similar, but the overall survival of patients with *SMARCA4*/BRG1-deficient thoracic sarcomas trended toward worse survival in comparison among all three groups (Figure 2a). In pairwise comparisons of survival between the study subgroups, the overall survival of patients with tumors with *SMARCA4*/BRG1 loss was significantly worse than those with retained *SMARCA4*/BRG1 immunoexpression ($P = 0.02$) and approached significance when compared with the overall survival of the patients in the thymic carcinoma group ($P = 0.06$; Figures 2b and c). There was no difference in survival between patients with thymic carcinoma and those with retained *SMARCA4*/BRG1 immunoexpression ($P = 0.84$).

Discussion

In this study, we have demonstrated that *SMARCA4*/BRG1 deficiency characterizes a subset of undifferentiated thoracic tumors with rhabdoid morphology and aggressive behavior. Sarcomas with rhabdoid morphology in the chest appear more likely to show *SMARCA4* inactivation, often with co-inactivation of *SMARCA2,* rather than inactivation of *SMARCB1*, and have a propensity for the mediastinum, although they also occur in the pleuropulmonary parenchyma. This work expands on the prior studies in French and Japanese cohorts of *SMARCA4*-deficient thoracic sarcomas[22,23] by further contributing to the clinicopathological characterization of these tumors. Similar to the findings of Yoshida *et al*,[22] our cohort showed a male predominance with a wide age range (second to eighth and fourth to seventh decades in the Japanese and our cohorts, respectively). However, in both the Japanese and French cohorts, these sarcomas occurred in younger patients (medians of 39 and 42 years, respectively) compared with our cohort (median 59 years). Our findings indicate that it is important to consider *SMARCA4*-deficient thoracic sarcoma in the differential diagnosis of tumors showing suggestive morphologic features in patients of all ages, as in our experience, this entity is

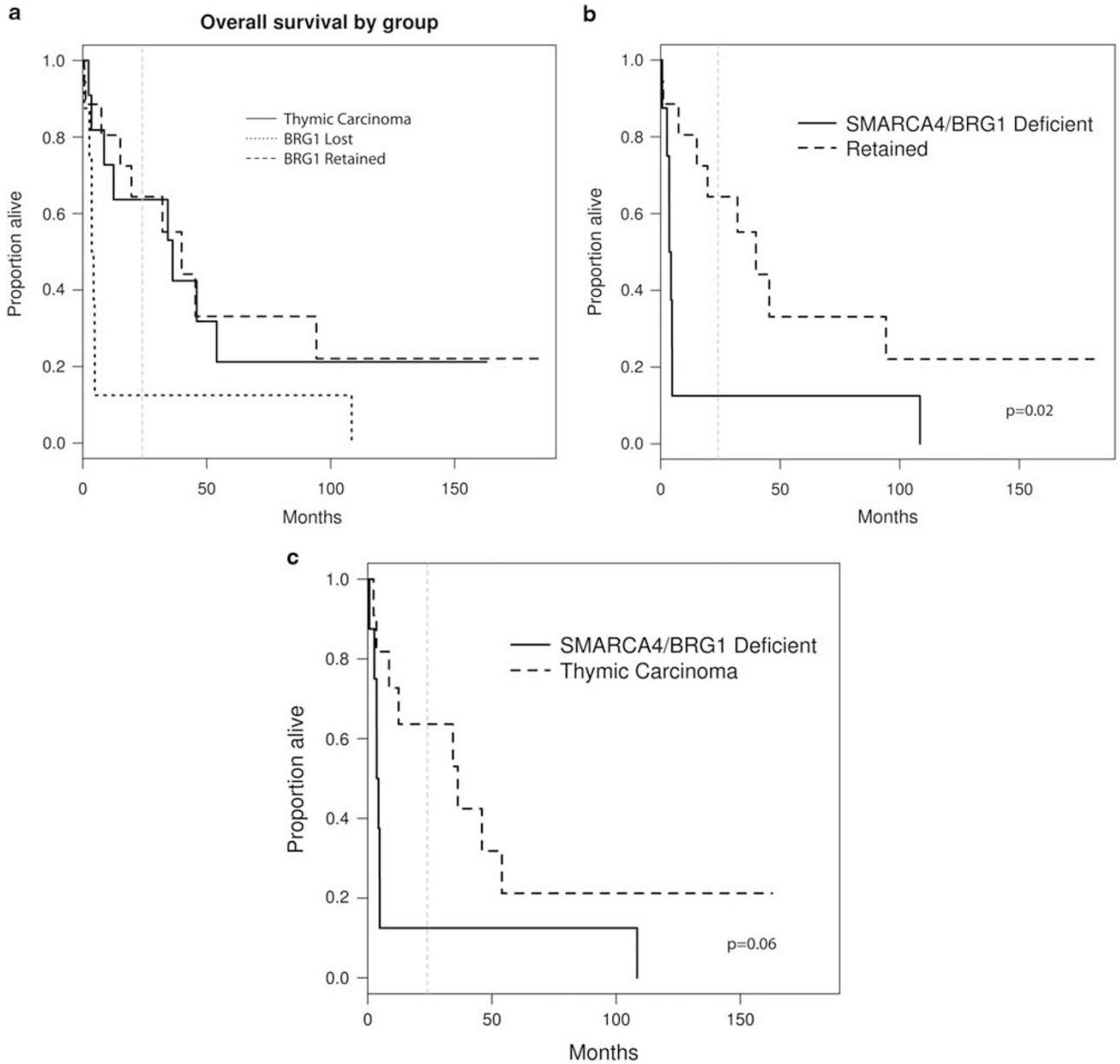

Figure 2 Overall survival of patients with BRG1-deficient tumors trends toward worse survival when comparing overall survival of patients with thymic carcinoma, BRG1-retained tumors, and BRG1-deficient tumors (**a**). In pairwise comparisons of survival between study subgroups, the overall survival of patients with BRG1-deficient tumors was significantly worse than those with retained BRG1 ($P=0.02$) (**b**), and approaches significance when compared with the thymic carcinoma group ($P=0.06$; **c**).

not limited to young patients. In all the three cohorts, the tumors occurred predominantly in the mediastinum and showed a nonspecific immunophenotype with many cases showing very focal, but not diffuse, cytokeratin expression. The median survival in both the French and Japanese groups was 7 months and was similarly poor in our cohort at only 4 months. The findings of our study support and further validate the behavior of *SMARCA4*-deficient thoracic sarcomas as very aggressive, conferring an extremely poor prognosis.

An interesting question requiring further study is the relationship, or lack thereof, between *SMARCA4*-deficient thoracic sarcomas and *SMARCA4*-deficient lung carcinomas. Le Loarer *et al*[23] demonstrated that *SMARCA4*-deficient thoracic sarcomas are genetically distinct from lung carcinomas and are instead more closely related to MRTs and SCCOHTs. In their study, the *SMARCA4*-deficient thoracic sarcomas also showed co-deficiency in *SMARCA2,* while lung carcinomas showed isolated *SMARCA4* inactivation. Co-deficiency of these genes has also been identified in SCCOHT.[44] Herpel *et al*[24] found isolated *SMARCA4* loss in 10 of 316 (3.2%) differentiated lung carcinomas, but co-deficiency of *SMARCA4* and *SMARCA2* in only 0.1% (2 squamous cell carcinomas and 2 adenocarcinomas). All tested BRG1-deficient thoracic tumors in our study showed

co-inactivation of *SMARCA4* and *SMARCA2* at the protein level. This concomitant loss of both *SMARCA4* and *SMARCA2* expression, in addition to the undifferentiated rhabdoid morphology and immunophenotype seen in these tumors, support that the tumors described herein fall into the category of *SMARCA4*-deficient thoracic sarcomas rather than lung carcinomas with *SMARCA4* inactivation. SOX2 immunohistochemistry has been proposed as a marker for distinguishing *SMARCA4*-deficient thoracic sarcomas from carcinomas with *SMARCA4* inactivation.[23] In our study, we performed SOX2 staining in a subset of cases with BRG1 loss and retained BRG1 expression. SOX2 was diffusely positive in all the tested cases with BRG1 loss, and negative in the tested cases with retained BRG1 expression, although our number of tested cases was small. However, its use as a diagnostic marker in this setting remains to be established, as it is not specific and may be expressed in several carcinomas that could enter the differential diagnosis, including a high percentage of squamous cell carcinomas and high-grade neuroendocrine carcinomas, as well as rare lung adenocarcinomas.[45–48] Yoshida *et al* also observed SOX2 immunostaining in 4 of 12 *SMARCA4*/BRG1-deficient lung carcinomas, although only one was diffuse.[22] A recent study demonstrated that Claudin-4 immunohistochemistry may be helpful in distinguishing SWI/SNF complex-deficient undifferentiated tumors/sarcomas from the various carcinomas that show loss of *SMARCA4* expression.[49] The clinical significance of this distinction in the thorax remains to be determined, as lung carcinomas with SMARCA4 loss also have a poor prognosis,[50–52] and direct comparative studies have not been performed. However, as discussed below, emerging evidence suggests that tumors with *SMARCA4* and *SMARCA2* co-deficiency may respond more favorably to targeted therapy than those with isolated *SMARCA4* loss.

Currently, it is not entirely clear whether *SMARCA4*-deficient thoracic sarcomas represent truly unique *de novo* neoplasms, or possibly a form of 'dedifferentiation' from a prior lung carcinoma with or without *SMARCA4* inactivation. This is not a novel concept, as INI-1/*SMARCB1* loss has been rarely noted to occur as a secondary phenomenon in malignancies of various types, imparting a 'composite extrarenal rhabdoid tumor' morphology.[53–55] Tumors with *SMARC*-intact differentiated areas juxtaposed with dedifferentiated components deficient in a SWI/SNF protein have also been reported in several organs.[20,56–58] Thus, the fundamental classification of these tumors as sarcoma *vs* carcinoma is still a matter of debate. Some of the mutations detected by Yoshida *et al* in their cohort of *SMARCA4*-deficient thoracic sarcomas are common in lung adenocarcinoma (eg, *KRAS, TP53*), and there was an increased number of smokers and patients with emphysema in their cohort.[22] The distribution of metastases observed in our study is also very similar to the pattern expected for lung carcinoma (mediastinal lymph nodes, adrenal, bone, and brain), although that is not a specific feature. One might argue for classification of *SMARCA4*-deficient thoracic tumors as carcinomas based on the focal keratin expression that is often observed; however, focal keratin expression is also commonly seen in other tumors with *SMARCA2* or *SMARCB1* loss that are generally accepted as sarcomas. In addition, as mentioned above, genetic data indicate that these tumors are distinct from lung carcinomas with *SMARCA4* inactivation.[23] The propensity for the mediastinum, overrepresentation of younger patients, and distinctive monotonous morphology (as opposed to the usual pleomorphism of carcinoma) also support the concept of these tumors as *de novo* and distinct malignancies. Therefore, the nature of the relationship of these tumors to lung carcinoma and/or smoking remains to be determined. It is also possible that the early studies of this entity have comprised a mix of 'true' *SMARCA4*-deficient sarcomas as well as poorly differentiated carcinomas with *SMARCA4* inactivation, as pathologists are refining the ability to identify and properly classify these rare tumors.

In addition to prognostic implications, identification of *SMARCA4*-deficient thoracic sarcomas is becoming important for clinical management as targeted therapy begins to emerge. Currently, targeted EZH2 inhibitors are being developed for the treatment of tumors with abnormalities in the SWI/SNF complex proteins. EZH2 is a histone methyltransferase that acts as the catalytic subunit of the polycomb repressor complex (PRC), a transcriptional regulator involved in cancer metastasis and cell proliferation. The SWI/SNF complex regulates the PRC, and if this regulation is disturbed by mutations in the proteins of the SWI/SNF complex, uninhibited PRC activity leads to tumor progression and metastasis. Targeted EZH2 inhibitors disable the PRC complex and stop uninhibited cell proliferation.[59,60] Currently, there is a clinical trial with an EZH2 inhibitor available for patients with *SMARC*-deficient tumors,[37] including *SMARCA4*-deficient thoracic sarcomas demonstrating rhabdoid morphology. In addition, emerging data suggest that co-deficiency of *SMARCA4* and *SMARCA2* expression confers synthetic lethality to EZH2 inhibitors as preclinical models of *SMARCA2*- and *SMARCA4*-deficient SCCOHT, MRT and lung adenocarcinomas have shown selective killing with EZH2 inhibition.[61–63] Although clinical trial data are not yet available, knowledge of the *SMARCA2* status in a tumor may prove to be important not only for distinguishing these sarcomas from *SMARCA4*-deficient carcinomas but also as a predictive biomarker for response to therapy.

As *SMARCA4*-deficient thoracic sarcomas usually present at an advanced stage and are often unresectable, many will be only sampled via minimally invasive small biopsies. In these situations, BRG1

and BRM immunostaining may be critical for understanding the molecular mechanism of these tumors and for selecting the appropriate patients for targeted therapy, even with scant samples that may contain insufficient quantity of tumor for more advanced molecular testing.

In conclusion, *SMARCA4*-deficient thoracic sarcomas have distinctive morphological features that can be identified by pathologists. They are histologically similar to tumors with INI-1 loss and show a high-grade rhabdoid appearance, often with cytokeratin expression in rare tumor cells. BRG1 and/or BRM immunohistochemistry is useful to confirm the diagnosis, and an immunohistochemical panel is helpful for the exclusion of morphologic mimics. The identification of *SMARCA4*-deficient thoracic sarcomas is important both prognostically and therapeutically. These tumors behave aggressively and have a prognosis worse than other poorly differentiated thoracic tumors. The development of targeted therapy provides additional clinical relevance for the identification of *SMARCA4*-deficient thoracic sarcomas by pathologists.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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