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ARTICLE

RNA expression profiling reveals PRAME, a potential immunotherapy target, is frequently expressed in solitary fibrous tumors

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Abstract

Solitary fibrous tumors are a type of translocation-associated sarcoma with up to 30% rates of metastasis and poor response to conventional chemotherapy. Other translocation-associated sarcomas have been shown to display elevated expression of various cancer-testis antigens which may render them susceptible to immunotherapy strategies such as cancer vaccines and adoptive T-cell therapy. After an RNA sequencing assay brought the cancer-testis antigen *Preferentially Expressed Antigen In Melanoma* (*PRAME*) to our attention as possibly being upregulated in aggressive *TERT* promoter-mutated solitary fibrous tumors, we used tissue microarrays to asses PRAME expression in a large series of previously characterized solitary fibrous tumors, with correlation to various clinicopathologic features, as well as with tumor-infiltrating macrophages and the associated signal regulatory protein α (SIRPα)-CD47 regulatory checkpoint. We found that PRAME was expressed in 165/180 solitary fibrous tumors, with high expression seen in 58%, irrespective of *TERT* promoter status. Elevated PRAME expression was more frequent in primary intrathoracic solitary fibrous tumors and correlated with older age at primary diagnosis. Elevated PRAME was also associated with features suggestive of immune evasion, including lower numbers of antigen-presenting CD163+ and CD68+ macrophages, and expression of the "don't eat me" receptor CD47 on tumor cells. Taken together, these features suggest that strategies targeting PRAME with or without concomitant SIRPα-CD47 axis inhibition may represent a potential future therapeutic option in aggressive solitary fibrous tumor.

Introduction

Solitary fibrous tumors are indolent translocation-associated sarcomas with reported metastatic rates of 10–30% [1–4]. While the majority of patients are cured by primary surgical resection, there are few good systemic therapeutic options for those patients with tumors that do go on to metastasize. Conventional cytotoxic chemotherapy is relatively ineffective, and although strategies targeting angiogenesis in these vasculature-rich tumors showed some early promise [5, 6], a more recent solitary fibrous tumor-specific phase 2 trial with pazopanib showed variable efficacy [7–10], with ~50% partial response as the best reported outcome [7].

Currently, the underlying molecular biology leading to aggressive behavior in solitary fibrous tumor is poorly understood. While some have suggested that the specific exon fusion types of the pathognomonic *NGFI-A-binding protein 2—Signal transducer and activator of transcription 6* (*NAB2-STAT6*) gene fusion might affect tumor behavior [11], this finding has not been validated in subsequent series [12, 13]. We and others have shown that *Telomerase*

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Reverse Transcriptase (*TERT*) promoter mutations are associated with aggressive behavior in solitary fibrous tumor [14–16], and *TP53* has also been shown to be mutated in some aggressive tumors [16–18]. However, the mechanism by which *TERT* promoter mutations promote aggressive behavior in solitary fibrous tumor is not defined. Studies have suggested that TERT overexpression in cancer is associated with an increase in cancer stem cells [19], which have different gene expression profiles compared to more differentiated cells [20–22].

In order to better understand the potential effects of *TERT* promoter mutations on signalling pathways and differentiation in solitary fibrous tumors, we compared the gene expression profiles of solitary fibrous tumors with wild-type *TERT* promoters to those with *TERT* promoter mutations.

Upon identification of the potentially therapeutically targetable cancer-testis antigen *PRAME*, also known as *Preferentially Expressed in Melanoma antigen*, as the most highly overexpressed gene in *TERT*-promoter mutated solitary fibrous tumors, we performed further studies to investigate expression at the protein level in solitary fibrous tumors. Further, as expression of cancer-testis antigens is associated with mechanisms to evade immune detection, we investigated the correlation between PRAME expression with the presence of antigen-presenting cells and CD47 (anti-phagocytotic cell surface marker of self) together with its receptor SIRPα.

Materials and methods

Ethics approval and specimen acquisition

All studies were conducted after approval by the appropriate institutional ethics boards (Mount Sinai Hospital REB 17-0223-E). Fresh frozen solitary fibrous tumor tissues (Cohort A) were retrieved from the musculoskeletal oncology biorepository, and corresponding formalin-fixed paraffin-embedded blocks and slides were retrieved from the pathology archives of Mount Sinai Hospital, Toronto, ON. Clinicopathologic data and tissue microarrays from solitary fibrous tumor specimens from our previously published series (Cohort B) [1, 24–26] were utilized for protein analysis and clinicopathologic correlative studies.

RNA sequencing (Cohort A)

mRNA was extracted from eight fresh frozen solitary fibrous tumor specimens using the RNAeasy minikit (74104; Qiagen). RNA sequencing was performed by The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada using an Illumina HiSeq2500 platform and paired-end reads (125 bp) after library preparation using the NEBNext Ultra II Directional RNA Library prep kit for Illumina (E7760; New England Biolabs). Reads were aligned to reference genome gencode_v19, downloaded from https://data.broadinstitute.org/Trinity/CTAT_RESOURCE_LIB/. Reads were generated in FASTQ format and quality assessed using FASTQC v.0.11.2 (RRID:SCR_014583), and adaptors trimmed using TRIM Galore (RRID:SCR_011847). *NAB2-STAT6* gene fusions were detected using the STAR-Fusion pipeline [27]. To assess read distribution, positional read duplication, and confirm read strandedness, the RSeQC package was used [28]. STAR alignments were processed to extract raw read counts for genes using htseq-count v.0.6.1 (HTSeq) [29].

DNA sequencing

TERT promoter mutation testing on formalin-fixed paraffin-embedded solitary fibrous tumor tissues from Cohort B was previously reported [14]. TERT promoter mutation testing on 8 frozen solitary fibrous tumor specimens from Cohort A was performed using a similar protocol, as follows: Total cellular DNA was extracted from fresh frozen solitary fibrous tumor tissues using the DNAeasy isolation kit (69504; Qiagen) according to the manufacturer's protocol. The *TERT* promoter amplicon of 163 bp spanning hot spot mutations at positions 1,295,228 and 1,295,260 on chromosome 5 was amplified using forward primer 5′ CAGCGCTGCCTGAAACTC and the reverse primer 5′ GTCCTGCCCCTTCACCTT23 and the Amplitaq gold 360 polymerase chain reaction (PCR) master mix (4398876; ThermoFisher). PCR was performed on 100 ng DNA in a total volume of 25 µl, with initial denaturation at 95 °C for 7 min, followed by 45 cycles with denaturation at 95 °C for 30 s, annealing at 62 °C for 25 s, and extension at 72 °C for 1 min. The amplification product was purified using the QIAquick PCR clean-up kit (28104; Qiagen) according to the manufacturers' protocol. Bidirectional Sanger sequencing was performed by ACGT Corporation, Toronto, Canada using an Applied BioSystems/Life Technologies 3730xl capillary electrophoresis DNA sequencer (3730 S; ThermoFisher).

NAB2-STAT6 fusion identification by RT-PCR (Cohort B)

mRNA was extracted from formalin-fixed paraffin-embedded tissues and subjected to real-time PCR for selected common *NAB2-STAT6* fusion types at the qPCR CoRE, Icahn School of Medicine at Mount Sinai, New York, USA. In brief, RNA was isolated from 10 µm formalin-fixed paraffin-embedded tissue curls (4/case), using Invitrogen Pure link FFPE kit (K156002; ThermoFisher) following the

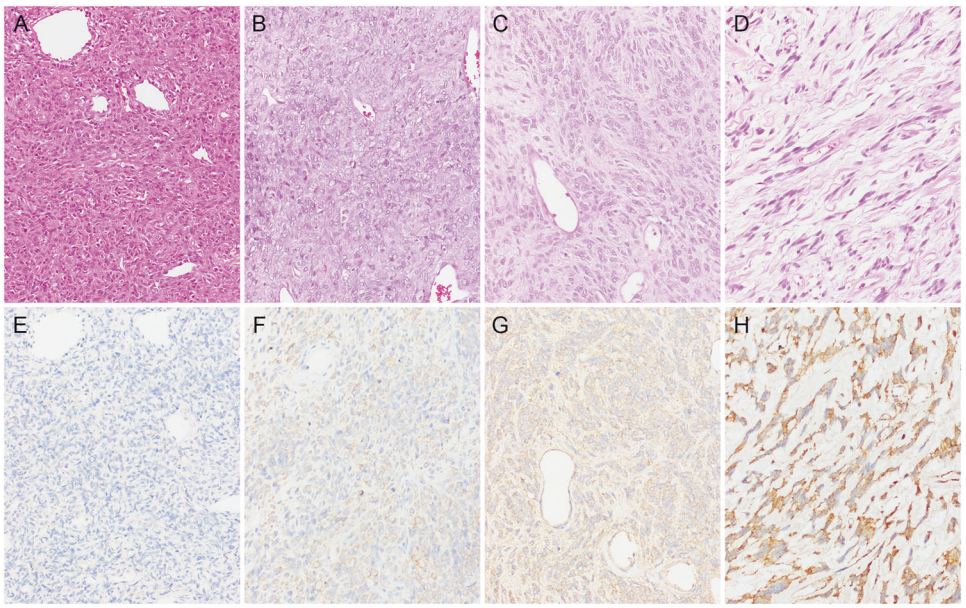

Fig. 1 Scoring of PRAME immunohistochemistry in solitary fibrous tumors with corresponding H&E sections. A&E. No labeling. B&F. Weak labeling. C&G. Moderate labeling. D&H. Strong labeling.

manufacturer's protocol. cDNA was synthesized from total RNA with AffinityScript™ Multi-Temp RT (600105; Agilent) with oligo dT18 as primer. Previously published primers for NAB2 exons 4, 6, and 7 and STAT 6 exon 2, 16, and 17 were utilized to detect the following NAB2-STAT6 fusion types (4–2, 6–17, 7–17, 6–16, 7–16, 7–2) [11], and amplification performed using a real-time reverse transcriptase PCR. For real-time PCR PlatinumTaq DNA polymerase (10966026; ThermoFisher) and a SYBR green (S7563; ThermoFisher) containing buffer were used. Real-time PCR was performed using a thermocycler (ABI7900HT; Applied Biosystems). The PCR conditions used were: 95 °C for 2 min, 40 cycles of 95 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s. The RNA levels for the house keeping gene β-actin were also assayed in all samples as an internal control.

Tissue microarrays (Cohort B)

The construction and clinicopathologic features of the two solitary fibrous tumor tissue microarrays used in this study have been previously described [24, 30]. In brief, the two arrays comprise a total of 209 solitary fibrous tumor from 178 patients, including 142 primary, 50 metastastic, 13 locally recurrent tumors, and 4 of unknown status.

Immunohistochemistry

Immunohistochemistry for PRAME on tissue microarrays from Cohort B and whole tissue sections from Cohort A was conducted using anti-PRAME antibody (clone H10, 1:150, Santa Cruz Biotechnology) on a Leica Bond Rx autostainer (Leica Biosystems). Immunohistochemical studies were optimized using normal testicle which also was used as tissue control when performing the assays. PRAME expression was scored as 0 negative; 1+ weak cytoplasmic intensity, 2+ moderate cytoplasmic staining intensity, and 3+ strong cytoplasmic staining intensity (Fig. 1). The extent of immunohistochemical staining, when present, was diffuse. Immunohistochemical studies for CD163, CD68, signal regulatory protein α (SIRPα), and CD47 performed on one tissue microarray were previously reported [31].

Statistical analysis

Differential gene expression between TERT promoter wild type and mutant solitary fibrous tumors was analysed using the edgeR R package v.3.22.3 (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html) [32]. The data set was filtered to retain only genes with fragments per kilobase million (FPKM) > 2 in at least 2 samples. The method used for normalizing the data was trimmed mean of M values (TMM), implemented by the calcNormFactors(y) function. All samples were normalized together. The exact test functionality in edgeR was used for the differential expression test.

Receiver operating characteristic (ROC) curve analysis was performed to identify a correlation between the average immunohistochemical staining intensity of PRAME and *TERT* promoter mutation status and identify if an optimal "strong" staining cut-off existed. Correlations between clinicopathologic variables and PRAME staining were performed using a cut-off of 2+ average PRAME staining intensity, which was defined as "high". Fisher's exact test was utilized for categorical variables (e.g. site, tumor status), or the Mann–Whitney non-parametric test for

continuous variables (e.g., patient age, tumor size, mitotic count). The Mann–Whitney test was also used to compare the density of CD163+ or CD68+ macrophages and strong PRAME expression, while Fisher's exact test was used to compare positive/negative SIRPα or CD47 expression in PRAME low- or high- expressing solitary fibrous tumors. Kaplan–Meier analysis was used to asses for an association between PRAME expression and overall survival, disease-specific death, and the incidence of first metastasis. For all comparisons based on PRAME immunohistochemical staining, an alpha of <0.05 was considered significant.

Results

Identification of PRAME in solitary fibrous tumor

We performed RNA sequencing on eight available fresh frozen solitary fibrous tumor samples (Cohort A) as part of an initial exploratory analysis investigating gene expression and mutations correlating with *TERT* promoter mutation status. There were 3 cases with hotspot *TERT* promoter mutations (C228T) and 5 cases with wild type TERT. Comparison of gene expression data between *TERT* promoter mutant and *TERT* promoter wild-type cases revealed 103 genes with differential expression (FDR < 0.05; Supplemental Table 1). Among these, the most highly overexpressed gene in *TERT* promoter mutant solitary fibrous tumors relative to those with wild-type TERT was *PRAME* (LogFC −8.73, FDR = 0.037). Immunohistochemical study for PRAME on formalin-fixed paraffin-embedded whole tissue sections from the eight tumors showed absent expression in four cases and diffuse weak (1+) cytoplasmic expression in the other 4. There was no observable correlation between PRAME immunohistochemistry and either *PRAME* mRNA expression or *TERT* promoter mutation status, although interpretation was limited by the small sample size.

Given the small size of this exploratory data set, and the uncertain correlation between mRNA and protein expression, we wished to further characterize PRAME expression in solitary fibrous tumors using immunohistochemistry on existing solitary fibrous tumor tissue microarrays (Cohort B). We found that PRAME was frequently expressed in solitary fibrous tumors, with 165/180 (92%) successfully scored cases showing at least weak expression and 105/180 (58%) cases showing high expression (2+ to 3+).

Clinicopathologic correlates of PRAME overexpression in solitary fibrous tumor

We previously reported the presence of 29% *TERT* mutations in the 209 solitary fibrous tumors in Cohort B [14], and among 180 cases with data on PRAME staining, there were 99 with wild-type *TERT* promoters, and 43 with *TERT* promoter mutations. *TERT* promoter mutation status was not available in the remaining 38 cases. Although our initial mRNA data suggested that overexpression of *PRAME* might correlate with the presence of *TERT* promoter mutations, we found no correlation between average PRAME immunohistochemical staining intensity and the presence of *TERT* promoter mutations (ROC analysis, area under curve = 0.56, data not shown).

In other sarcomas such as liposarcoma, high PRAME expression is associated with high tumor grade, worse prognosis and advanced disease [33, 34]. We found that high PRAME expression was frequent in metastatic solitary fibrous tumors (31/44, 70%) compared to primary solitary fibrous tumors (67/121, 55%) or locally recurrent tumors (6/13, 46%). However, this difference did not reach statistical significance ($p = 0.15$; Table 1).

PRAME expression varied by the tumor site of origin with 37/47 (79%) primary intrathoracic solitary fibrous tumors showing at least 2+ PRAME expression, compared to 30/74 (41%) solitary fibrous tumors of all other sites combined ($p < 0.0001$). Likewise, PRAME expression in

Table 1 Clinicopathologic correlates of PRAME expression across all solitary fibrous tumors.

	PRAME low	PRAME high	*P* value
Tumor status (*n*)			0.15
Primary	54	67	
Local recurrence	7	6	
Metastasis	13	31	
Mitotic figures (median /10 HPF)	2	3	0.27
Necrosis (*n*)			0.057
Absent	54	61	
Present	20	43	
Nuclear pleomorphism (*n*)			0.24
Low	27	49	
Moderate	36	44	
High	8	20	
Tumor cellularity (*n*)			0.36
Low	3	8	
Moderate	27	30	
High	41	65	
NAB2-STAT6-fusion type			**0.037**
Exon 4–2	9	27	(4–2 vs all others)
Exon 6–16/6–17	8	12	
Other/unknown	41	46	
Not tested	17	20	

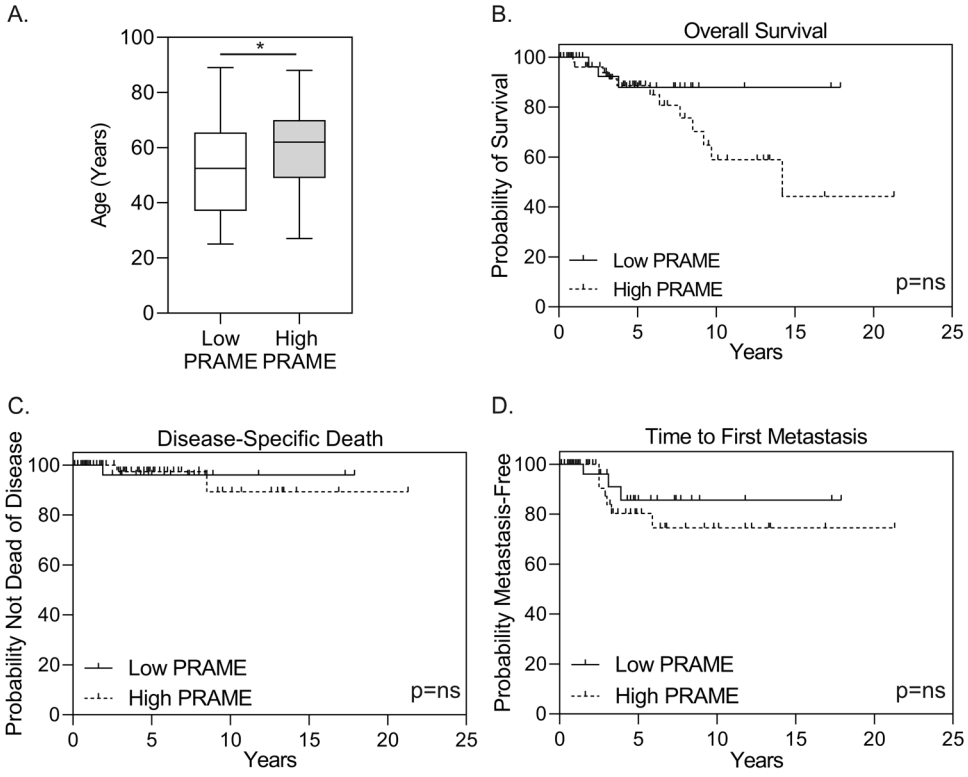

Fig. 2 Clinical correlates of high PRAME expression. **a** Correlation of high PRAME with patient age at primary tumor diagnosis. **b** Overall survival by PRAME status. **c** Death from disease by PRAME status. **d** Incidence of first metastasis by PRAME status. *$p < 0.05$, ns not significant ($p > 0.4$).

primary tumors correlated with older age at diagnosis, with a median age of 62 years for primary tumors with 2+ PRAME expression, compared to 53 years for tumors from patients with low PRAME expression ($p = 0.01$; Fig. 2a).

There was no significant correlation between PRAME expression and tumor necrosis (68% vs 53%, $p = 0.06$), patient sex, mitotic count, tumor cellularity, nuclear pleomorphism, primary tumor size, or primary tumor metastatic risk score (Table 2).

Prognostic implications of elevated PRAME in solitary fibrous tumor

Because expression of cancer-testis antigens has been reported to be a risk factor for worse oncologic outcomes in other translocation-associated sarcomas such as myxoid liposarcoma and synovial sarcoma, we investigated whether PRAME expression might also have prognostic implications in solitary fibrous tumors. However, we found no significant differences in time to first metastasis, overall survival, or disease-specific survival between patients with high or low PRAME expression in primary solitary fibrous tumor (Fig. 2b–d).

Tumor infiltrating macrophages and PRAME

As expression of cancer-testis antigens may be immunogenic, unless tumors employ strategies to escape immune detection, we investigated the density of tumor infiltrating CD68+ and CD163+ macrophages as surrogates for

Table 2 Clinicopathological correlates of PRAME expression in primary solitary fibrous tumors.

	PRAME low	PRAME high	*P* value
Primary site (*n*)			**<0.0001** (intrathoracic vs all other sites)
Intrathoracic	10	37	
Abdomen	10	10	
Extremities	10	8	
Meninges	10	5	
Trunk	6	5	
Head and neck	8	2	
Patient age (median, years)	53	62	**0.016**
Tumor size (median, cm)	7.0	8.7	0.48
Patient sex (*n*)			0.21
F	29	28	
M	25	39	
Risk score (Demicco 2017) (*n*)			1
Low	21	31	
Intermediate	11	18	
High	7	11	

overall antigen presenting cell density in a subset of cases from Cohort B [31]. We found that the overall density of both CD68+ macrophages/mm^2 and CD163+ macrophages/mm^2 was decreased in tumors with high vs low PRAME expression respectively (CD68 median density 84 vs 215, $p = 0.0048$; CD163 median density 60 vs 142, $p = 0.021$; Fig. 3a, b). Moreover, tumors with elevated PRAME expression were also more frequently positive for CD47, an immune checkpoint regulator which acts as a "don't eat me signal" for macrophages, compared to tumors with low PRAME expression (62% vs 35%, $p = 0.034$; Fig. 3c). There was no correlation between PRAME expression and SIRPα expression on tumor-infiltrating macrophages (Fig. 3d).

Discussion

TERT promoter mutations have been associated with aggressive behavior in solitary fibrous tumors and other solid tumors, including melanoma [35, 36] and various carcinomas such as urothelial carcinoma [37]. Even though *TERT* promoter mutations are thought to promote carcinogenesis via telomere length maintenance and telomerase reactivation [38], we previously found no difference in telomere length between solitary fibrous tumor with and without *TERT* promoter mutations [14]. However, other studies showed that TERT can promote cancer stemness, invasion, and metastasis by alternative mechanisms [19]. For example, in gastric cancer models, TERT overexpression was associated with increased invasiveness in vitro and colonization in vivo; effects that were independent of telomere lengthening [39]. TERT has also been reported to form complexes with beta-catenin and c-MYC to affect target gene transcription in these pathways, including genes associated with tissue invasion, in carcinoma models [19]. Other studies found that TERT overexpression in cancer results in increased cancer stem cells [19], which have different gene expression profiles compared to more differentiated cells [20–22], including factors involved in self-renewal and lineage differentiation, such as cancer-testis antigens [23]. We therefore questioned whether *TERT* promoter mutations might have similar consequences in solitary fibrous tumors.

Our initial exploratory analysis in a small set of frozen tumors identified *PRAME* as being highly overexpressed in solitary fibrous tumors with *TERT* promoter mutations. However, we were unable to confirm this association using immunohistochemical evaluation of PRAME protein

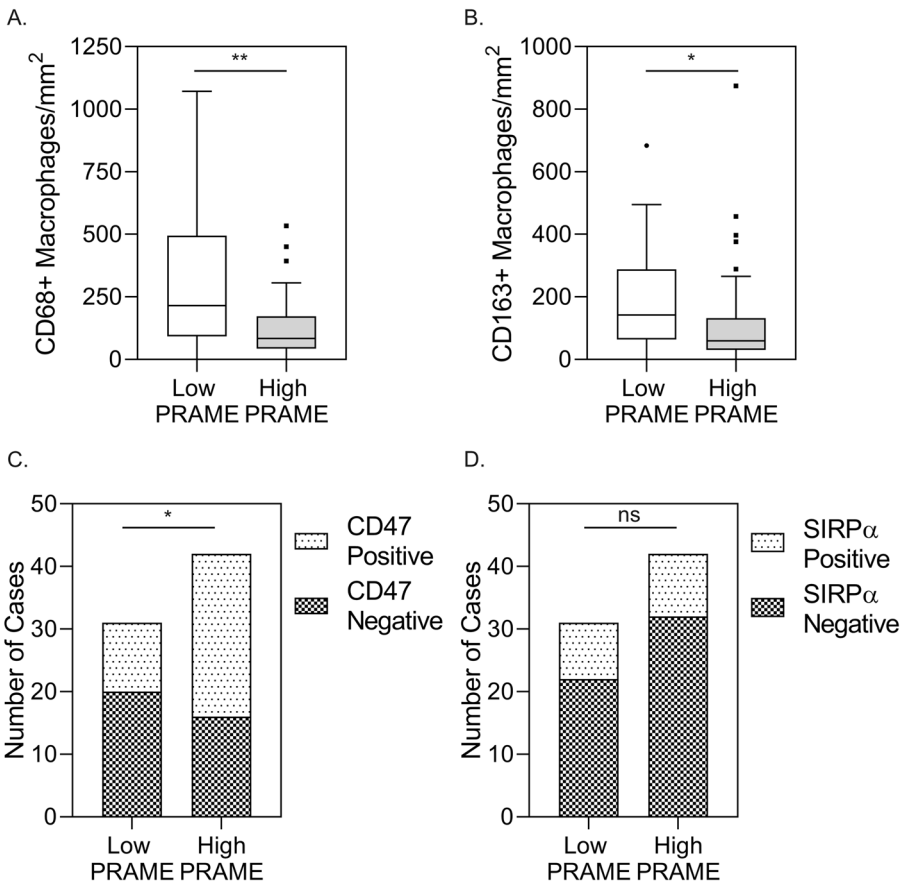

Fig. 3 Correlation of PRAME expression with tumor-associated macrophages. **a** High PRAME correlates with a lower density of CD68+ macrophages. **b** High PRAME correlates with a lower density of CD163+ macrophages. **c** Correlation of PRAME with CD47 expression on tumor cells. Positive indicates the presence of staining in any tumor cells, although in all positive cases at least 20% of cells expressed CD47. **d** Correlation of PRAME and SIRPα expression in tumor-associated macrophages. Positive indicates the presence of at least once macrophage with SIRPα expression. $^{**}p < 0.005$, $^{*}p < 0.05$, ns not significant.

expression in either formalin-fixed paraffin-embedded tissues from the original 8 samples subjected to mRNAseq, or in a larger series of solitary fibrous tumors. Instead, we found that PRAME expression was widespread in solitary fibrous tumors, including primary and metastatic tumors.

Solitary fibrous tumors respond poorly to conventional cytotoxic chemotherapy, and antiangiogenic targeted agents offer only limited improvements in outcome [7]. The identification of PRAME suggests a possible new therapeutic target for the management of solitary fibrous tumor. While PRAME expression can be very heterogeneous in pleomorphic sarcomas, possibly limiting its utility as a target in these tumors [40], we identified consistent staining between tissue cores from the same tumor in solitary fibrous tumors, as well as in whole tissue sections, and expression within positive cases was uniform, suggesting that heterogenous expression of PRAME may be less of a concern in solitary fibrous tumors, and therefore these may be more responsive to targeted therapy.

PRAME, first described as a melanoma antigen [41], is known to repress retinoic acid receptor signaling, and to repress transcription of genes involved in growth arrest, differentiation, and apoptosis in melanoma models [42]. It belongs to a group of cancer-testis antigens, which are highly expressed during embryogenesis but become largely restricted to the testis in normal mature tissues. There are over 250 cancer-testis antigen genes reported to date (http://www.cta.lncc.br) [43], which are thought to serve a variety of functions in the regulation of cell processes, including proliferation and apoptosis. Reactivation of expression is seen in a variety of cancers including in sarcomas, particularly those that are translocation-associated [23]. For example both New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1), and PRAME are commonly expressed in synovial sarcoma [40, 44, 45], myxoid liposarcoma, and sometimes in osteosarcoma [46], while membrane-associated phospholipase A1-β (LIPI) and X Antigen Family Member 1 (XAGE-1) have been reported in Ewing sarcoma [47, 48]. The expression of these antigens has been used to develop either vaccine based or adoptive T-cell immunotherapy with chimeric T-cell receptor immunotherapies [46], such as engineered T cells with T-cell receptors targeting NY-ESO-1 in synovial sarcoma [49–51]. A similar approach could be employed toward treating patients with advanced or metastatic solitary fibrous tumors.

To date, there have been few studies investigating the potential responsiveness of solitary fibrous tumors to immunotherapy. Immune checkpoint markers such as PD1/PDL1 are infrequently expressed in solitary fibrous tumor, and like other translocation-associated sarcomas, solitary fibrous tumor tends to exhibit only sparse tumor-infiltrating lymphocytes [52]. These features suggest that solitary fibrous tumors may not be ideal candidates for a lymphocyte immune checkpoint inhibitor-based approach to immunotherapy. However, cancer-testis antigens such as PRAME represent an attractive target for immunotherapy against solitary fibrous tumor given that it has been shown to have strong immunogenicity, and a restricted expression profile [46].

Tumors with PRAME expression are also known to develop mechanisms to escape immune surveillance, and possibly regulate innate immune responses [53], including downregulation of MHC I and an increase in FOXP3+ suppressor T cells in urothelial carcinoma [54], and downregulation of antigen presenting genes in leiomyosarcoma and dedifferentiated liposarcoma [40]. We found that in solitary fibrous tumors, PRAME expression correlated with decreased CD68+ and CD163+ macrophages, as well as with more frequent CD47 expression on tumor cells. The finding of CD47 expression is significant in that it functions as a "don't eat me" signal to immune cells and normally functions as a marker of self [55, 56]. It binds to the ligand SIRPα (signal regulator protein alpha) on macrophages and inhibits phagocytosis [57]. These findings suggest targeted immunotherapies involving PRAME expression in solitary fibrous tumors may also benefit from concurrent targeting of the CD47-SIRPα pathway.

PRAME has also been reported to be prognostic in some tumors. For example, its expression is associated with metastatic potential in melanoma [41], osteosarcoma [34], myxoid liposarcoma [33], esophageal squamous cell carcinoma [58], and gastric cancer [59], among others, whereas in dedifferentiated liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma/myxofibrosarcoma, PRAME expression showed no correlation with prognosis [40]. While PRAME was frequently seen in metastatic solitary fibrous tumors in our series, we found no significant differences in overall survival or metastasis-free interval in patients with high vs low PRAME expression in the primary tumor. This may be due to the relatively short follow-up times available for most patients and low event rates associated with solitary fibrous tumor, limiting the power of this finding. Interestingly, PRAME was associated with intrathoracic primary tumor site. The reasons behind this are not clear, though various series have also reported genomic and protein expression differences in solitary fibrous tumors by clinicopathological features [11, 30]. As intrathoracic solitary fibrous tumors are typically associated with exon 4–2/3 *NAB2-STAT6* fusions and older age at presentation compared to solitary fibrous tumors of other sites, we likewise identified weak correlations between PRAME expression and patient age and *NAB2-STAT6* fusion type.

Our study was limited by the small size of the exploratory data set, and by the lack of an independent validation

cohort to confirm the observed correlation between PRAME overexpression and clinicopathologic features. As this was an exploratory analysis, we opted not to perform adjustments to the p-values in our evaluation of clinicopathologic correlates with PRAME expression to correct for multiple comparisons testing, as it was our belief that this would be too conservative and overly reduce the power to detect subtle differences in our fairly small dataset, resulting in minimization of false positives at the expense of potentially excess false negatives. Had stringent Bonferroni corrections for multiple comparisons testing been applied, only the correlations between PRAME expression and density of CD68+ macrophages across all tumors, and the correlation in primary tumors between PRAME and site would be considered significant.

Our study also highlights the common problem in biomarker discovery of poor correlation between expression of mRNA and protein. While the specific mechanisms for the unexpected discrepancy we saw between mRNA and immunohistochemical studies for PRAME in Cohort A have yet to be elucidated, frequent causes of differential mRNA and protein expression include regulation of translation or protein turnover resulting in decreased protein synthesis or more rapid turnover. We can also not exclude the potential for artefactual bias due to RNA degradation in frozen tissues during storage, or due to changes in the tumor banking protocols over time at our center, particularly as 3 out of 4 of the lowest *PRAME* mRNA levels were seen in cases older than 10 years, while the 4 higher *PRAME* mRNA expression levels were all in cases banked in the last 10 years.

In conclusion, we found widespread expression of PRAME in solitary fibrous tumors, particularly those arising intrathoracically. While PRAME expression did not significantly correlate with outcome measures, elevated PRAME was associated with decreased intratumoral macrophages and frequent tumor cell expression of CD47, suggesting a downregulation of pathways involved in immune surveillance and antigen presentation in these tumors. The widespread expression of PRAME, and frequent high-level expression in metastases may represent a potential therapeutic target for immunotherapy modalities such as adoptive T-cell transfer in advanced solitary fibrous tumors. More extensive studies are needed to further pursue this avenue of investigation and validate the exploratory findings presented in this study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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