HHS Public Access

Author manuscript

Mod Pathol. Author manuscript; available in PMC 2023 December 01.

Published in final edited form as:

Mod Pathol. 2022 December ; 35(12): 1900–1909. doi:10.1038/s41379-022-01148-x.

The genetic landscape of *SMARCB1* alterations in SMARCB1-deficient spectrum of mesenchymal neoplasms

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Abstract

SMARCB1 biallelic inactivation resulting in SMARCB1/INI1 deficiency drives a wide range of malignancies, including many mesenchymal tumors. However, the specific types of *SMARCB1* alterations and spectrum of cooperating mutations among various types of sarcomas has not been well investigated. We profiled *SMARCB1* genetic alterations by targeted DNA sequencing and fluorescence in situ hybridization (FISH) in a large cohort of 118 soft tissue and bone tumors, including SMARCB1-deficient sarcomas (78, 66%): epithelioid sarcomas, epithelioid peripheral nerve sheath tumors, poorly differentiated chordomas, malignant rhabdoid tumors, and soft tissue myoepithelial tumors, as well as non-SMARCB1-deficient sarcomas (40, 34%) with various *SMARCB1* genetic alterations (mutations, copy number alterations). SMARCB1 loss by immunohistochemistry was present in 94% *SMARCB1* pathogenic cases. By combined sequencing and FISH assays, 80% of SMARCB1-deficient tumors harbored homozygous (biallelic) *SMARCB1* loss, while 14% demonstrated heterozygous *SMARCB1* loss-of-function (LOF) alterations, and 6% showed no demonstrable *SMARCB1* alterations. FISH and sequencing were concordant in the ability to detect *SMARCB1* loss in 48% of cases. Epithelioid sarcomas most commonly (75%) harbored homozygous deletions, while a subset showed focal intragenic deletions or LOF mutations (nonsense, frameshift). In contrast, most soft tissue myoepithelial tumors (83%) harbored *SMARCB1* nonsense point mutations without copy number losses. Additionally, clinically significant, recurrent co-occurring genetic events were rare regardless

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AUTHOR CONTRIBUTIONS

JKD performed study design, data acquisition, data analysis and interpretation, writing and revision of the paper. SS, WT, BN, PC, LHW, MVO, and MG performed data acquisition and critical review of the paper. CRA performed study design and conception, analysis and interpretation of data, writing, review and revision of paper. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

This study was approved by the Memorial Sloan Kettering Cancer Institute Institutional Review Board.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41379-022-01148-x.

of histotype. By sequencing, extended 22q copy number loss in genes flanking the *SMARCB1* locus (22q11.23) occurred in one-third of epithelioid sarcomas and the majority of poorly differentiated chordomas. Poorly differentiated chordomas and soft tissue myoepithelial tumors showed significantly worse overall and disease-free survival compared to epithelioid sarcomas. Overall, *SMARCB1* LOF alterations predominate and account for SMARCB1 protein loss in most cases: majority being biallelic but a subset were heterozygous. In contrast, *SMARCB1* alterations of uncertain significance can be seen in diverse sarcomas types and does not indicate a SMARCB1-deficient entity

INTRODUCTION

SMARCB1 (located on chromosome 22q11.23) encodes for INI1/SMARCB1/BAF47/sSNF5 protein which is a core subunit of the ubiquitously expressed SWI/SNF (BAF) chromatin remodeling complex. Loss of SMARCB1 function destabilizes the BAF complex, thereby perturbing its chromatin occupancy and opposing function of polycomb-mediated promoter repression[1]. SMARCB1 deficiency is seen in up to 20% of human malignancies[2, 3], including many soft tissue and bone tumors often displaying epithelioid or rhabdoid morphology[4–7]. Loss of SMARCB1 expression is seen in the majority of epithelioid sarcomas (distal and proximal types)[8, 9], 50–70% of epithelioid malignant peripheral nerve sheath tumors (eMPNST)[9, 10], 50% of epithelioid schwannomas (eSCHW)[11], 30% of soft tissue myoepithelial tumors (STME)[9, 12], majority of poorly differentiated chordomas (PDCHO)[13, 14], and almost all malignant rhabdoid tumors (MRT)[15, 16]. In these studies, SMARCB1 loss was largely evidenced by loss of immunohistochemical expression of SMARCB1, or in a few studies, detection of homozygous or heterozygous *SMARCB1* deletions using fluorescence in situ hybridization (FISH) or microarray-based comparative genomic hybridization (aCGH). However, the specific types and distribution of *SMARCB1* genetic alterations among the various SMARCB1-deficient sarcomas have not been studied in detail.

To better characterize the nature and spectrum of *SMARCB1* alterations in mesenchymal tumors, we profiled a large cohort of 118 soft tissue and bone sarcomas by targeted DNA sequencing and FISH, focusing on SMARCB1-deficient sarcomas, correlating *SMARCB1* copy number (homozygous/heterozygous deletion, amplification) and mutational status with histotypes, SMARCB1 protein immunohistochemical expression, extent of chromosome 22q copy number losses, and co-occurring genomic alterations.

MATERIALS AND METHODS

Case selection and study cohort

After approval from the Institutional Review Board, cases were selected from the Pathology Department archives of Memorial Sloan Kettering Cancer Center (MSKCC) from 2013 to 2021. Inclusion criteria were: 1) soft tissue and bone tumors for which SMARCB1 deficiency has been well established in the literature to be pathogenic, i.e., proximal type epithelioid sarcoma (EPS-P), distal type epithelioid sarcoma (EPS-D), eMPNST, eSCHW, PD-CHO, MRT, and STME; 2) other soft tissue and bone tumors displaying *SMARCB1*

genetic alterations: deletion, amplification, point mutations, etc.; 3) all cases included have already had targeted DNA sequencing performed on formalin-fixed paraffin embedded (FFPE) tissue [105 cases by clinical MSK-IMPACT (103 with matched germline control, 2 with pooled FFPE control), 13 cases by research DNA sequencing using similar platform]. A total of 118 cases were included. Note that all STME in this study were malignant tumors, and thus may also be referred to as “myoepithelial carcinomas”. Immunohistochemistry (IHC) using antibody clone 25/BAF47 (BD Bioscience, catalog# 612110, 1:200 dilution) for SMARCB1 was performed in all cases where SMARCB1 deficiency is pathogenic (criteria #1) and a subset of the other cases (criteria #2) when archival tissue could be obtained. SMARCB1 IHC was available in 95 (80%) cases.

Fluorescence in situ hybridization (FISH)

SMARCB1 FISH testing was performed for 57 (48%) of cases. Details of probe hybridization conditions and signal detection were described previously[12]. Custom BAC probes for *SMARCB1* were designed and used to detect the presence of large homozygous or heterozygous deletions of *SMARCB1* at chromosome 22q11.23. Telomeric probe against *EWSR1* at chromosome 22q12 served as a reference. Normal copy number pattern was defined when two copies of the *SMARCB1* gene were detected, with a 1:1 ratio to the control probe (i.e., telomeric-*EWSR1* or 22q11). Heterozygous deletion was defined as only one copy of the gene of interest being present compared to the reference control probe on 22q (ratio 1:2). Homozygous deletion of *SMARCB1* was interpreted when both copies of the gene were lost, compared to the control probes, either telomeric-*EWSR1* or 22q11. A monosomy pattern (or large deletion) was defined if one allele copy of both the gene of interest and control were lost, with a ratio of 1:1.

Targeted DNA sequencing for mutational and copy number profiling

Detailed descriptions of MSK-IMPACT workflow and data analysis, a hybridization capture-based targeted DNA next-generation sequencing (NGS) assay for solid tumor were described previously[17]. All mutational and copy number calls were generated by the standard MSK-IMPACT pipeline[17]. Pathogenic (mostly loss-of-function) genetic alterations included copy number deletion, intragenic deletion, nonsense mutation, frameshift insertion/deletion, most splice site mutations, and missense mutations annotated as oncogenic or likely oncogenic by OncoKb[18]. Non-pathogenic or passenger mutations included in-frame insertion/deletion and missense mutations of unknown significance. Copy number amplification and deletion are defined as gains and losses of gene-level copy number greater than two-fold in the tumor relative to pooled FFPE normal based on NGS. Intragenic deletion denotes deletions that span one or more exons of a gene, with distinct and abrupt loss of pileup reads visualizable on integrated genome browser (IGV) but may or may not result in less than 2-fold change of gene-level copy number.

Data analysis was performed using R version 4.1.0. MSK-IMPACT data were imported using R packages “gnomeR” version 1.0.0 and “cbioportalR” version 0.0.0.9000. Mutations and gene-level copy number alterations were visualized and summarized using the R package “ComplexHeatmap” version 2.8.0[19]. Cohort-level copy number segmentation data were summarized and visualized using the R package “GenVisR” version 1.24.0[20].

Survival analysis

Survival analysis by comparison of hazard ratios using log rank *P* testing and visualization of Kaplan–Meier curves were performed using R packages “survminer” version 0.4.9 and “survival” version 3.2.13. Clinical charts were manually reviewed to document date of initial presentation, disease progression, and survival status. Median time (in years) to disease progression was defined as the time interval between initial presentation (presence of tumor seen radiographically or on physical examination) and the first instance of tumor recurrence or distant metastases after initial surgical resection and/or chemoradiation therapy with radiographically negative evidence of residual tumor.

RESULTS

Spectrum of SMARCB1 genomic alterations and correlations with various histotypes

We classified our cohort of 118 cases into: 1) *SMARCB1* pathogenic (78, 66%): soft tissue and bone entities for which SMARCB1 deficiency has been well established to be pathogenic per published studies and also had DNA sequencing data available: EPS-P (30), EPS-D (14), eMPNST (11), eSCHW (7), PD-CHO (7), MRT (3), and STME (6); 2) other soft tissue and bone tumors where *SMARCB1* genetic alterations were identified by targeted sequencing but likely represent non-pathogenic or passenger mutations, including SNV or intragenic deletions (27, 23%) and amplifications (13, 11%). Figure 1A summarizes the spectrum of *SMARCB1* genetic alterations and corresponding histotypes.

Among the 78 SMARCB1-deficient pathogenic cases, all but four (74, 95%) showed loss of SMARCB1 nuclear expression at the protein level by immunohistochemistry. The 4 cases with retained SMARCB1 were all eSCHW, with otherwise typical histologic features. Fifty-nine (79.7%) of 74 SMARCB1-deficient pathogenic cases with SMARCB1 loss harbored homozygous (biallelic) *SMARCB1* loss: mostly large homozygous deletions detected by FISH (Fig. 1B), or homozygous deletions detected by copy number profiling, and a small subset by the presence of two LOF point mutations (nonsense, frameshift, etc.) present in trans (different alleles) (2 cases) or one LOF mutation plus heterozygous deletion by FISH (2 cases).

Additionally, we found that not all SMARCB1-deficient pathogenic cases demonstrated biallelic *SMARCB1* inactivation, despite loss of SMARCB1 expression at the protein level (Fig. 1A). Ten (13.5%) of 74 SMARCB1-deficient pathogenic cases with SMARCB1 protein loss (4 EPS-P, 3 STME, 1 PD-CHO, 1 MRT, 1 eSCHW) demonstrated only heterozygous loss-of-function point mutations. Five (6.8%) of 74 SMARCB1-deficient pathogenic cases with SMARCB1 protein loss (3 EPS-D, 1 EPS-P, 1 eMPNST) showed no demonstrable *SMARCB1* genetic alterations in *SMARCB1* or genes encoding for other SWI/SNF subunits. In total, 15 (20%) out of 74 cases with SMARCB1 loss showed no demonstrable biallelic loss based on molecular testing.

The compared sensitivity between the two assays applied (DNA sequencing vs FISH) showed that among the 53 cases of SMARCB1-deficient pathogenic cases for which FISH was performed and SMARCB1 loss was proven by IHC, FISH and sequencing were concordant in 28 (48%) cases. *SMARCB1* abnormalities were not detected by FISH but

present via sequencing in 14 (27%) cases: as copy number deletion in 4 cases, as intragenic deletion in 3 cases, and as loss-of-function mutations in 7 cases. On the other hand, *SMARCB1* alterations was missed by sequencing but detected by FISH in 10 (19%) cases. In one case of eMPNST, neither sequencing nor FISH detected *SMARCB1* alterations, despite loss of SMARCB1 by IHC. Combined, when SMARCB1 was lost by IHC, the sensitivity of sequencing and FISH in detecting *SMARCB1* abnormalities was 93% (69/74).

The breakdown of *SMARCB1* alterations by tumor types in the SMARCB1-deficient pathogenic category was as follows (Table 1): epithelioid sarcomas, both EPS-P and EPS-D, most frequently harbored homozygous deletions detected by FISH and/or copy number profiling (33, 75%). Within EPS-P, 7 cases (21%) harbored heterozygous intragenic deletions or loss-of-function mutations (nonsense, frameshift). For PD-CHO, 4 (57%) of 7 cases showed homozygous deletions detected by FISH and/or copy number profiling, 2 (28%) cases showed intragenic deletion, and 1 case showed only *SMARCB1* frameshift deletion. For eMPNST, 7 (63%) of 11 cases showed homozygous deletions detected by FISH and/or copy number profiling, 2 (18%) cases showed bialleleic LOF mutations, and 1 case showed intragenic deletion. For eSCHW, 1 (14%) case showed homozygous deletion and 3 (43%) cases showed heterozygous deletion by copy number profiling, 1 showed a monoallelic nonsense mutation. For MRT, 2 (66%) of 3 cases showed homozygous deletion by copy number profiling, 1 showed a monoalleleic nonsense mutation. On the contrary, the majority of STME (5, 83%) harbored nonsense mutations in *SMARCB1* without copy number losses (monoallelic alteration), while 1 case showed copy number deletion.

Additionally, we identified a cohort of 40 heterogenous sarcoma histotypes that harbored *SMARCB1* genetic alterations, including copy number changes and heterozygous non-LOF mutations. Since SMARCB1 deficiency has not been recognized as the oncogenic driver in these histotypes, this group was considered separately from the SMARCB1 pathogenic category. This was supported by the fact that among the 15 cases tested by SMARCB1 IHC, only 1 case (synovial sarcoma) showed loss of SMARCB1. As a subset of these cases showed *SMARCB1* amplification, which has not yet been studied before, we further subdivided this cohort into *SMARCB1*-altered but nonamplified and *SMARCB1*-amplified cases. The first group included 27 cases (23% of total cohort) that harbored *SMARCB1* copy number losses as well as *SMARCB1* mutations (missense and in-frame mutation) of uncertain significance (non-LOF). These tumor types included angiosarcomas, conventional MPNST, osteosarcomas, spindle cell rhabdomyosarcomas, myxofibrosarcomas, and sarcoma not otherwise specified. The second group included 13 cases (11% of total cohort) with *SMARCB1* amplifications, and the histotypes were angiosarcoma, soft tissue leiomyosarcoma, sarcoma not otherwise specified, etc. Eight (61%) of these cases also harbored copy number amplifications at *CRKL* and *MAPK1* (genes flanking *SMARCB1* on chromosome 22q).

Co-occurring genomic alterations in SMARCB1-deficient sarcomas

Next, we investigated the types and distribution of co-occurring (secondary) genetic events in SMARCB1-deficient sarcomas (cases with SMARCB1 loss at the protein level). We focused only on recurrent events (occurring more than once) that were likely oncogenic

(per OncoKb annotations)[18]. Overall, oncogenic, recurrent co-occurring genetic events were rare in SMARCB1-deficient sarcomas: 11 (14%) out of 74 cases of SMARCB1-deficient pathogenic cases—with mostly 1–2 alterations per case. These co-occurring alterations occurred in: *TP53*, *RB1*, *CDKN2A/2B*, *ARID1A/B*, and *BRCA2*. Overall, there was no obvious segregation of specific co-occurring genetic alterations by histotypes among SMARCB1-deficient sarcomas (Fig. 2A).

For five cases of EPS-P, longitudinal sequencing was available from multiple tumors from the same patient over time; we tracked whether there were additional genomic alterations acquired in subsequent recurrence or metastases. Figure 2B illustrates the acquisition of co-occurring mutations in *RB1* and *ID3* in two cases, respectively, and copy number amplifications in *AKT2, KIT/PDGFRA*, and losses in *RB1* and *PARK2* in four cases, respectively, in the subsequent recurrent/metastatic specimen that were not identified in the primary tumor. Mutations in *ARID1B*, *TP53* and *BRCA2* were present in both the initial and subsequent samples. We did consider a potential confounder that may account for falsely negative findings: differences in % tumor purity across sequentially sequenced samples from the same patient. Fortunately, tumor purity was comparable if not higher in the primary sample than the subsequent recurrent or metastatic samples in this cohort, mostly because the primary samples were usually resection specimens while the subsequent samples were often small biopsies.

Extended 22q loss in SMARCB1-deficient sarcomas

Since *SMARCB1* is located on chromosome 22q, we examined the extent of 22q loss in SMARCB1-deficient sarcomas. Figure 3A isa copy number segmentation plot of the SMARCB1-deficient pathogenic cohort showing varying proportion of copy number losses at the genes flanking *SMARCB1* (22q11.23), from centromeric to telomeric side: *CRKL* (22q11.21), *MAPK1* (22q11.21), *CHEK2* (22q12.1), *NF2* (22q12.2), *EP300* (22q13.2), with *SMARCB1* showing the “deepest” (highest proportion with) copy number loss. Figure 3B illustrates the cases of SMARCB1-deficient sarcomas with extended 22q copy number loss beyond *SMARCB1* gene. Overall, among SMARCB1-deficient sarcomas where *SMARCB1* deletions were detected by sequencing and/or FISH, extended 22q copy number loss was seen in 9 of 25 (36%) EPS-P, 4 of 11 (36%) EPS-D,5 of 6 (83%) PD-CHO, 1 of2 (50%) MRT, 1 of 2 (50%) STME, and 1 of 7 (14%) eMPNST.

Outcome and survival

Among the various histotypes of SMARCB1-deficient sarcomas, PD-CHO and STME showed significantly worse overall (OS) and disease-free survival (DFS) compared to EPS-P and EPS-D: median OS for PD-CHO and STME were 3.00 and 1.28 years, respectively, compared to 7.25 and 8.10 years for EPS-P and EPS-D, respectively (log-rank $P = 0.013$). Median DFS for PD-CHO and STME were 0.73 and 1.01 years, respectively, compared to 2.78 and 5.48 years for EPS-P and EPS-D, respectively (log-rank $P = 0.023$) (Fig. 4A, B). The number of cases for eMPNST and MRT with survival data were too small for statistical comparison.

There was no significant impact on OS or DFS based on the presence of absence of co-occurring genetic alterations (possibly related to the low number of cases with co-occurring genetic hits), whether *SMARCB1* alterations were homozygous or heterozygous, or whether there was extended chromosome 22q deletions across all SMARCB1-deficient histotypes or within epithelioid sarcoma (Supplementary Fig. 1–3).

DISCUSSION

The mechanisms behind how *SMARCB1* biallelic inactivation within an otherwise quiet genome drives the pathogenesis of a wide spectrum of both benign and highly aggressive mesenchymal neoplasms remain unresolved. In order to gain further insight into this clinical heterogeneity, we performed genomic profiling of a large cohort of soft tissue and bone tumors, focusing on the interrelationship between the types of *SMARCB1* genetic alterations (copy number and/or mutational status), SMARCB1 protein expression, coexisting additional genomic abnormalities and histotypes.

Among our cohort of SMARCB1-deficient sarcomas, in all but 5 cases (69, 93%) showing SMARCB1 loss of expression at the protein level, *SMARCB1* copy number deletions or LOF mutations were detected by either one or both methods. However, among these 69 cases, 10 (14%) showed only monoallelic LOF mutations and 5 (7%) had no demonstrable alterations by both methods applied. The incidence and types of *SMARCB1* alterations show significant heterogeneity among histotypes. Table 1 summarizes this data from the published literature compared to the current study. Overall, for epithelioid sarcoma[8, 12, 21, 22] and PD-CHO[13, 14], our data are largely consistent with prior findings that *SMARCB1* deletions predominate. In MRT, *SMARCB1* point mutations/intragenic deletions ranged from 55–60% in somatic cases and 71% in germline cases, whereas we observed 1 of 3 MRT with a nonsense point mutation[15, 23, 24]. Interestingly, among epithelioid sarcomas, intragenic deletions or loss-of-function *SMARCB1* mutations (nonsense, frameshift, splice site) were only observed in EPS-P but not EPS-D. Also, four cases (29%) of EPS-D versus just one case (3%) of EPS-P showed no *SMARCB1* alterations despite SMARCB1 protein loss. On the other hand, for eMPNST and eSCHW, we detected a higher percentage of cases with *SMARCB1* deletions compared to point mutations/intragenic deletions compared to the study by Schaefer 2019[25]. Finally, for STME, *SMARCB1* point mutations/intragenic deletions predominate in our study (5 of 6). Although SMARCB1 deficiency has been reported in a variant of extraskeletal myxoid chondrosarcoma (EMC)[26], of the 15 cases with DNA sequencing data available at our institution, we did not identify any cases with *SMARCB1* deletions or mutations, and thus we did not include EMC in our study cohort.

We speculate that in a small subset of cases, germline LOF *SMARCB1* alterations might be missed by our MSK-IMPACT platform, which only detects somatic alterations. However, *SMARCB1* germline alterations are extremely rare outside of MRT[12]. Moreover, cases with copy number neutral loss of heterozygosity on 22q with a *SMARCB1* LOF mutation may technically appear heterozygous. Further, the relatively low frequency of intragenic deletion detected in our study compared to Sullivan et al may be due to reduced sensitivity of targeted DNA sequencing compared to the gold standard method of multiplex ligation-dependent probe amplification MLPA, which was used in their study to detect

intragenic deletion[8]. Additionally, *SMARCB1* gene expression could be perturbed at a post-transcriptional or epigenetic level. For example, studies have shown that *SMARCB1* gene expression in epithelioid sarcomas is regulated by specific microRNAs on both mRNA and protein levels, rather than promoter hypermethylation[27, 28]. Conversely, in synovial sarcomas, SMARCB1 protein is known to be reduced but not completely lost in about 70% of cases without corresponding reduction in *SMARCB1* mRNA levels[29]. This was postulated to be the result of a post-transcriptional mechanism where the SS18/SSX oncogenic fusion disrupts the SWI/SNF (BAF) complex by displacing wildtype SS18 from the BAF complex[30]. This may account for the single case of synovial sarcoma in our study that showed "loss" of SMARCB1 by IHC, which may be more accurately referred to as reduction in protein expression rather than loss.

Our study also investigated for the first time the sensitivity of FISH versus sequencing in detecting *SMARCB1* alterations in SMARCB1-deficient cases, showing concordant findings in 48% of cases for which FISH was available. FISH was used as a complementary method to detect large, arm-level copy number deletions of *SMARCB1*, which may not be reported in standard clinical workflow. Reasons that could account for the discrepant results between these two methods are likely related to the low resolution of FISH to detect copy number neutral cases, such as loss-of-function *SMARCB1* mutations or intragenic deletions (spanning one or more exons of a gene). By profiling the genes flanking *SMARCB1* on chromosome 22q, we also demonstrated that in about two-third of cases, *SMARCB1* was the only gene deleted on chromosome 22q. Thus, it is likely that FISH may not be able to detect some of these cases without extended 22q deletion. Overall, in our cohort, LOF *SMARCB1* alterations predominate. Importantly, the significant number of cases with *SMARCB1* genetic alterations in the absence of SMARCB1 loss at the protein level—mostly point mutations of uncertain significance (monoallelic missense or in-frame mutations)—suggests that the presence of a *SMARCB1* alteration detected by molecular testing does not necessarily imply a SMARCB1-deficient entity. Indeed, these *SMARCB1* mutations occurred in various sarcoma histotypes driven by known oncogenic drivers (such as fusions, gene amplifications), which typically are not associated with SMARCB1 loss. The question remains if these *SMARCB1* monoallelic LOF alterations represent either passenger mutations or are functional in the context of co-occurring driver events. Among this group, a subset (11%) of cases showed *SMARCB1* amplifications. In at least 61% of these cases, copy number amplifications were seen at genes flanking *SMARCB1* on chromosome 22q, indicating that *SMARCB1* amplification could be a bystander event due to broad copy number gains on chromosome arm 22q.

Additionally, we demonstrated that clinically significant, recurrent co-occurring (secondary) genetic events were rare in SMARCB1-deficient sarcomas of various histotypes, regardless of outcome. This extremely low mutation rate has been well documented in malignant rhabdoid tumors[15], but not in other mesenchymal neoplasms driven by the same SMARCB1 deficiency. This is analogous to fusion-driven sarcomas, which tend to harbor relatively "quiet" genome with low tumor mutation burden[31]. These infrequent co-occurring events involved genes that are commonly altered in sarcomas: *TP53*, *RB1*, *CDKN2A/B*;[32] *CDKN2A* deletions were also previously observed in epithelioid sarcomas[21]. However, in contrast to a prior study showing higher tumor mutation burden (TMB) in epithelioid

sarcomas compared to MRT, we did not observe a significantly higher TMB in epithelioid sarcomas. This could be due to less precise TMB estimates for low to moderate TMB samples for targeted, panel-based NGS compared to whole exome or whole genome sequencing[33], the latter of which was used in the aforementioned study for TMB estimation[21]. In this same study, they observed *CDKN2A/B* deletion in 2 of 7 epithelioid sarcomas; we observed *CDKN2A/B* deletion in only 1 case of epithelioid sarcoma (out of 44 cases), a frequency no higher than other SMARCB1-deficient tumors (1 of 11 eMPNST, 1 of 7 PD-CHO). Further, an aCGH study showed *CDKN2A* deletion in a case of conventional chordoma but not in poorly differentiated chordoma (one case), which only showed 22q loss (spanning *SMARCB1* locus)[34]. By contrast, a targeted NGS study reported 31% (4 of 16) of eMPNST and 20% (1 of 5) eSCHW harboring *CDKN2A* deletion or nonsense mutation[25]. On the other hand, we observed only *TP53* and *ARID1A* alterations in STME, and *RB1* alterations in epithelioid sarcoma only, in contrast to a case report of a STME showing *RB1* deletion[35]. Overall, studies on co-occurring genetic alterations in SMARCB1-deficient sarcomas are largely limited to small case series and single case reports, therefore the true incidence of co-occurring genetic alterations in these tumors remains unclear. Nevertheless, in our cohort, the presence of co-occurring genetic alterations did not correlate with worse OS or DFS. Studies have demonstrated compound heterozygous mutations in more than one BAF complex subunits and intra-complex co-dependencies between different subunits of the BAF chromatin remodeling complex, e.g., SMARCA4-ARID2, SMARCA4-ACTB, etc[2, 36]. On the MSK-IMPACT panel, the subunits of BAF complexes that were targeted are as follows: *ARID1A*, *ARID1B*, *ARID2*, *PBRM1*, *SMARCA2*, *SMARCA4*, *SMARCB1*, *SMARCD1*, *SMARCE1*. Of these, the only subunit that co-occurred with *SMARCB1* in our study was *ARID1A/B*.

Within EPS-P, longitudinal sequencing of recurrence/metastatic tumors in comparison to the initial sample showed that during tumor progression, acquisition of copy number alterations was frequent (4 of 5 cases) while mutations were less commonly acquired in subsequent recurrence/metastases (2 of 5 cases). Further, mutations in *ARID1B*, *TP53* and *BRCA2*, present in both the initial sample and subsequent recurrence/metastases, appeared to represent early genetic events, and the tumors maintained a relatively low overall tumor mutation burden even in late-stage disease. Interestingly, extended 22q copy number loss in genes flanking the *SMARCB1* locus (22q11.23) occurred in approximately one-third of epithelioid sarcomas and STME but in the majority of PD-CHO. There were no significant differences in the frequencies of coexisting mutations or extended 22q loss between EPS-P and EPS-D.

There is a paucity of large studies in the literature that specifically investigate the correlation between *SMARCB1* loss and myoepithelial tumor histology (specifically, benign *vs* malignant myoepithelial neoplasms). This is also compounded by the inconsistent nomenclature adopted in the literature, where “soft tissue myoepithelial tumor” or “myoepithelioma-like tumor” are used to signify malignant myoepithelial neoplasms[2], whereas other authors prefer the term “myoepithelial carcinoma” when referring to such neoplasms to distinguish them from benign myoepithelial tumors (i.e., myoepitheliomas)[38, 39]. Nonetheless, the limited case series and case reports published thus far, as well as the STME cases in the current study, have demonstrated that SMARCB1

loss are limited to malignant myoepithelial neoplasms[9, 12, 37–39]. For example, in a study of myoepithelioma-like tumors of the vulvar region (MLTVR), SMARCB1 IHC was lost in all 9 cases of MLTVR, but retained in all 11 cases of myoepitheliomas[37]. To our knowledge, SMARCB1 loss has not been reported in benign myoepithelial tumors[4, 37].

In summary, we demonstrated that in the majority of SMARCB1-deficient sarcomas, SMARCB1 protein loss could be accounted for by the presence of biallelic *SMARCB1* deletion or LOF mutations. Further, SMARCB1 IHC is a highly sensitive and specific surrogate for diagnostically and clinically relevant *SMARCB1* pathogenic alterations. However, a minority of cases only showed heterozygous or no detectable *SMARCB1* copy number alterations or mutations. Recurrent co-occurring genetic events are relatively rare, indicating that SMARCB1 deficiency is the primary oncogenic driver. A subset of cases also harbored extended 22q deletion. Among SMARCB1-deficient sarcomas, PD-CHO and STME showed worse prognosis than epithelioid sarcomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We gratefully acknowledge the members of the Molecular Diagnostics Service in the Department of Pathology and would like to acknowledge the Center Core grant (P30 CA008748) and the Marie-Josee and Henry R. Kravis Center for Molecular Oncology for use of MSK-IMPACT data. We acknowledge Lei Zhang for her assistance with FISH assays. We would also like to thank Dr. Sharon Weiss for generously providing us with cases of epithelioid schwannoma and epithelioid malignant peripheral nerve sheath tumor as part of the study cohort. This work was supported by P50 CA217694 (SS, WT, CRA), P30 CA008748 (SS, WT, CRA, MVO), Kristin Ann Carr Foundation (CRA).

DATA AVAILABILITY

The raw data generated are not publicly available due to lack of access to indefinite hosting capabilities, but are available from the corresponding author on reasonable request.

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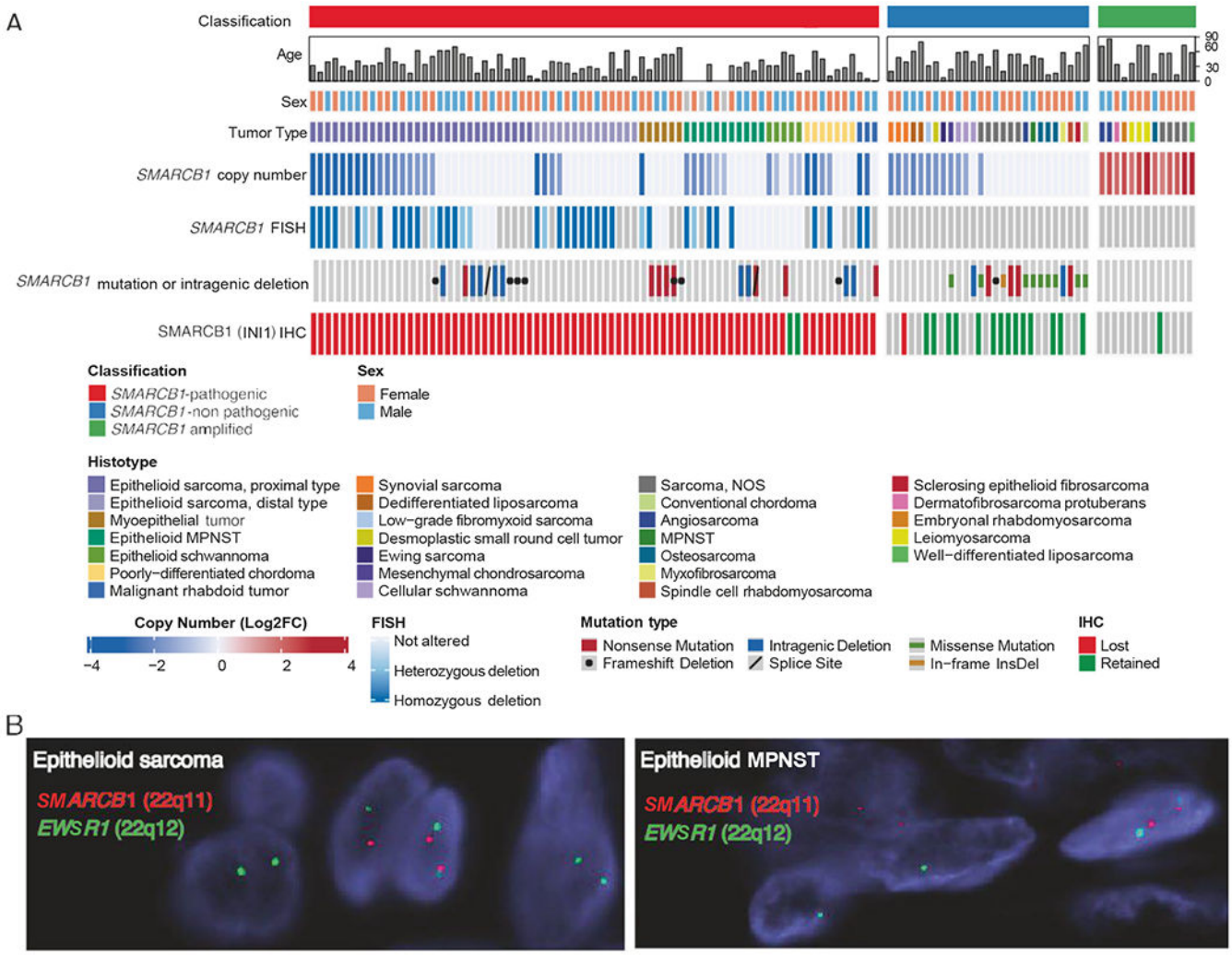

Fig. 1.

Spectrum of SMARCB1 genomic alterations and histotypes. A Oncoprint showing various soft tissue and bone tumors with *SMARCB1* copy number alterations detected by MSK-IMPACT or FISH and *SMARCB1* point mutations detected by MSK-IMPACT, their corresponding histotypes, SMARCB1 (INI1) IHC status, with patient age and sex, categorized into: SMARCB1-deficient/pathogenic sarcomas (red), *SMARCB1*-altered but likely non-pathogenic sarcomas, including *SMARCB1*-mutated or -deleted (blue) and *SMARCB1*-amplified sarcomas (green). B FISH showing homozygous deletion of *SMARCB1* (22q11, red probe) in a case of epithelioid sarcoma (left panel) and a case of epithelioid malignant peripheral nerve sheath tumor (MPNST) (right panel). *EWSR1* (22q12, green probe) serves as the reference probe. Unaffected (non-neoplastic) nuclei show 2 red signals plus 2 green signals. In affected (SMARCB1-deficient) nuclei, depending on the extent of 22q deletion, there could be 0 red signals plus 2 green signals, or 0 red signals plus 1 green signal.

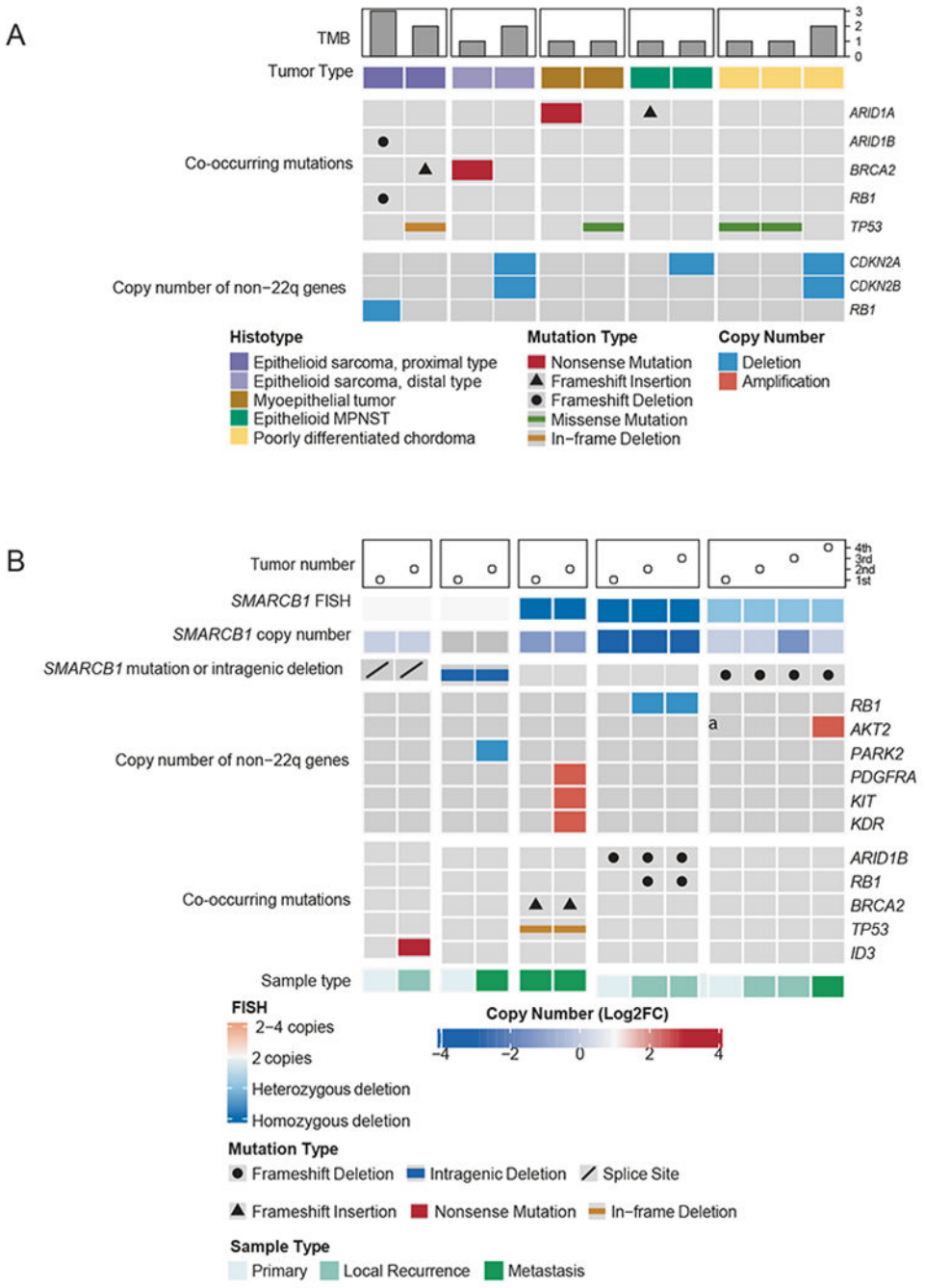

Fig. 2.

Co-occurring genomic alterations in SMARCB1-deficient sarcomas. A Oncoprint showing recurrent, clinically significant (by OncoKB annotation) co-occurring genetic alterations in SMARCB1-deficient sarcomas and their corresponding histotypes. TMB: tumor mutation burden. B Oncoprint showing acquisition of co-occurring mutations or copy number alterations in subsequent recurrence or metastases among proximal-type epithelioid sarcoma and their *SMARCB1* genetic alterations (copy number, loss-of-function mutations). This

plot only included cases with *SMARCB1* deletion detected by sequencing but not those detected by FISH.

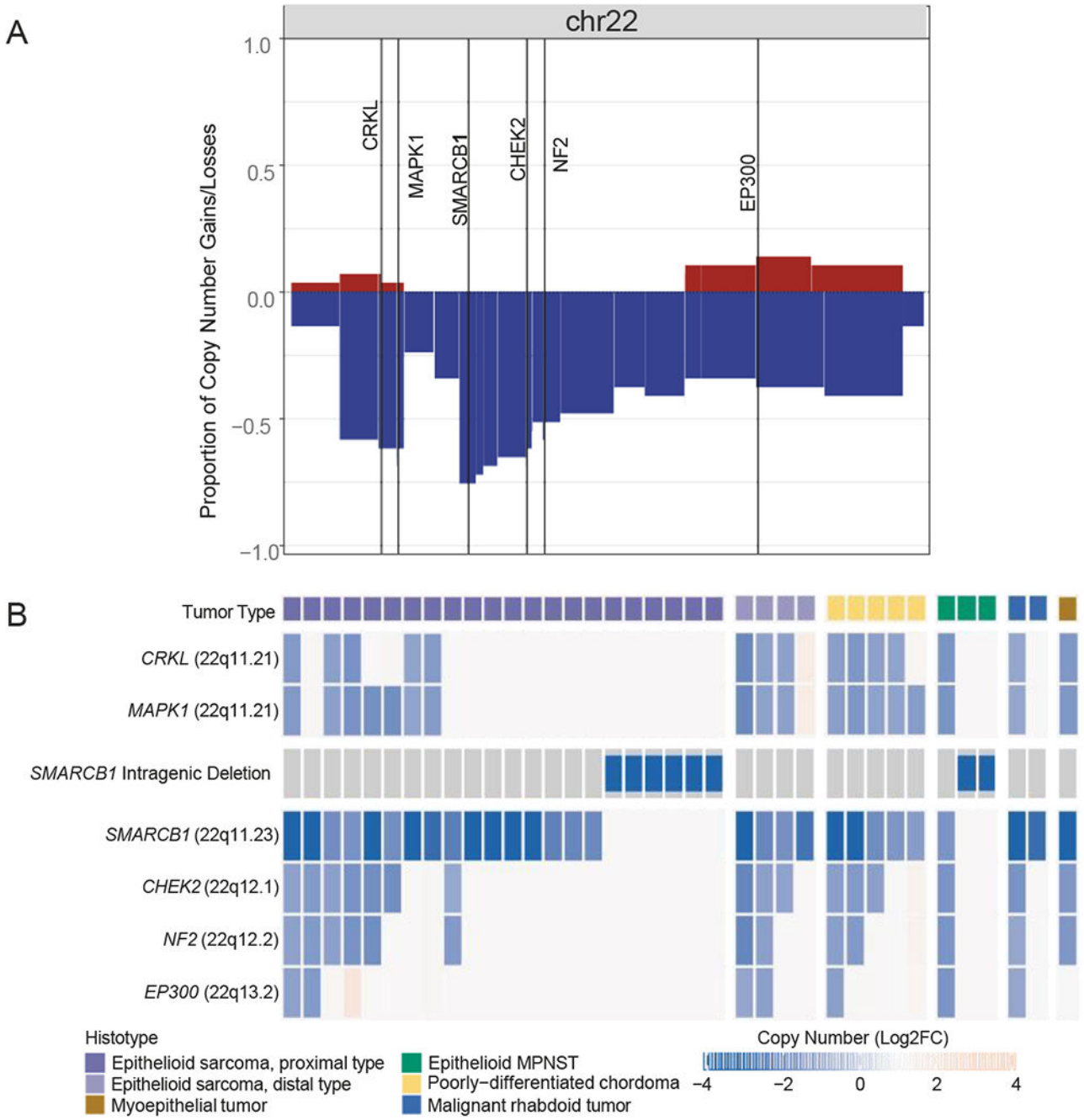

Fig. 3.

Extended 22q loss in SMARCB1-deficient sarcomas. A Cohort-level copy number segmentation plot showing proportion of copy number alterations on chromosome 22q, highlighting the genes flanking *SMARCB1* (22q11.23), from centromeric to telomeric: *CRKL* (22q11.21), *MAPK1* (22q11.21), *CHEK2* (22q12.1), *NF2* (22q12.2), *EP300* (22q13.2), among SMARCB1-deficient sarcomas. B Oncoprint showing SMARCB1-deficient sarcomas and corresponding tumor types with or without copy number alterations at the flanking genes, *CRKL*, *MAPK1*, *CHEK2*, *NF2*, and *EP300*.

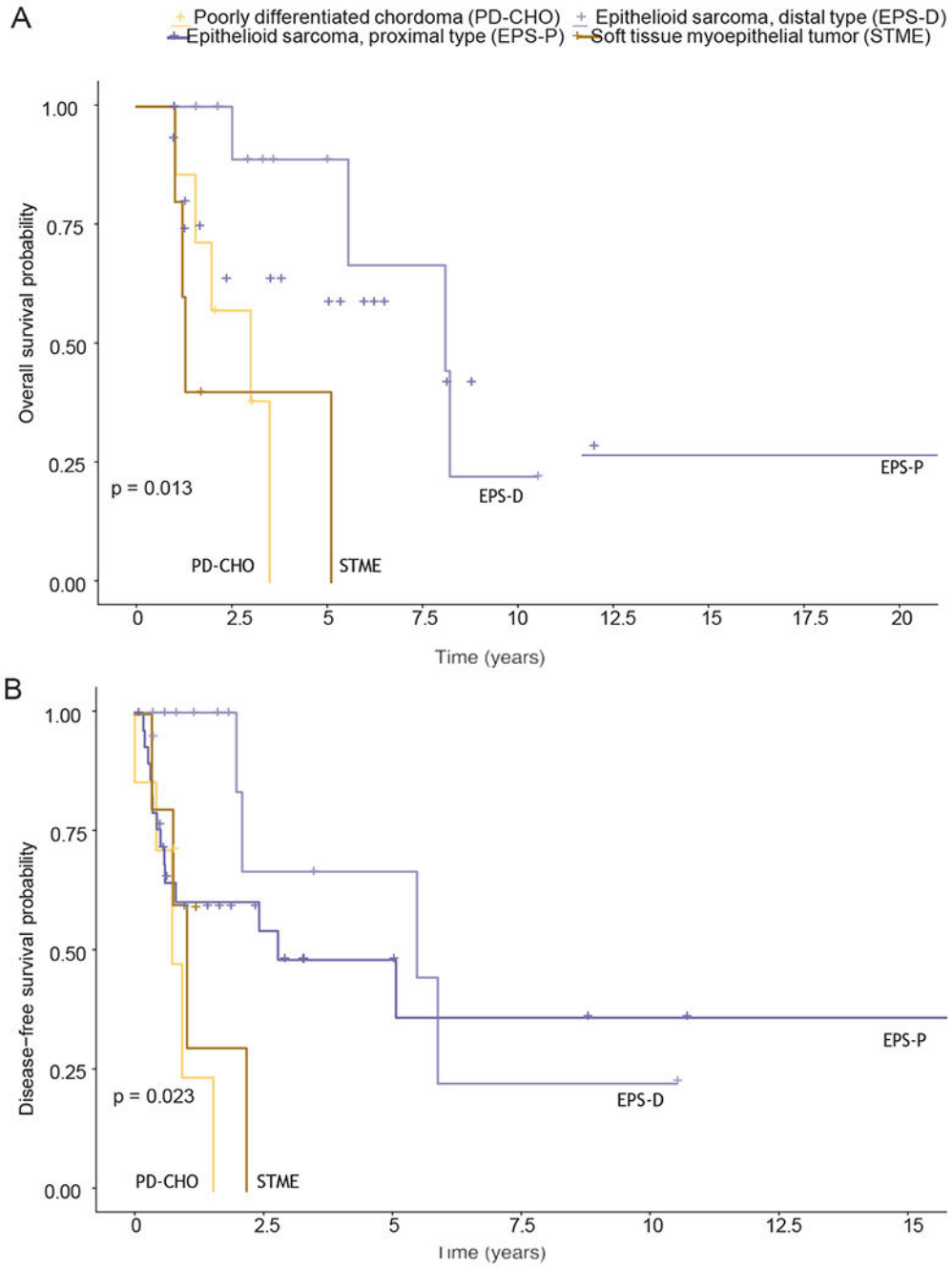

Fig. 4.

Survival of various histotypes of SMARCB1-deficient sarcomas. Kaplan Meier plots showing A overall survival and B disease-free survival of proximal-type epithelioid sarcoma ($n = 30$), distal-type epithelioid sarcoma ($n = 15$), soft tissue myoepithelial tumors ($n = 6$), and poorly differentiated chordomas ($n = 7$) [log-rank P value].

Table 1.

Incidence and types of *SMARCB1* alterations across different SMARCB -deficient histotypes in published studies compared to current study.

Tumor type	Studies		*SMARCB1* homozygous deletions	*SMARCB1* heterozygous deletions	*SMARCB1* intragenic deletions and/or mutations	Total number of cases
Epithelioid sarcoma	Published studies	Sullivan[8]	8 (67%)	2 (16%)	2 (16%) – homozygous intragenic deletions	12
		Le Loarer[12] 2014	36 (90%)			40
		Folpe[38]	3 (60%)	2 (40%)		5
		Jamshidi[21]	4 (40%)	4 (40%)		10
	Current study		33 (75%)		7 (16%)	44
Poorly differentiated chordoma	Published studies	Mobley[13]		3 (75%)		4
		Owosho[14]	8 (89)			9
	Current study		4 (57%)		3 (43%)	7
Malignant rhabdoid tumor	Published studies	Jackson[23]	24 (47%)	11 (22%)	28 (55%)	51
		Eaton[24]	3 (9%) — germline	9 (26%)-germline	25 (71%)-germline	35-germline
			26 (40%)-somatic		39 (60%)-somatic	65-somatic
		Lee[15]	25 (78%)			32
	Current study		2 (67%)		1 (33%)	3
Epithelioid malignant peripheral nerve sheath tumor	Published studies	Schaefer[25]	5 (31%)		7 (44%)	16
	Current study		7 (64%)		3 (27%)	11
Epithelioid schwannoma	Published studies	Schaefer[25]	2 (40%)		3 (60%)	5
	Current study		1 (14%)	3 (43%)	1 (14%)	7
Soft tissue myoepithelial tumor	Published studies	Le Loarer[12]	3 (60%)			5
		Folpe[38]	2 (50%)	1 (25%)	1 (25%) – homozygous intragenic deletions	3
	Current study		1 (17%)		5 (83%)	6

Mod Pathol. Author manuscript; available in PMC 2023 December 01.