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Recurrent *SMARCB1* Inactivation in Epithelioid Malignant Peripheral Nerve Sheath Tumors

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Abstract

Epithelioid malignant peripheral nerve sheath tumors (EMPNST) are characterized by diffuse S-100 and SOX10 positivity, frequent immunohistochemical loss of *SMARCB1* expression (70%), and rare association with neurofibromatosis type 1. Some cases arise in a pre-existing epithelioid schwannoma (ESCW), which also show *SMARCB1* loss in 40% of cases. To date, little is known about the genomic landscape of this distinctive variant of malignant peripheral nerve sheath tumor. The aim of this study was to use targeted next-generation sequencing to identify recurrent genomic aberrations in EMPNST and a subset of ESCW, including the basis of *SMARCB1* loss. 16 EMPNSTs (13 *SMARCB1*-lost, 3 *SMARCB1*-retained) and 5 ESCWs with *SMARCB1* loss were selected for the cohort. Sequencing identified *SMARCB1* gene inactivation in 12/16 (75%) EMPNST and all 5 (100%) ESCW through homozygous deletion (N=8), nonsense (N=7), frameshift (N=2), or splice site (N=2) mutations; 2 EMPNSTs harbored 2 concurrent mutations each. *SMARCB1* immunohistochemistry status and *SMARCB1* alterations were concordant in 20/21 of the sequenced tumors. Additional genetic alterations in a subset of EMPNST included inactivation of *CDKN2A* and gain of chromosome 2q. Among *SMARCB1*-wild-type EMPNSTs there were single cases each with *NF1* and *NF2* mutations. No cases had *SUZ12* or *EED* mutations. In summary, we identified recurrent *SMARCB1* alterations in EMPNST (and all 5 *SMARCB1*-negative ESCWs tested), supporting loss of *SMARCB1* tumor suppressor function as a key oncogenic event. *SMARCB1*-retained EMPNSTs lack *SMARCB1* mutations and harbor different driver events.

Keywords

Epithelioid malignant peripheral nerve sheath tumor; epithelioid schwannoma; *SMARCB1*; next-generation sequencing; neurofibromatosis type 1

INTRODUCTION

Epithelioid malignant peripheral nerve sheath tumor (EMPNST) is a distinctive variant that differs from conventional malignant peripheral nerve sheath tumor (MPNST) by its characteristic features of strong and diffuse positivity for S-100 protein and SOX10, rare association with neurofibromatosis type 1, occasional origin in a pre-existing schwannoma, and loss of expression of the tumor suppressor SMARCB1 (INI1, SNF5, BAF47) in up to 70% of cases.¹⁻³ Most patients are affected during adulthood (median age, 44 years) with an equal sex distribution, and tumors commonly arise as superficial masses in the extremities and trunk (although some may be deep-seated or in visceral sites).^{1, 3}

Histologically, EMPNST exhibits a lobulated growth pattern within a variably myxoid or fibrous stroma, being comprised of a generally uniform but clearly atypical population of round, polygonal, or ovoid cells with round vesicular nuclei, prominent nucleoli, and abundant amphophilic or palely eosinophilic cytoplasm. While conventional MPNST is associated with neurofibromatosis type 1 in about 50% of cases, EMPNST has only rarely been reported to arise in the setting of neurofibromatosis type 1. EMPNST may occasionally arise in a pre-existing schwannoma or neurofibroma,¹ and precursor schwannomas include both conventional and epithelioid types. Interestingly, a subset of epithelioid schwannomas (ESCW) (~42%) also show loss of SMARCB1 expression.^{4, 5} ESCW shows similar morphologic features to EMPNST, except cytologic atypia is absent or minimal and significant mitotic activity or necrosis are lacking. While the distinction between most cases of ESCW and EMPNST is usually straightforward, there likely exists a morphologic continuum and a subset of peripheral nerve sheath tumors cannot be classified reliably as benign or malignant.⁵

The mechanism of loss of expression of the tumor suppressor SMARCB1 (encoded by *SMARCB1* located at 22q11.23) in EMPNST is currently unknown. Silencing of *SMARCB1* by miRNAs, specifically miR-206, -381, and 671-5p, has been reported in various malignant mesenchymal neoplasms including a subset of EMPNST,⁶ but little is known about the genomic landscape of EMPNST and whether they exhibit alterations affecting key drivers implicated in the oncogenesis of conventional MPNST, such as *NFI*, *CDKN2A*, or genes encoding components of the polycomb repressive complex 2 (i.e., *SUZ12* or *EED*).^{7, 8}

The aim of this study was to evaluate a cohort of EMPNSTs (and, for comparison, a small subset of SMARCB1-lost ESCW) by large panel-targeted next-generation sequencing to identify recurrent genomic alterations, specifically those targeting the *SMARCB1* tumor suppressor, and to correlate the findings with SMARCB1 immunohistochemical expression.

MATERIALS AND METHODS

Case Selection

This study was performed with the approval of the Institutional Review Board at Brigham and Women's Hospital. Cases were retrieved from the consult files of one of the authors (C.D.M.F.) and the surgical pathology files of Brigham and Women's Hospital. In total, 23

epithelioid peripheral nerve sheath tumors diagnosed between 2007 and 2018 were selected, which included 18 EMPNSTs (14 SMARCB1-lost and 4 having retained expression of SMARCB1), and 5 ESCWs with SMARCB1 loss. Five cases (#15, 17, 19, 20, and 21, Table 1) were included in prior studies in which diagnostic criteria have been described.^{1, 5}

Immunohistochemistry

SMARCB1 immunohistochemistry was performed for all cases on 4- μ m-thick formalin-fixed paraffin-embedded whole tissue sections using a mouse monoclonal antibody (1:200 dilution; clone 25/BAF47; citrate buffer and EDTA pretreatment; BD Biosciences, Carlsbad, CA, USA) and the Envision Plus detection system (Dako). Appropriate positive and negative controls were used throughout. Tumors were considered “SMARCB1-lost” (i.e. negative) when there was loss of nuclear expression in >95% of tumor nuclei.

Targeted Next-Generation Sequencing

Next-generation sequencing was performed utilizing our institutional targeted sequencing platform, OncoPanel, which interrogates the exonic sequences of 447 cancer-associated genes for mutations and copy number variations, and 191 introns across 60 genes for gene rearrangements^{9, 10}. DNA extraction from formalin-fixed paraffin-embedded tissue sections of tumor (QIAamp DNA mini kit, Qiagen, Valencia, CA), construction of hybrid-capture libraries, sequencing using the Illumina HiSeq 2500 (Illumina, San Diego, CA), and sequence data analysis was performed as previously described.¹⁰ Sequencing was performed on tumor DNA only, without a paired non-neoplastic tissue section. UVA mutational signature was assessed using an approach modified from Brash,¹¹ requiring that 60% of mutations are C>T substitutions at dipyrimidine sites or 5% of total mutations being CC>TT dinucleotide substitutions. All detected alterations (including single nucleotide variants, copy number alterations, and translocation calls) were reviewed manually and annotated.

21 total cases underwent successful targeted next-generation sequencing, with mean tumor percentage was 67% (range, 50-75%), and mean target coverage 325x (range, 162-429). Two cases of EMPNST (1 SMARCB1-lost, 1 SMARCB1-retained) failed due to low sequencing quality metrics and were excluded from the study.

RESULTS

Clinicopathologic findings

Table 1 summarizes the clinicopathologic findings of the 21 cases diagnosed as epithelioid peripheral nerve sheath tumors that were sequenced successfully in the study cohort. The 16 cases of EMPNST included 10 male and 6 female patients with an age range of 19-69 years (median, 43), and were located on the trunk (N=9), extremity (N=6), and urinary bladder (N=1). By SMARCB1 immunohistochemistry, 13 EMPNSTs showed loss of expression and 3 were positive/retained. The 5 ESCW cases (all SMARCB1-lost by immunohistochemistry) included 2 men and 3 women, age ranging from 27-64 years (median, 42); tumor sites were extremity (N=4) and trunk (N=1). All EMPNSTs and ESCWs showed strong and diffuse expression of S-100 protein.

Fifteen EMPNSTs represented primary tumors, and 2 were metastases. One patient who presented with an axillary mass (case #2) had a history of 2 prior, separate primary EMPNSTs located in the subclavicular region and thigh, 12 and 5 years before, respectively, suggestive of a germ-line predisposition syndrome. Another patient with a pleural metastasis (case #9) had a history of a primary EMPNST located in the forearm 2 years earlier.

Targeted Next-Generation Sequencing Results

Table 2 summarizes the sequencing results for the 21 successfully sequenced cases, which included 16 EMPNSTs and 5 ESCWs.

Overall, *SMARCB1* alterations were detected in a total of 17/21 (81%) sequenced cases, including 12 EMPNSTs and 5 ESCWs. The distribution of single nucleotide variants and copy number variants within the *SMARCB1* (NM_003073) coding region is shown in Figure 1.

SMARCB1 gene inactivation was identified in 12/16 (75%) EMPNSTs, all of which showed immunohistochemical loss of SMARCB1 expression (Fig. 2), and in all 5 (100%) SMARCB1-lost epithelioid schwannomas (Fig. 3). Specifically, *SMARCB1* inactivation resulted from homozygous deletion (N=8), nonsense (N=7), frameshift (N=2) or splice site (N=2) mutations. One EMPNST (case #10) harbored 2 heterozygous *SMARCB1* nonsense mutations (p.R40* and p.Y248*) consistent with biallelic *SMARCB1* inactivation, as well as an additional *NF1* frameshift mutation (p.E2195fs). Another EMPNST (case #11) harbored a concurrent *SMARCB1* nonsense mutation (p.R53*) and localized hemizygous deletion of exons 5-9. Only one EMPNST with SMARCB1 loss (case #9) lacked detectable *SMARCB1* genomic aberrations. No *SMARCB1* alterations were observed in SMARCB1-retained EMPNSTs. Overall, SMARCB1 immunohistochemical status and *SMARCB1* alterations were concordant in 15/16 (94%) EMPNSTs and 20/21 (95%) of sequenced tumors overall.

Four of 16 (31%) EMPNSTs (including 2 SMARCB1-lost cases) harbored *CDKN2A* inactivation, through homozygous deletion (N=3) or nonsense (N=1) mutation; additionally, one ESCW showed homozygous deletion of *CDKN2A* (Table 2). Among the SMARCB1-retained EMPNSTs, inactivating mutations of *NF1* (case #14) or *NF2* (case #16) were detected in one case each, as well as concurrent inactivating mutations of *TP53* and *CDKN2A* in another case (case #15) (Table 2). Notably, case #14 arose in association with neurofibroma in a patient who did not have a known clinical diagnosis of neurofibromatosis type 1, and case #16 arose in a conventional schwannoma (Fig 4). Of note, genomic aberrations targeting *SUZ12* or *EED* encoding components of the polycomb repressive complex 2 were consistently absent in the cohort.

The mean number of mutations per Mb in the EMPNSTs was 4.6 (range, 2.3- 9.1 per Mb), which was similar to the mean in ESCWs (3.8 per Mb (range, 0.8-6.1/Mb)) (Table 2). No UVA mutational signatures were detected in the remaining 21 cases, including case #13. Case #13 was originally diagnosed descriptively as most suggestive of metastatic malignant melanoma but was included in our analysis based on having SMARCB1 loss and absent expression of melanocytic markers MART-1 and PNL2. This tumor was a vertebral bone metastasis in a patient with widespread lymphadenopathy, initially suggestive of metastatic

disease of an unknown primary. *SMARCB1* homozygous deletion was detected in this case, which in conjunction with the immunohistochemical features support the diagnosis of EMPNST.

Chromosomal copy number alterations (Fig. 5) were relatively more frequent in EMPNSTs (median, 2.5 cytogenetic events per tumor; mean, 3.2 cytogenetic events per tumor; range, 0-10) compared to ESCWs (median, 1 cytogenetic event per tumor; mean, 1.4 cytogenetic events per tumor; range, 0-4). The most frequent chromosomal aberrations were loss of chromosome 22q (i.e., either complete or segmental loss or copy number neutral loss of heterozygosity), which was observed in 12 cases (8/16; 50%) EMPNSTs and 4/5 (80%) ESCWs). Gain of chromosome 2 or 2q was second most frequent and was observed in 9 total cases, including 8/16 (50%) EMPNSTs (all 8 *SMARCB1*-lost) and 1/5 (20%) ESCW (Fig. 5).

Discussion

SMARCB1 (INI1, SNF5, BAF47) is a member of the SWI/SNF chromatin remodeling complex, which is involved in epigenetic regulation of gene transcription. Pathogenic alterations in various subunits of the SWI/SNF complex have been identified in >20% of human cancers, and these alterations in the SWI/SNF complex have been shown to lead to global disruptions in transcriptional regulation.¹² The spectrum of *SMARCB1*-deficient neoplasms is continuously expanding and includes numerous benign and malignant mesenchymal and non-mesenchymal neoplasms. *SMARCB1* loss is therefore not tumor-specific *per se*, and is a feature of malignant rhabdoid tumor (the prototypical *SMARCB1*-deficient neoplasm driven by recurrent *SMARCB1* genetic inactivation),¹³ as well as many other neoplasms including epithelioid sarcoma,¹⁴ a subset of myoepithelial tumors,¹⁵ extraskeletal myxoid chondrosarcoma,¹⁵ poorly differentiated chordoma,¹⁶ renal medullary carcinoma,¹⁷ and *SMARCB1*-deficient sinonasal carcinoma,¹⁸ among others. ESCW and EMPNST are known to show frequent loss of *SMARCB1* expression in ~40% and 70% of cases, respectively, and our findings demonstrate that *SMARCB1* immunohistochemical status correlates highly with genetic *SMARCB1* inactivation.

The high rate of *SMARCB1* gene inactivation (75%) in EMPNST in our study suggests that loss of *SMARCB1* tumor suppressor function is probably essential and also permissive for the development of these tumors. The inactivating mutations were either mononucleotide mutations or localized homozygous or hemizygous deletions occurring across the entire coding region of the gene. One mutation (p.R40*), which has been reported previously in rhabdoid tumor¹⁹ and chordoma,²⁰ was detected in 2 EMPNSTs (cases #4 and #10) and in one ESCW (case #20), supporting the hypothesis that *SMARCB1* inactivation is an early event in the development of both EMPNSTs and ESCWs. *SMARCB1* inactivation was identified in all but one *SMARCB1*-lost EMPNST (case #9). The mechanism of *SMARCB1* loss remains unexplained in this case; it is possible that this tumor harbors a structural variant that is not detected by our targeted sequencing panel or that *SMARCB1* is downregulated epigenetically, perhaps by previously suggested mechanisms.⁶ *SMARCB1* inactivation is unlikely to have any prognostic significance, given that most cases of EMPNST are associated with overall less aggressive behavior.¹ Distinct driver events were

detected in the *SMARCB1*-wild-type EMPNSTs, namely alterations targeting tumor suppressors implicated in conventional MPNST development and progression. This included inactivating events targeting *NF1* (case #14, arising in neurofibroma), *NF2* (case #16, arising in schwannoma), and *TP53* and *CDKN2A* (case #15). Given that neurofibromas and schwannomas show frequent inactivation of *NF1* and *NF2*, respectively, it is not surprising that the two EMPNST arising in these benign precursors showed *NF1* and *NF2* mutations. While these examples seem to be driven by pathogenetic events distinct from EMPNST with *SMARCB1* inactivation, the genetic mechanisms fostering transformation to malignancy with epithelioid morphology remains unknown. There were no morphologic differences between these *SMARCB1*-retained tumors and EMPNSTs with *SMARCB1* loss.

Conventional MPNSTs often develop from a benign precursor neurofibroma and acquire a sequence of genomic events that promote malignant progression. Inactivation of *NF1* is usually the first event in the development of neurofibroma, and is followed by *CDKN2A* inactivation in the progression to atypical neurofibroma/early malignant peripheral nerve sheath tumor.²¹ Subsequent inactivation of the polycomb repressive complex 2 through mutations in either *SUZ12* or *EED*^{7, 8} results in loss of the chromatin mark trimethylation at lysine 27 of histone 3 (H3K27me3), which can be detected by immunohistochemistry in about half of all conventional MPNST, increasingly at higher grades.^{22, 23} Based on our findings, EMPNST appears to lack canonical aberrations involving *SUZ12* and *EED*, and *NF1* mutations are largely absent (apart from the single case arising in a neurofibroma), which suggests that these tumors follow a different sequence of events in malignant progression. However, similarly to conventional MPNST, *CDKN2A* inactivation was the second most common alteration following *SMARCB1* inactivation, and was present in EMPNSTs with (2/12) and without (2/3) *SMARCB1* alterations.

Losses of chromosome 22q were the most common chromosomal aberrations, and were present in half of all EMPNSTs in our cohort (as well as 4/5 ESCWs), all of which had *SMARCB1* alterations. Notably, *SMARCB1* is located on 22q, and chromosome 22q aberrations (either through hemizygous loss or copy number neutral-loss of heterozygosity) may represent mechanisms of a second-hit. Chromosome 2 or 2q gain was the second most frequent chromosomal aberration and was identified in half of EMPNSTs and one ESCW, the biologic significance of which is currently uncertain.

ESCW and EMPNST share common morphologic and immunohistochemical features, and this present study also highlights very similar genomic features between these two entities. There were no major differences in the number of mutations and copy number alterations between EMPNST and ESCW, thus it seems likely that *SMARCB1* inactivation is an early event in the development of both neoplasms. These findings suggest that at least some of these tumors may represent a biologic continuum, and there may be an event that defines malignancy that remains to be identified. Given that there are no significant genetic differences between ESCW and EMPNST, appreciable cytologic atypia remains the sole criterion for malignancy.

EMPNST shows consistently strong and diffuse expression of S-100 protein and SOX10, a feature (in conjunction with epithelioid morphology) that allows distinction from

conventional MPNST, which only shows expression of these markers in ~30% of cases (and, if present, staining is usually limited in extent). However, EMPNSTs may be difficult to distinguish from amelanotic malignant melanoma on morphologic and immunohistochemical grounds. SMARCB1 immunohistochemistry may be helpful in distinguishing EMPNST from malignant melanoma in most scenarios. Malignant melanoma shows intact SMARCB1 expression,¹⁴ however up to one-third of EMPNST also retain SMARCB1 expression. While heterozygous *SMARCB1* missense mutations have previously been reported in a small subset of melanomas (including p.R40*), SMARCB1 expression was retained as the mutations did not appear to be completely inactivating;²⁴ in this latter study, 8 melanoma cases were found to have *SMARCB1* missense mutations; 5 had *TP53* mutations, 2 had *BRAF* p.V600E mutations, and 4 had activating *MAPK* mutations. In another study, loss of SMARCB1 expression was reported in malignant melanomas, with expression loss in metastatic cases and reduced expression in advanced primary tumors, although these cases did not undergo genetic analysis.²⁵ In challenging cases with equivocal clinicopathologic features, negativity for melanocytic markers (MART-1 and HMB-45) and loss of SMARCB1 expression would favor the diagnosis of EMPNST and molecular confirmation may be helpful to confirm the presence of *SMARCB1* inactivation, as illustrated by case #13 in our study. This case was originally favored to represent metastatic malignant melanoma but was re-classified as EMPNST based on the presence of *SMARCB1* homozygous deletion and absence of the UVA mutational signature. Given the overlapping morphologic and immunohistochemical features of EMPNST and malignant melanoma, close correlation with clinical presentation and disease course remains essential.

In conclusion, we identified *SMARCB1* gene inactivation in 81% of the epithelioid peripheral nerve sheath tumors in our cohort, supporting loss of *SMARCB1* tumor suppressor function as a key oncogenic driver in EMPNST and some cases of ESCW. *SMARCB1* inactivation is likely an initiating event, and the observation that ESCW are precursors to EMPNST in some cases indicate that there are unidentified events that promote malignant progression. SMARCB1-positive/*SMARCB1*-wildtype EMPNSTs harbor various other driver events that inactivate tumor suppressors with roles in conventional peripheral nerve sheath tumors.

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Figure 1.

SMARCB1 mutations detected in 12 epithelioid malignant peripheral nerve sheath tumors (blue) and 5 epithelioid schwannomas (green). Inactivating *SMARCB1* mutations were mononucleotide alterations and intragenic deletions (lines indicate deleted exons); 7 cases had homozygous (hom) deletions, one case a hemizygous (hemi) deletion. Mutations are described according to cDNA and protein sequence mutations. # and ^ denote concurrent mutations identified in the same tumor, respectively.

Figure 2.

Case #4: Epithelioid malignant peripheral nerve sheath tumor (A) with nuclear atypia, prominent nucleoli, and frequent mitoses showing loss of SMARCB1 expression (A, inset) resulting from a homozygous *SMARCB1* nonsense mutation (c.118C>T;p.R40*; allele fraction 0.47) (B). An identical mutation was found in another epithelioid malignant peripheral nerve sheath tumor and in an epithelioid schwannoma. Case #6: Epithelioid malignant peripheral nerve sheath tumor (C) with loss of SMARCB1 expression (C, inset) harboring a localized homozygous deletion of *SMARCB1* exon 2 (top panel, arrow) (D) resulting in marked decrease in exon 2 reads (bottom panel arrow) compared to retained read levels in normal tissue.

Figure 3.

Case #18: Epithelioid schwannoma (A) harboring loss of SMARCB1 expression (A, inset) resulting from a hemizygous *SMARCB1* frameshift mutation (c.779_782del;p.Q260fs*; allele fraction 0.78) (arrow) (B). This case had concurrent segmental loss of the *SMARCB1* locus at 22q. Case #19: Epithelioid schwannoma (C) with loss of SMARCB1 expression (C, inset) harboring a localized homozygous deletion of *SMARCB1* exons 1-4 (top panel, arrow) (D) causing marked decrease in exon 1-4 reads (bottom panel arrow) compared to retained read levels in normal tissue.

Figure 4.

Case #14: One epithelioid malignant peripheral nerve sheath tumor arose in a neurofibroma (A), with malignant areas showing epithelioid morphology, cytologic atypia, and hypercellularity (B). This tumor retained SMARCB1 expression (B, inset), was *SMARCB1*-wild-type, and had inactivating mutations of *NF1*. Case #16: Epithelioid malignant peripheral nerve sheath tumor arising in association with a conventional schwannoma (C-D). SMARCB1 expression was retained (D, inset) corresponding with wild-type *SMARCB1* status; this tumor had an *NF2* inactivating mutation.

Figure 5.

Overview of cytogenetic events in 21 epithelioid peripheral nerve sheath tumors.

Chromosome arms from 1p through Xq are indicated at the top. Loss of 22q (harboring the *SMARCB1* locus at 22q11.23) and gain of 2q and were the most frequently observed aberrations. CNN-LOH: Copy number neutral loss of heterozygosity; CNV: Chromosomal copy number variations; EMPNST: Epithelioid malignant peripheral nerve sheath tumor; ESCW: Epithelioid schwannoma; (+): Retained expression; (-): Lost expression.

Table 1.

Clinicopathologic findings in 21 epithelioid peripheral nerve sheath tumors.

Case no.	Diagnosis	Age	Sex	Status	Site	Association	SMARCB1 IHC
1	EMPNST	66	F	Prim	Abdominal wall	None	Lost
2	EMPNST	35	M	Prim	Axilla	None	Lost
3	EMPNST	67	F	Prim	Chest wall	None	Lost
4	EMPNST	54	F	Prim	Back	None	Lost
5	EMPNST	25	M	Prim	Thigh	None	Lost
6	EMPNST	43	F	Prim	Arm	None	Lost
7	EMPNST	19	F	Prim	Arm	None	Lost
8	EMPNST	35	F	Prim	Knee	None	Lost
9	EMPNST	35	M	Met	Pleura [#]	None	Lost
10	EMPNST	28	M	Prim	Chest wall	None	Lost
11	EMPNST	37	M	Prim	Urinary bladder	None	Lost
12	EMPNST	69	M	Prim	Knee	None	Lost
13	EMPNST [^]	43	M	Met	Vertebral column	None	Lost
14	EMPNST	47	M	Prim	Chest wall	Neurofibroma	Retained
15 [*]	EMPNST	46	M	Prim	Calf	None	Retained
16	EMPNST	59	M	Prim	Back	Schwannoma	Retained
17 [*]	ESCW	28	F	Prim	Calf	None	Lost
18	ESCW	44	F	Prim	Forearm	None	Lost
19 [*]	ESCW	27	M	Prim	Arm	None	Lost
20 [*]	ESCW	42	F	Prim	Chest wall/breast	None	Lost
21 [*]	ESCW	64	M	Prim	Knee	None	Lost

EMPNST: Epithelioid malignant peripheral nerve sheath tumor; ESCW: Epithelioid schwannoma; F: Female; M: Male; Met: Metastasis; Prim: Primary;

^{*} Case reported previously (1; 4);

Patient with history of primary epithelioid malignant peripheral nerve sheath tumors in the subclavicular region (12 years before) and thigh (5 years before);

[#] Primary tumor: forearm (2 years before);

[^] Initially diagnosed as malignant melanoma, re-classified as epithelioid malignant peripheral nerve sheath tumor.

Table 2.
Results of targeted next-generation sequencing in 21 epithelioid peripheral nerve sheath tumors.

Case no.	Diagnosis	SMARCB1 IHC	Tumor (%)	Mutations/ Mb	SMARCB1 genomic aberrations	Other relevant aberrations	Copy number alterations
1	EMPNST	Lost	75	3.802	c.978C>G;p.Y326* (VAF 0.41)	-	Gain: 5, 6; loss: 22q
2	EMPNST	Lost	65	6.844	c.559delG;p.V187fs (VAF 0.27)	-	Loss: 22q
3	EMPNST	Lost	75	6.844	c.94-22_111del (VAF 0.18)	-	Gain: 2, 5, 7, 8, 21q (seg); CNN-LOH: 22q
4	EMPNST	Lost	75	3.042	c.118C>T;p.R40* (VAF 0.47)	-	-
5	EMPNST	Lost	60	3.042	Hom del (ex 4-5)	-	Gain: 2, 22q (seg); loss: 22q (seg)
6	EMPNST	Lost	75	6.083	Hom del (ex 2)	-	Gain: 2, 8, 21q; CNN-LOH: 22q
7	EMPNST	Lost	70	7.604	c.94-4_111del (VAF 0.2)	-	Gain: 2q; CNN-LOH: 22q
8	EMPNST	Lost	50	2.281	Hom del (ex 1-9)	<i>CKDN24</i> hom del; <i>KMT2D</i> c.7960delA;p.S2654fs (VAF 0.39)	-
9	EMPNST	Lost	65	9.125	-	-	Gain: 2q, 8, 10, 21q
10	EMPNST	Lost	50	3.802	c.118C>T;p.R40* (VAF 0.28); c.744C>G;p.Y248* (VAF 0.25)	<i>NF1</i> c.6577_6578delG;p.E2195fs (VAF 0.21)	Gain: 2q, 5p (seg), 5q (seg), 8p (seg), 8q (seg), 21q (seg)
11	EMPNST	Lost	75	3.802	c.158C>T;p.R53* (VAF 0.37); hemi del (ex 5-9)	-	Gain: 2, 5, 6q (seg), 16p (seg), 17q (seg), 20q; loss: 17q (seg), 18q, 22q (seg)
12	EMPNST	Lost	70	4.562	Hom del (ex 1-9)	<i>CKDN24</i> hom del	Gain: 6; loss: 9, 22q (seg)
13	EMPNST	Lost	70	3.802	Hom del (ex 1-5)	-	Gain: 2
14	EMPNST	Retained	70	3.042	-	<i>NF1</i> c.4431-3_4431-2delinsTT (VAF 0.27); <i>NF1</i> c.6855C>A;p.Y228* (VAF 0.27); <i>CDKN24</i> hom del	Gain: 3p (seg), 4q (seg), 6q (seg), 7q, 9q, 16q (seg), 17q (seg); loss: 7p, 10q (seg), 11p, 14p (seg)
15	EMPNST	Retained	70	2.281	-	<i>TP53</i> c.431A>C;p.Q144P (VAF 0.06); <i>CDKN24</i> c.148C>T;p.Q50* (VAF 0.06)	Loss: 9p, 17p
16	EMPNST	Retained	50	3.802	-	<i>NF2</i> c.600-15_601del (VAF 0.07)	-
17	ESCW	Lost	70	3.802	c.544C>T;p.Q182* (VAF 0.56)	-	Gain: 2, 17q (seg); CNN-LOH: 22q, 11
18	ESCW	Lost	70	0.760	c.779_782del;p.Q260fs (VAF 0.78)	-	Loss: 22q (seg)
19	ESCW	Lost	70	3.802	Hom del (ex 1-4)	<i>CDKN24</i> hom del	-
20	ESCW	Lost	70	6.083	c.118C>T;p.R40* (VAF 0.68)	-	Loss: 22q (seg)
21	ESCW	Lost	70	4.562	Hom del (ex 1-9)	-	Loss: 22q (seg)

VAF: Variant allele fraction; CNN-LOH: Copy number neutral loss of heterozygosity; EMPNST: Epithelioid malignant peripheral nerve sheath tumor; ESCW: Epithelioid schwannoma; ex: Exon; Hom del: Homozygous deletion; Hemi del: Hemizygous deletion; IHC: Immunohistochemistry; Mb: Megabase; seg: Segmental.