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ARTICLE

Histologic and genomic features of breast cancers with alterations affecting the SWI/SNF (SMARC) genes

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Abstract

The SWI/SNF family of proteins is a multisubunit ATPase complex frequently altered in human cancer. Inactivating mutations in SWI/SNF-related matrix-associated actin-dependent regulator of chromatin (SMARCs) underpin a subset of tumors such as the malignant rhabdoid tumor and small cell carcinoma of the ovary, hypercalcemic type. Here, we investigated the genotypic and phenotypic characteristics of breast cancers harboring somatic genetic alterations affecting genes of the SMARC family. We analyzed a series of 6026 primary and metastatic breast cancers subjected to targeted-capture sequencing. SMARC core subunit (*SMARCA4*, *SMARCB1*, and *SMARCA2*) alterations were identified in <1% of all breast cancers, consisting of 27 primary and 30 recurrent/metastatic tumors. The majority of *SMARC* alterations were monoallelic mutations (47/57, 82%) and thus categorized into two groups: Class 1 alterations consisting of potentially pathogenic mutations and rearrangements and Class 2 alterations consisting of missense mutations and small in-frame deletions of unknown significance. Biallelic events in a SMARC gene were present in a minority of cases (10/57, 18%). Histologic patterns in the form of rhabdoid, composite rhabdoid, sarcomatoid or anaplastic features were observed in a subset of Class 1 primary and metastatic tumors (7/57, 12%). SMARC protein was preserved in nearly all tumors analyzed with immunohistochemistry (26/30, 87%). Four Class 1 tumors demonstrated altered SMARC protein expression in the form of loss (1/30, 3%) or mosaic pattern (3/30, 10%). Complete loss of SMARCA2 (BRM) was observed in a sole tumor with composite rhabdoid morphology, and biallelic hits in the *SMARCA2* gene. The genomic landscape of both primary Class 1 and 2 breast cancers did not reveal any characteristic findings. In summary, SMARC alterations likely contribute to the biology of a rare subset of breast cancers in the form of biallelic or pathogenic alterations in SMARC, as evidenced by SMARC-deficient phenotype or altered expression of SMARC protein.

Introduction

The switch/sucrose nonfermentable (SWI/SNF) complex is a highly conserved, ATP-dependent complex with a role in chromatin remodeling and recruitment of cofactors involved in tissue and lineage-specific gene expression [1, 2]. Mutations in any member of the SWI/SNF complex have been identified in up to 20% of all human cancers [3, 4].

A subset of tumors with alterations in the core member of the SWI/SNF complex, i.e., SWI/SNF-related matrix-associated actin-dependent regulator of chromatin (SMARCs), demonstrate strong genotypic–phenotypic correlations [5–13]. Malignant rhabdoid tumor (MRT) was the initial example of these tumors with its namesake morphology and biallelic inactivation of *SMARCB1* [5, 6]. Subsequently, biallelic S*MARCA4* mutations came to define small cell carcinoma of the ovary, hypercalcemic type [7]. At present, SWI/SNF genotype–phenotype relationships have been reported across multiple organ systems including the central nervous system, soft tissue, thoracic and the gynecologic tract [8–13]. The histologic appearance has also expanded beyond rhabdoid, as tumors harboring SWI/SNF alterations are often poorly differentiated

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and can exhibit basaloid, sarcomatoid or even anaplastic appearances [14–16]. While some tumors are driven by SMARC genetic alterations (i.e, MRT), others may acquire SMARC alterations as a potential dedifferentiation phenomenon, as shown in rare examples of high-grade endometrial cancer [14].

Immunohistochemical analysis of loss of expression of BRG1 and INI1 in tumor cells has become an important tool in recognizing SWI/SNF neoplasms [17–20]. Furthermore, identifying SWI/SNF tumors may have therapeutic relevance, as tumors with aberrant SWI/SNF proteins may be vulnerable to new chemotherapy regimens targeting the Enhancer of zeste homolog 2 (EZH2)/Polycomb Repressive Complex 2 [21, 22].

Whilst our knowledge about SMARCs in other organ systems has expanded, studies in breast cancer are limited. Although technically not a core SWI/SNF subunit, the most commonly mutated SWI/SNF component in breast cancer is *ARID1A*. *ARID1A* mutations have recently been implicated as a mediator of endocrine therapy resistance in metastatic ER-positive breast carcinoma (BC) [23]. The role of the core/ATPase SWI/SNF family members (*SMARCA4*, *SMARCA2*, and *SMARCB1*) in breast neoplasia, however, remains to be elucidated.

The purpose of our study was to determine the frequency of inactivating genetic alterations affecting SMARC genes in breast cancer and characterize their clinicopathologic characteristics, histologic features, and repertoire of genetic alterations.

Materials and methods

Cases

Following approval by our Institutional Review Board, we interrogated 3196 primary breast cancers and 2830 metastatic breast cancers previously subjected to MSK-Integrated Mutation Profiling of Actionable Targets Assay (MSK-IMPACT) [24] for alterations in the SWI/SNF complex core genes included in the MSK-IMPACT panel (*SMARCB1*, *SMARCA2*, and *SMARCA4*). We identified 27 primary and 30 recurrent/metastatic breast cancers sequenced on MSK-IMPACT platforms targeting all coding regions of up to 468 cancer-related genes. We retrieved the genetic information including non-synonymous mutations, rearrangements, amplifications and homozygous deletions using cBioPortal [25]. Clinicopathologic information for all cases including patient age, family history, BRCA1/2 status, tumor size, and hormone receptor status was obtained from the medical records.

Histologic review

All tumors with genetic alterations targeting *SMARC* core subunit genes were reviewed by four pathologists (CJS, EB, DSR, and HYW) according to the criteria put forward by the World Health Organization [26]. Given the known association of genetic alterations affecting SMARC genes with a distinctive phenotype in other organ systems [5–16], we conducted a focused analysis for the identification of rhabdoid, anaplastic and sarcomatoid morphologies. For determination of rhabdoid phenotype, tumors evaluated assessing five previously described histologic criteria: [27] (i) cytoplasmic inclusions, (ii) cytoplasm abundance, (iii) eccentric nuclei, (iv) prominent nucleoli, and (v) cellular dyscohesion. Tumors with some but not all histologic criteria were designated as composite rhabdoid.

Immunohistochemistry

Immunohistochemical assessment of BRG1 (*SMARCA4*) was performed using a Leica Bond-3 automate stainer platform (Leica, Buffalo Grove, IL). In brief, following heat-based antigen retrieval, tissue sections were incubated for 30 min with the monoclonal antibody clone G7 for BRG1 (Santa Cruz, Dallas, Texas) at a 1:250 dilution or the monoclonal antibody clone D9E8B for BRM (SMARCA2; Cell Signaling) at a 1:1000 for 30 min for 30 min. The primary antibodies were detected using the Refine Detection kit (Leica). *SMARCA4/SMARCA2*-deficient tumors and normal breast tissue/lymphocytes were used as controls. INI1 (*SMARCB1*) immunohistochemistry was performed using a BenchMark ULTRA platform (Roche, Basel, Switzerland), following antigen retrieval with a cell conditioning solution (CC1, Roche). Tissue sections were incubated with the monoclonal antibody clone 25/BAF47 for INI1 (BD Biosciences, San Jose, California) at a 1:200 dilution and detected using the OptiView DAB detection system (Roche). INI1-deficient tumors and normal breast tissue served as controls.

We classified tumors as having retained, loss or mosaic expression (i.e., partial loss observed in a subset of cells and diminished staining intensity) of proteins encoded by SMARC genes as previously described [18, 19, 28].

Grouping of SMARC mutations

The SMARC genetic alterations in the primary and metastatic tumor BC datasets were classified into two categories as previously described [29], namely (i) Class 1, including monoallelic loss-of-function mutations, i.e., truncating, frameshift, or essential splice-site mutations), hotspot

mutations or potentially pathogenic rearrangements and (ii) Class 2, including missense mutations or in-frame indels of unknown significance. A tumor was considered to harbor biallelic inactivation in a SMARC gene if a Class 1 alteration was coupled to loss of heterozygosity (LOH) of the wild type allele.

Statistical analysis

Statistical comparison of clinical parameters between groups was performed using R v1.2. Fisher's exact test was used for comparisons between categorical variables, and Student's *t* test was employed for comparisons between continuous variables. All tests were two-sided and p values < 0.05 were considered statistically significant.

Results

SMARC alterations in primary breast carcinomas

We identified 27 out of 3196 (0.8%) previously sequenced primary BCs with somatic alterations in SWI/SNF core ATPase subunits (*SMARCA4*, *SMARCA2*, and *SMARCB1*). Our cohort included 19 tumors with alterations in *SMARCA4* (70%), 7 with *SMARCB1* (26%), and 1 with *SMARCA2* (4%, Fig. 1). Tumors were then categorized into Class 1 (likely pathogenic alterations) and Class 2 (likely passenger alterations). Less than half of primary BCs (11/27, 41%) fell into the Class 1 category, with the remaining tumors classified as Class 2 (16/27, 59%). Five of 27 (19%) tumors showed biallelic hits in the form of a Class 1 alteration and concomitant LOH. Fifteen tumors (15/27, 53%) harbored missense mutations, followed by rearrangements (9/27, 32%), frameshift deletion (1/27, 4%), splice-site mutation (1/27, 4%), and in-frame deletion (1/27, 4%; Fig. 1 and detailed in Supplementary Table 1). Notably, homozygous deletions were not identified across *SMARC*-altered primary BCs.

Clinicopathologic characteristics of SMARC-altered primary BCs

Patients with Class 1 primary carcinomas (Table 1) presented at an earlier median age (52 vs. 58.5 years) and a larger median tumor size (2.0 cm vs. 1.6 cm). Patients showed similar rates of family history of breast cancer (73% vs. 64%) and *BRCA1* or *BRCA2* germline mutations (25% vs. 33%) between groups. Among primary BCs in Class 1, 36% were ER+, 18% were HER2+ and 36% were ER−HER2−. Among Class 2 tumors 62% were ER+, 13% were HER2+ and 25% were ER−HER2−. No statistically significant differences in hormone receptor subgroup were identified.

Histopathologic and immunohistochemical features of SMARC-altered primary BCs

Given the genotypic–phenotypic correlation described in SMARC-deficient/altered tumors in other anatomic sites [6–13], we sought to determine whether BCs harboring inactivating genetic alterations affecting SMARC genes would display distinct histologic features. We identified a distinctive phenotype exclusively in Class 1 tumors (6/11, 55%), comprised of rhabdoid/composite rhabdoid morphology (3/11, 18%), marked anaplastic features (2/11, 18%), and sarcomatoid features (1/11, 9%, Fig. 2A–F and Table 1). Notably, these observed phenotypes have been reported in SMARC-deficient tumors in other anatomic locations [5–16]. Biallelic hits in a respective SMARC gene were seen in 3 of 6 tumors with distinctive morphology. By contrast, all Class 2 tumors were invasive ductal carcinomas of no special type (16/16, 100% vs. 6/11, 54%, Fischer's exact test, p value < 0.05, Table 1). The majority of Class 1 and 2 carcinomas were histologic grade 3 (91% vs. 75%).

Considering the diagnostic value of antibodies directed against SMARC subunits in other organ systems [16–19], we assessed the expression of SMARC proteins in BCs harboring *SMARC* alterations by immunohistochemistry in tumors with available material. Overall, most Class 1 (8/10, 80%) and Class 2 primary BCs (9/9, 100%) displayed diffuse and strong protein expression irrespective of the underlying molecular alterations.

We identified only two Class 1 primary BCs (2/10, 20%) with SMARC genotypic-phenotype correlations. Case 5, occurring in a 73-year-old female, was an ER-positive/HER2 positive tumor with composite rhabdoid morphology and complete loss of BRM protein (Fig. 2G). The tumor had biallelic hits in the *SMARCA2* gene due to a *SMARCA2-EWSR1* rearrangement disrupting the first 28 exons (including both helicase and SNF2 domains) of the *SMARCA2* gene and concomitant LOH. The tumor was node-negative, histologic grade 3 and measured 2 cm in size. Case 9, occurring in a 37-year-old female, was an ER-/HER2- tumor with sarcomatoid morphology and partial loss/mosaic pattern of INI1 (Fig. 2H). A monoallelic *SMARCB1* rearrangement: t (2;22) (q37.3; q11.23) involving a breakpoint in Intron 1 with disruption of *SMARCB1* beyond exon 2 was present. The tumor was histologic grade 3 and measured 4.6 cm prior to neoadjuvant therapy. A complete pathologic response was observed in the tumor, without lymph node involvement.

Somatic landscape of SMARC-altered primary BCs

Next, we sought to determine whether the repertoire of genetic alterations of primary Class 1 and Class 2 BCs

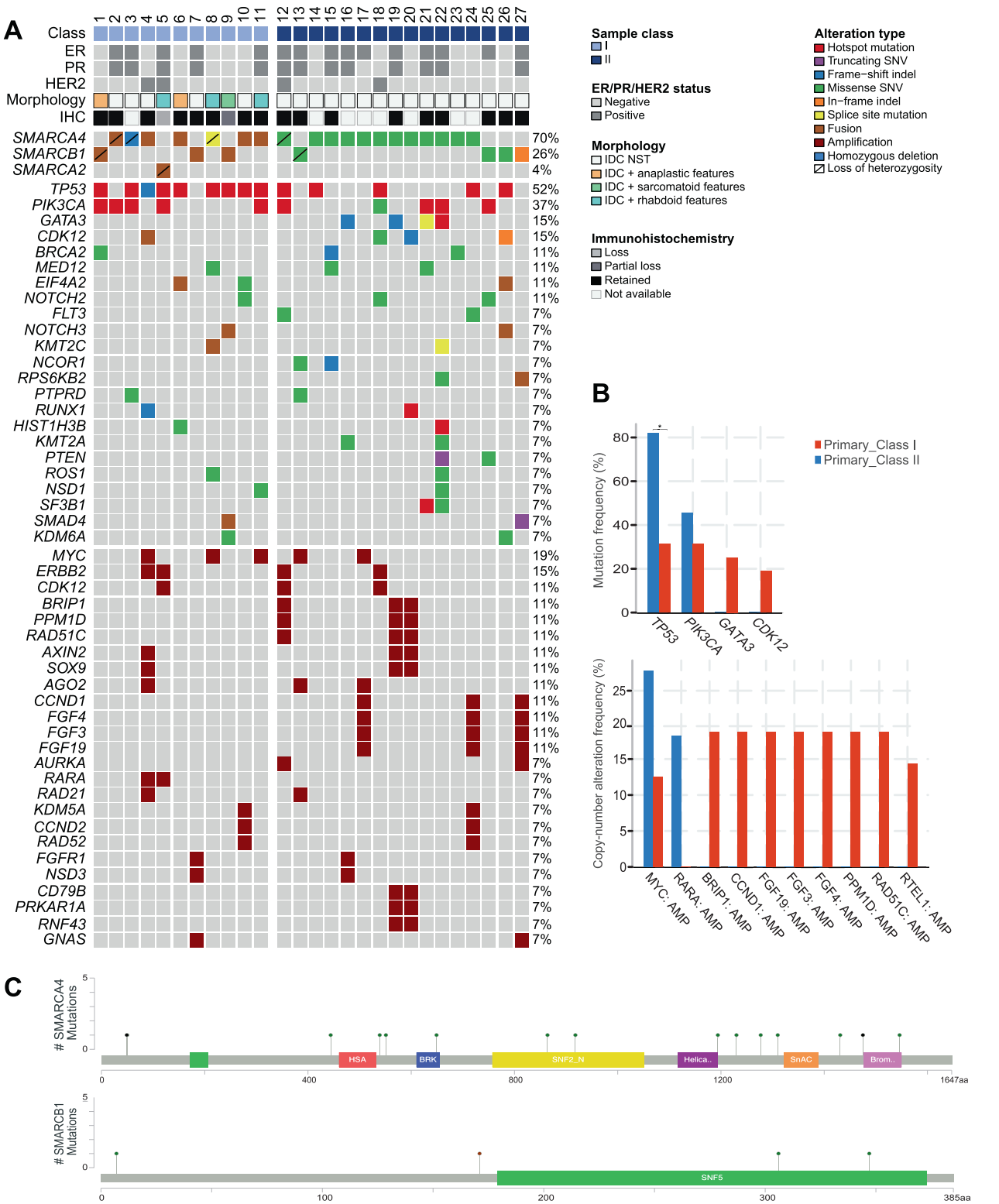

Fig. 1 Summary of primary breast cancers with *SMARC* alterations. (**A**) cases are shown in columns and genes with genetic alterations in rows. Clinicopathologic characteristics and Class designation are depicted in phenotype bars (**B**) comparison of somatic mutations and copy number alterations between Class 1 and 2 (**C**) distribution of *SMARCA4* and *SMARCB1* alterations. IDC (invasive ductal carcinoma), NST (no special type), and AMP (amplification).

Table 1 Clinicopathologic features of SMARC-altered breast carcinomas.

	Primary Class 1 SMARC Alterations ($n = 11$)	Primary Class 2 SMARC Alterations ($n = 16$)	*P* value, Fisher's Exact Test	Metastatic Class 1 SMARC Alterations ($n = 15$)	Metastatic Class 2 SMARC Alterations ($n = 15$)	*P* value, Fisher's Exact Test
Median age (range)	52 (37–73)	58.5 (37–80)		50 (31–67)	49.5 (34–76)	
Family history of breast cancer	(8/11, 73%)	(9/14, 64%)	1.0	(11/13, 87%)	(8/12, 67%)	0.38
Germline BRCA1 or 2	(2/8, 25%)	(3/9, 33%)	1.0	(1/10, 10%)	(1/9, 11%)	1.0
Median tumor size (range) (cm)	2.0 (0.8–4.6)	1.6 (0.8–6.5)				
Hormone receptors						
ER+HER2−	(4/11, 36%)	(10/16, 62%)	0.25	(7/15, 47%)	(10/15, 66%)	0.46
ER−HER2−	(5/11, 45%)	(4/16, 25%)	0.68	(6/15, 40%)	(4/15, 27%)	0.69
HER2+	(2/11, 18%)	(2/16, 13%)	1.0	(2/15, 13%)	(1/15, 7%)	1.0
Histology						
IDC-NST	(5/11, 45%)	(16/16, 100%)	<0.05	(14/15, 93%)	(15/15, 100%)	1.0
IDC with SMARC phenotype	(6/11, 55%)	(0/16, 100%)		(1/15, 7%)	(0/15, 0%)	1.0
Rhabdoid features	(3/11, 27%)			(1/15, 7%)	(0/15, 0%)	
Sarcomatoid features	(1/11, 9%)					
Anaplastic features	(2/11, 18%)					
Histologic grade						
2	(1/11, 9%)	(4/16, 25%)	0.62			
3	(10/11, 91%)	(12/16, 75%)	0.62			
SMARC protein expression						
Retained	(8/10, 80%)	(9/9, 100%)	0.47	(2/6, 33%)	(6/6, 100%)	0.45
Lost	(1/10, 10%)	(0/9, 0%)	1	(0/6, 0%)	(0/6, 0%)	1
Mosaic	(1/10, 10%)	(0/9, 0%)	1	(2/6, 33%)	(0/6, 0%)	0.45

Summary of clinicopathologic data and tumor characteristics in primary and metastatic SMARC-altered breast carcinoma.

ER Estrogen receptor, *HER2* human epidermal growth factor receptor 2, *NED* no evidence of disease, *AWD* alive with disease, *DOD* dead of disease, *IDC* invasive ductal carcinoma, *NST* no special type, *LVI* lymphovascular invasion.

differed (Fig. 1A–C). The genes most frequently affected across both groups were *TP53* and *PIK3CA*. *TP53* alterations were present in 52% of all tumors, with a higher frequency in Class 1 tumors (81% vs. 31%, Fisher's exact test, p value < 0.05, Fig. 1B). The enrichment of *TP53* alterations in Class 1 tumors are likely attributed to an imbalance of TNBC/HER2+ tumors between the groups. *PIK3CA* events were the second most common alteration, occurring in 37% of tumors with similar frequencies in Class 1 and Class 2 tumors (45% vs. 31%). Notably, the majority of Class 1 tumors showed oncogenic/likely oncogenic alterations in *TP53* (9/11, 82%, hotspot mutations ($n = 8$) or frameshift indels ($n = 1$)), *PIK3CA* (5/11, 45%, hotspot mutations), or combined *TP53/PIK3CA* alterations (4/11, 36%). Oncogenic/likely oncogenic rearrangements were present in 9% of Class 1 (1 each) tumors involving the following genes: *CDK12*, *EIF4A2*, *KMT2C*, *NOTCH3*, and SMAD4. A *RUNX1* frameshift indel was also present in a one Class 1 tumor. *GATA3* events were numerically enriched in Class 2 tumors (4/16; 25%) relative to Class 1 (0/11). No statistically significant differences in copy number alterations were observed in the two groups.

SMARC alterations in metastatic breast carcinomas

Thirty out of 2830 (1%) sequenced metastatic/recurrent BCs harbored SMARC alterations, including 26 tumors with *SMARCA4* events (87%) and 3 tumors with *SMARCB1* events (10%; Fig. 3 and Supplementary Table 2). One tumor showed combined *SMARCA4/SMARCB1* alterations (1/30, 3%). Tumors were equally divided between Class 1 and 2 groups with biallelic hits in a single SMARC gene present in 5 of 30 tumors (17%). Similar to the primary setting, homozygous deletion of a SMARC gene was not observed. Missense mutations were the most common events (14/30, 47%). The remaining events were as follows:

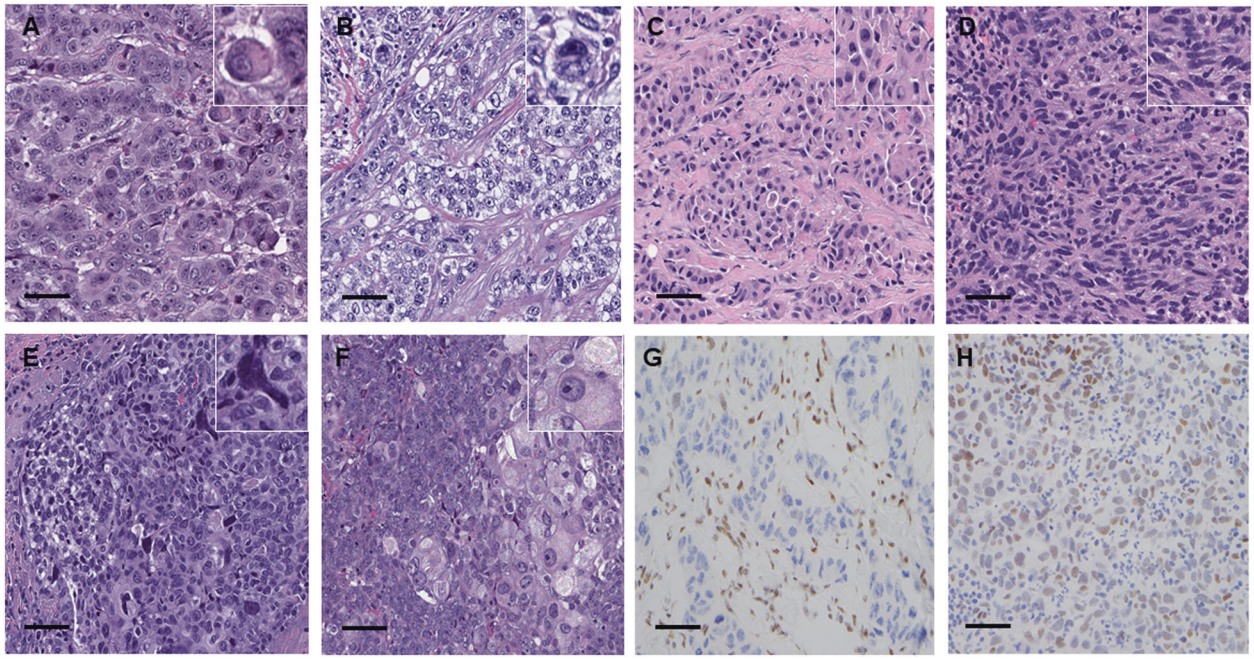

Fig. 2 Morphologic spectrum of primary Class 1 SMARC-altered tumors. (**A**) Case 5 with classic rhabdoid features including abundant cytoplasm, eccentric/prominent nucleoli and eosinophilic inclusions (**B**) Case 8 with composite rhabdoid features including round, monomorphic cells with abundant cytoplasm, prominent nucleoli and scattered rhabdoid cells (**C**) Case 11 with classic rhabdoid features including cells with abundant cytoplasm and crescentic nuclei (**D**) Case 9 with sarcomatoid features (**E**, **F**) Case 1 and 6 with marked anaplastic features. Immunohistochemical micrographs of primary Class 1 SMARC-altered tumors (**G**) Case 5 showing complete loss of BRM (*SMARCA2*; lymphocytes serving as the positive control) (**H**) Case 9 showing mosaic pattern of BRG1 (*SMARCA4*, Scale bar, 100 μm).

hotspot mutations (3/30, 10%), splice-site mutation (3/30, 10%), rearrangement (3/30, 10%), in-frame deletion (3/30, 10%), frameshift deletion (2/30, 7%), and truncating mutation (1/30, 3%). One carcinoma (3%) had combined truncating events in *SMARCA4/SMARCB1*.

Clinicopathologic features of SMARC-altered metastatic BCs

In the metastatic/recurrent cohort (Table 1), Class 1 and 2 patients presented at the same median age (50; range 31–67 vs. 49.5; 44–76). Metastatic tumors comprised the majority of the cohort (28/30, 93%), with two in breast recurrences (2/30, 7%). The most common sites of metastasis in order of frequency were bone (7/28, 25%), liver (7/28, 25%), skin/soft tissue (6/28, 21%), lymph node (5/28, 18%), and lung/pleura (3/28, 11%, Supplementary Table 2). 87% of patients with Class 1 tumors had a family history of breast cancer compared to 67% for patients with Class 2 tumors. *BRCA1* or *BRCA2* germline mutations were seen in 10% and 11% patients, respectively.

Metastatic/recurrent Class 1 tumors were ER+HER2− (7/15, 47%), HER2+ (2/15, 18%) and triple negative (6/15, 40%); Class 2 tumors were ER+HER2− (10/15, 66%), HER2+ (7%), and triple negative (4/15, 27%). Similar to the primary setting, SMARC-altered carcinomas were found across all hormone receptor subtypes despite numerical enrichment in TNBC/HER2+ subtypes in Class 1.

Histopathologic and immunohistochemical features of SMARC-altered metastatic BCs

We next examined the histologic features of metastatic tumors. Rhabdoid morphology was identified in a single Class 1 metastatic tumor (1/15, 7%) harboring a *SMARCA4* exon 13 splice-site mutation. Similar composite rhabdoid features were seen upon review of the primary tumor (Supplementary Fig. A, B). Distinct morphologic characteristics were not identified in the remainder of Class 1 and Class 2 metastatic tumors.

Despite the higher frequency of Class 1 alterations in the metastatic cohort, retention of SMARC protein was present in most tumors (10/12, 83%). Two of six Class 1 metastatic BCs exhibited a mosaic expression pattern relative to Class 2 (33% vs. 0%). Unfortunately, material was available to perform IHC in only 2 of 5 tumors harboring biallelic hits in a SMARC gene. No distinct differences in morphology, immunophenotype or genotype were seen based on the site of metastasis in our study.

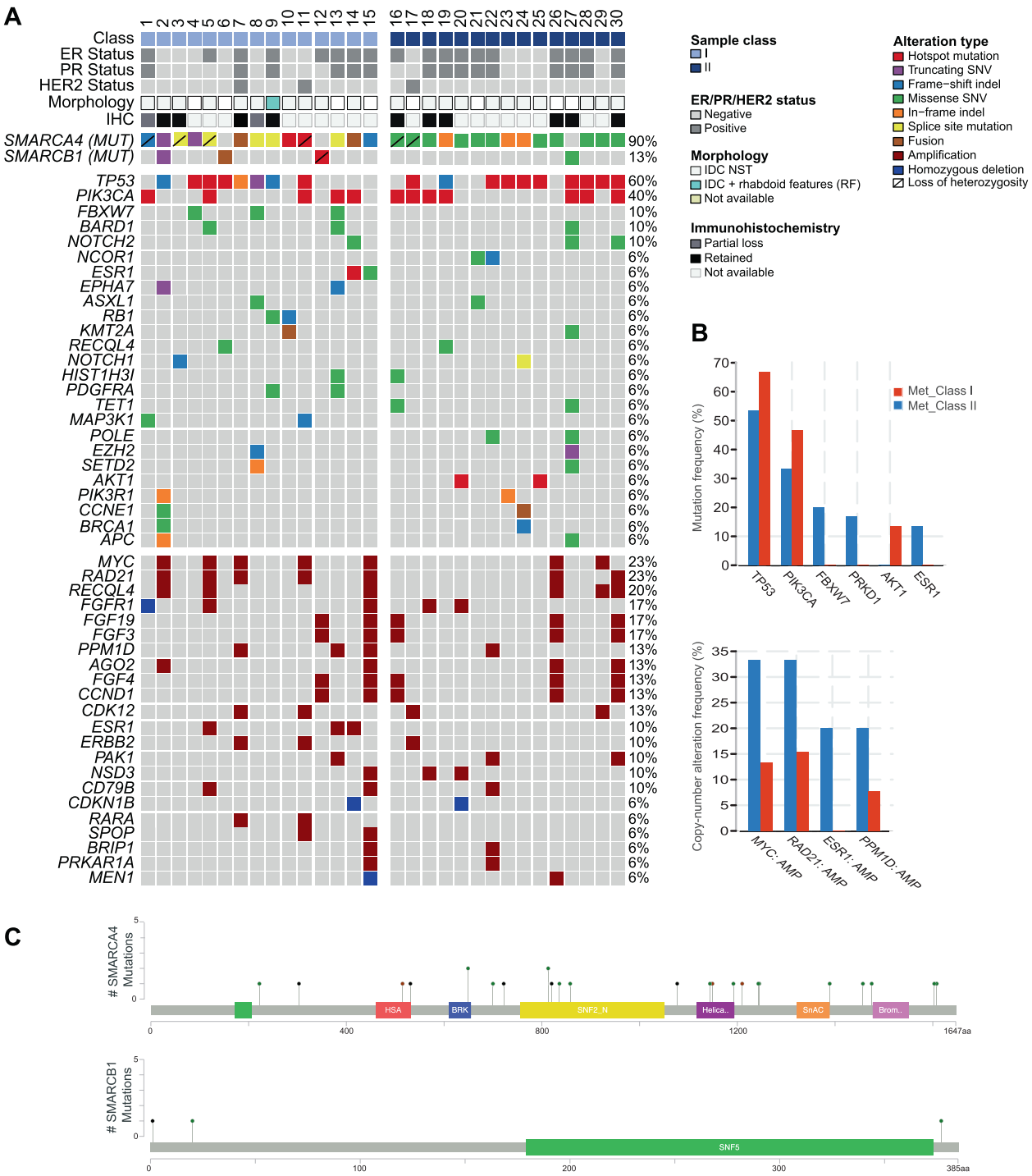

Fig. 3 Summary of metastatic breast cancers with SMARC alterations. (**A**) cases are shown in columns and genes with genetic alterations in rows. Clinicopathologic characteristics and Class designation are depicted in phenotype bars (**B**) comparison of somatic mutations and copy number alterations between Class 1 and 2 (**C**) distribution of *SMARCA4* and *SMARCB1* alterations.

Somatic Landscape of SMARC-altered metastatic BCs

As a group, metastatic BCs harboring pathogenic *SMARC* alterations had comparable somatic profiles to those observed in the primary setting (Fig. 3A–C). Mutations in driver genes *TP53* (18/30, 60%) and *PIK3CA* (12/30, 40%) were the most common events. Unlike SMARC-altered primary BCs, *TP53* alterations were more equally distributed across Class 1 and 2 tumors (8/15, 53% vs. 10/15, 67%, Fig. 3B). The rate of *PIK3CA* alteration was also similar (5/15, 33% vs. 7/15, 46%). Class 1 metastatic BCs harbored oncogenic/likely oncogenic alterations in *TP53* (8/

15, 53%, consisting of hotspot mutations ($n = 4$), frameshift indels ($n = 2$), and truncating mutations ($n = 1$)) or *PIK3CA* (5/15, 33%, hotspot mutations). Oncogenic/likely oncogenic frameshift indels were present in 6% of Class 1 tumors (1 each) in the following genes: *RB1*, *EZH2*, *MAP3K1*, and *NOTCH1*. A *KMT2C* rearrangement was present in one case. *EPHA7* alterations were seen in 2 of 15 cases (13%) in the form of frameshift indel and truncating mutation, respectively. Notably, alterations in *ARID1A*, the most commonly mutated SMARC component in metastatic BC, were uncommon in the entire cohort (2/30, 6%, data not shown). No significant copy number alterations were identified between the two classes.

Discussion

In the present study, we have found that core SMARC genetic alterations are vanishingly rare in both primary and metastatic breast cancer, the majority comprised of monoallelic or passenger alterations. The finding is further reflected in The Cancer Genome Atlas, as SMARC core subunit alterations (*SMARCA4*, *SMARCB1*, and *SMARCA2*) were seen in 2% of all BCs (10/507) with only 0.4% (2/507) of alterations falling into the Class 1 category [30]. Only a subset of pathogenic mono or biallelic events (Class 1) resulted in phenotypic characteristics of SMARC-deficient tumors reported at other anatomic locations [5–16]. Taken together, these observations are consistent with the notion that other than *ARID1A*, which can be a mediator of resistance to endocrine therapy in ER-positive BCs, other SMARC genes are rarely mutated in this disease, and that the phenotypic features consistent with SMARC loss of function were restricted to a subset of tumors with loss-of-function mutations.

We identified high-grade BCs with a SMARC phenotype (including six primary tumors and one metastatic tumor) in the form of rhabdoid, composite rhabdoid, sarcomatoid, or anaplastic morphologies. All tumors fell into the Class 1 category with three tumors harboring biallelic hits in a SMARC gene, two of which showed altered IHC expression of BRM and mosaic expression of BRG1, respectively. A single tumor with composite rhabdoid features demonstrated complete BRM protein loss, and derived biallelic hits consisting of a *SMARCA2* rearrangement coupled with LOH. We did not identify the same morphologic spectrum in Class 2 tumors, consistent with the notion that these alterations were mere passenger mutations.

We observed three Class 1 BCs (including one primary tumor and two metastatic tumors) showing a mosaic (partial loss of expression) immunohistochemical pattern. This is in contrast to a recent study of 212 primary lung cancers with pathogenic *SMARCA4* alterations, in which 80% of tumors showed loss of BRG1 protein by IHC [28]. The mosaic staining pattern we observed was more akin to that seen in synovial sarcoma, as *SMARCB1*-altered tumors tend to show weak or diminished staining for INI1 protein [28, 31]. At least in BC, irrespective of *SMARC* alteration, the great majority of tumors expressed SMARC protein in a diffuse and strong manner. We surmise our findings are the result of few tumors harboring biallelic events in a SMARC gene (10/57) with limited material to perform IHC in these tumors (6/10).

Importantly, our study shows that many tumors with SMARC alterations do not show a second hit, consistent with our findings of altered SMARC expression in few cases. Hence, only a small minority of SMARC-altered tumors might have SWI/SNF dysfunction. This finding has possible therapeutic implications, given that EZH2 inhibitors are dependent on the loss of core SMARC proteins, which in turn upregulates EZH2 [20, 21]. EZH2 overactivation has been shown to be an important driver in both cancer initiation and metastasis [32].

Our study has important limitations. First, our analysis was limited to the SMARC genes included in the MSK-IMPACT panel. Moreover, the small size of our cohort precluded the assessment of SMARC alteration on clinical outcome. Despite these points, we provide an in-depth exploration of SMARC alterations in breast cancer.

In conclusion, the SMARC genes we have investigated likely contribute to the biology of a small minority of breast cancers, but only in exceedingly rare cancers with pathogenic alterations in SMARC did we observe altered protein expression or tumors with a SMARC-deficient phenotype.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions CJS and HYW conceived the work. CJS, FP, EDS, EB, and DSR analyzed the data. CJS, FP, EB, and HYW drafted the paper. HYW, EB, BW, and JRF supervised the work. All the authors read and approved the final version of the paper.

Compliance with ethical standards

Conflict of interest JSR-F reports receiving personal/consultancy fees from Goldman Sachs, REPARE Therapeutics and Paige.AI, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis, Genentech and InVicro, outside the scope of this study. All other authors declare no competing interests.

Ethics approval and consent to participate The retrospective data for the paper was derived from the MSK-IMPACT or MSK-ACCESS clinical sequencing data. The study was reviewed and approved by the 12-245 Data and Tissue Utilization Committee.

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