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PRAME EXPRESSION IN CANCER. A SYSTEMATIC IMMUNOHISTOCHEMICAL STUDY OF >5800 EPITHELIAL AND NONEPITHELIAL TUMORS.

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Abstract

Preferentially expressed antigen in melanoma (PRAME) is considered a useful marker in the differential diagnosis between malignant melanoma and its melanocytic mimics. Recently PRAME expression was documented in non-melanocytic tumors, but much of the data are based on mRNA studies. This investigation evaluated PRAME expression in the spectrum of normal tissues and >5800 human tumors using immunohistochemistry and EP461 monoclonal antibody. In normal tissues, PRAME was expressed in the testis and proliferative endometrium. In tumors, PRAME was variably expressed in malignancies of different lineages. Among epithelial tumors, >50% of PRAME-positive lesions were found among endometrial carcinomas (82%), uterine serous carcinomas (82%), uterine carcinosarcomas (60%), ovarian clear cell carcinomas (90%), ovarian serous carcinomas (63%), adenoid cystic carcinomas (81%), seminomas (78%), thymic carcinomas (75%) and basal cell carcinomas (62%). In mesenchymal and neuroectodermal malignancies, PRAME was frequently expressed in synovial sarcoma (71%), myxoid liposarcoma (76%), neuroblastoma (61%) and metastatic melanoma (87%). Also, PRAME was consistently expressed in 4 melanomas that lacked all melanoma markers including S100 protein and SOX10 but harbored typical for melanoma BRAF or NRAS driver mutations. However, strong, and diffuse PRAME immunoreactivity was seen in many types of non-melanocytic poorly differentiated carcinomas and sarcomas. Based on this study, PRAME is a relatively unspecific immunohistochemical marker, which limits its use in diagnostic surgical pathology. However, immunohistochemistry is a reliable and unexpensive method useful in detecting PRAME-positive malignancies for potential immunotherapy.

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Keywords

PRAME; immunohistochemistry; qPCR; uterine carcinomas; ovarian carcinomas; salivary gland adenoid cystic carcinoma; seminoma; basal cell skin carcinoma; synovial sarcoma; myxoid liposarcoma; neuroblastoma; mucosal melanoma; metastatic melanoma; dedifferentiated melanoma

INTRODUCTION

Preferentially expressed antigen in melanoma (PRAME) is a nuclear receptor and transcriptional regulator and a member of cancer testis antigen (CTA) family of proteins. In normal tissue, PRAME is expressed almost exclusively in the testis. [1] Physiologically, it acts as a repressor of retinoic acid receptor pathway and thereby regulates cell differentiation, growth, and apoptosis. [2,3] Because PRAME is recognized by tumor-reactive cytotoxic T cells, there may be rationale for anti-PRAME immunotherapy and need to assess PRAME immunopositivity in search of treatment targets. Currently, there are active phase I and II treatment trials for hematological malignancies and solid tumors involving vaccines and engineered T cells directed against PRAME. (ClinicalTrials.gov) Besides melanoma, PRAME expression has been occasionally detected in various malignant epithelial, neuroectodermal and soft tissue tumors. [4–19] Many PRAME expression studies tested mRNA and no large-scale systematic analysis of PRAME expression using immunohistochemistry (IHC) has been reported. (Supplemental Table 1) The aim of this study was to evaluate PRAME expression in a large cohort of human tumors and identify possible diagnostic applications of PRAME immunohistochemistry.

MATERIAL AND METHODS

This study evaluated >5800 well characterized human tumors and 70 normal tissues assembled in either multi-tissue blocks or tissue microarrays. [20,21] Analyzed tumor cohort included epithelial (n=3627) and mesenchymal, neuroectodermal, and lymphoid (n=2213) malignancies. In selected cases, diagnoses were corroborated by genetic studies including next generation sequencing (NGS), interphase fluorescence in-situ hybridization (FISH) gene rearrangement assays, detection of fusion gene transcripts by ArcherDx (ArcherDx, Boulder, CO), and NGS studies.

Two rabbit anti-PRAME antibodies, EP461 (for *in vitro* diagnostic (IVD) use in the USA; Cell Marque, Rocklin, CA) and E7I1B (Cell Signaling Technology, Danvers, MA) were evaluated. Immunohistochemical staining with prediluted EP461 was performed using Ventana BenchMark Ultra (CC1 solution for 40 minutes, primary antibody incubation for 1 hour, OptiView DAB IHC Detection Kit) (Ventana Medical Systems, Tucson, AZ), while staining with diluted 1:800 E7I1B was performed using Leica Bond-Max (ER2 buffer for 25 minutes, incubation with primary antibody for 30 minutes, BOND Polymer Refine Detection kit) (Leica Biosystems, Bannockburn, IL). Randomly selected arrays (total of 585 tumor sections) were stained with either antibody to compare their performance. Patterns of EP461 and E7I1B immunostainings were almost identical. However, the EP461 produced less background and thus was chosen for further analyses.

The specimens were evaluated for nuclear PRAME expression, and the percentages of positive cells were estimated. Cytoplasmic staining, a very rare phenomenon, was disregarded when not accompanied by nuclear reactivity. Tumors showing barely perceptible staining and/or staining in less than 5% of cells were considered negative. Diffuse staining was defined as immunopositivity of at least 80% of nuclei. PRAME IHC was validated by quantitative polymerase chain reaction amplification (qPCR) PRAME gene expression assays in 23 randomly selected cases including 17 positive tumors. Details of RNA extraction, reverse transcription and qPCR amplification are provided in Supplemental Data.

RESULTS

Normal tissues

Nuclear PRAME staining was consistently noted in the testis, in which spermatogonia were strongly positive while weak positivity was focally present in spermatocytes and spermatids. (Supplemental figure 1A) Diffuse and strong staining was also present in proliferative endometrial glands (Supplemental figure 1B) whereas secretory phase epithelium stained focally and with weak to moderate intensity. Focal and weak PRAME expression was observed in decidua basalis of the placenta and granulosa cells of the ovarian corpus luteum. Neither epithelial nor mesenchymal structures of the colonic mucosa, liver, kidney, prostate, breast, thyroid, adrenal and salivary glands were positive. Alveolar epithelium of the lung was completely negative and respiratory epithelium showed luminal staining limited to the apical cilia. Cytoplasmic staining was seen in sebaceous glands of the skin whereas other cutaneous structures were negative. Lymphoid organs including the spleen, thymus and lymph nodes were negative. No staining was present in the brain, peripheral ganglia, and nerves. Also, negative was the adipose and fibrous connective tissue as well as smooth and skeletal muscle.

Epithelial tumors

Immunostaining of PRAME in epithelial tumors is summarized in Table 1. High frequency of PRAME-positive tumors was seen in female reproductive cancers. Endometrial carcinoma, both endometrioid and serous subtype, showed PRAME-expression in 82% of cases with diffuse immunoreactivity in ca. 50% of tumors. (Supplemental figure 2A) PRAME expression was also common (31/52, 60%) in uterine carcinosarcomas, usually in both epithelial and sarcomatous areas. However, diffuse staining was observed only in 10% of cases. Among ovarian tumors, PRAME expression was nearly consistent in clear cell carcinomas with 90% of positive cases, half of them diffusely. (Figure 1A) Also, serous and undifferentiated ovarian tumors revealed PRAME immunoreactivity in 63% and 50% of cases, respectively. In contrast, breast carcinomas rarely expressed PRAME. Analysis of 223 ductal cancers yielded 32 positive tumors (14%), 12 of them with diffuse staining. (Supplemental figure 2B) A third of the positive cases were hormone receptor-negative and 5 were triple-negative tumors. PRAME expression was seen only in 9% (5/53) of breast lobular carcinomas. However, 4 lobular tumors including a case with pleomorphic features revealed diffuse staining pattern.

Eighty-one percent (26/32) of adenoid cystic carcinomas showed PRAME positivity with diffuse staining seen in 50% of cases. (Figure 1B) Approximately a fourth (7/32) of salivary duct carcinomas also were PRAME-positive, but usually in a non-diffuse manner. Other salivary gland tumors such as acinic cell carcinoma (n=14), oncocytoma (n=12) and Warthin tumor (n=19) were uniformly negative.

Head and neck squamous cell carcinomas of various primary sites expressed PRAME with comparable frequency (ca. 20%). In tonsillar carcinomas the staining was focal, ranging from 5 to 30% of nuclei with exception of weak diffuse staining in 2 cases. On the contrary, squamous carcinomas of the oral cavity (excluding the tongue) revealed diffuse PRAME staining in majority (71%) of positive cases. Among 8 tongue tumors diffuse PRAME staining was present in 5 cases, which were all non-keratinizing carcinomas.

Also, strong, and diffuse PRAME expression was seen in 62% (26/42) of basal cell skin carcinomas. Thirty percent (8/27) of Merkel cell carcinomas expressed PRAME with diffuse staining seen in 63% (5/8) of positive cases, but its intensity was usually moderate or weak.

In urological malignancies, PRAME was expressed in 78% (73/94) of seminomas with diffuse staining in 44% of examined cases. (Supplemental figure 2C) Diffuse PRAME staining was seen in spermatocytic tumors (n=3) (Figure 1C) and 1 of 3 PRAME-positive testicular choriocarcinomas. Thirty-five percent (12/34) of yolk sac tumors (pure or as components) expressed PRAME, with diffuse expression in 25% of positive tumors. (Supplemental figure 2D) However, PRAME positivity was exceptionally rare in embryonal carcinomas (n=68). Teratomas were generally negative, but two cases demonstrated focal PRAME expression in cuboidal/columnar epithelium. PRAME expression in kidney carcinomas was restricted to clear cell tumors, with strong preference to grade 3 and 4 tumors. (Figure 1D) Other renal tumors, including several rare entities, were negative. In urothelial carcinomas, 7 high-grade tumors were positive, and the expression was mostly seen in less than 25% of cells. However, 2 tumors revealed weak diffuse PRAME staining pattern. All prostatic adenocarcinomas were negative.

Lung carcinomas were PRAME-positive in 20 to 40% of cases and squamous cell carcinomas were most frequently positive. Sixty percent (9/15) of PRAME-positive squamous cell carcinomas stained diffusely, and 7 of them were of non-keratinizing subtype. (Supplemental figure 3A) PRAME-immunopositivity was lower in lung adenocarcinomas (20%, 20/100) and usually coincided with poorly differentiated or solid histomorphology. Twenty nine percent (11/38) of small cell lung cancers were positive and except 3 cases showed diffuse staining pattern. Malignant mesotheliomas were PRAME-negative with the exception of a poorly differentiated pleural example, which revealed PRAME expression in 30% of neoplastic cells.

Remarkable positivity was seen in 75% of thymic carcinomas (non-keratinzing squamous type). (Figure 2A) Immunopositivity in thymomas was considerably lower (16 of 41 tumors), generally weaker and more focal than in carcinomas. (Supplemental figure 3B)

In general, thyroid carcinomas lacked PRAME expression. However, 10% (3/30) of anaplastic carcinomas with sarcomatoid morphology showed PRAME immunoreactivity

(Figure 2B) which was diffuse in two cases. Among papillary (n=57) and poorly differentiated (n=15) carcinomas positive tumors one in each subtype were identified. However, all follicular (n=50) and medullary (n=47) carcinomas were negative. One third (18/54) of adrenocortical carcinomas were PRAME-positive with diffuse staining in 78% of cases. (Supplemental figure 3C) However, weak PRAME staining in < 30% of tumor cells was detected in 4 cases.

Among 83 pancreatic neuroendocrine tumors two PRAME-positive cases were identified. Both were G1 tumors with weak expression in 40% and 70% of cells, respectively. The large cell neuroendocrine carcinoma category contained mainly pulmonary and intestinal tumors. PRAME immunoreactivity was seen in 36% (9/25) of cases. In all tumors at least 50% of cells were positive and diffuse staining was seen in 7 cases.

PRAME expression was infrequent among tumors of the gastrointestinal tract. Gastric cancers were all negative. Eleven percent (4/38) of duodenal carcinomas showed PRAME expression. One of them, a poorly differentiated tumor, was diffusely positive. (Supplemental figure 3D) PRAME-positive colorectal carcinomas were few: only 10 were found among 951 tumors tested. (Figure 2C) Positive PRAME staining often coincided with poorly differentiated morphology and three of four diffusely positive cases demonstrated no or only minimal gland formation.

In pancreatic adenocarcinomas PRAME staining was rare and widespread only in one tumor (70% of cells) while the remainder stained focally (5 and 25% of cells) and the intensity was weak. Among 86 hepatocellular carcinomas 3 positive cases were identified, of which only one displayed PRAME expression in majority (70%) of cancer cells. However, 13% (15/117) of intrahepatic cholangiocarcinomas were positive, usually in <50% of cells.

Mesenchymal and neuroectodermal tumors

Immunostaining of PRAME in mesenchymal and neuroectodermal tumors are summarized in Table 2. Primary mucosal melanomas and melanoma metastases (mostly of cutaneous primary) showed high incidence (99% and 87%, respectively) of PRAME-positive tumors. The staining ranged from 5 to 100% of positive cells but was typically strong and diffuse. (Supplemental figure 4A) Four dedifferentiated melanomas (clinicopathological and molecular characteristics of all cases are presented in Supplemental Table 2) that lacked expression of melanocytic differentiation markers such as HMB45, Melan-A, S100, and SOX10 but harbor typical driver mutations (BRAF p.V600E, BRAF p.V600K or NRAS p.Q61L) revealed moderate to strong PRAME staining in 90–100% of cells. (Figure 2D) Other melanocytic tumors (congenital and blue nevi, melanotic Schwannian tumors) were consistently negative.

Only one (4%) clear cell sarcoma revealed PRAME expression, variable staining (weak to strong) was seen in 10% of cells. (Supplemental figure 4B) EWSR1 rearrangement was identified in that tumor by interphase FISH and NGS-targeted RNA sequencing detected EWSR1:ATF1 fusion transcripts. Also, a case of EWSR1:ATF1 fusion-positive clear cell sarcoma-like tumor of the gastrointestinal tract was PRAME-negative.

PRAME expression was detected in 70% of synovial sarcomas with more than half of tumors showing diffuse staining pattern. Eighty-three percent (10/12) of positive biphasic synovial sarcomas showed PRAME immunoreactivity in the epithelial structures (prominent in 7 cases) in addition to the staining of mesenchymal component. (Figure 3A) Staining characteristics were comparable when cohort was limited to cases with confirmed SS18-SSX fusion protein expression.

A relatively small subset (27%) of malignant peripheral nerve sheath tumors (MPNSTs) expressed PRAME. (Figure 3B) All PRAME-positive MPNSTs showed at least partial loss of H3K27me3 demonstrated by IHC.

Solitary fibrous tumors (SFTs) were negative (neither nuclear nor cytoplasmic staining was noted), but three of six SFT mimics with no STAT6 or CD34 positivity showed PRAME expression. (Figure 3 C, D) One of these cases was strongly positive for PRAME in 100% of cells, whereas the other two tumors were only weakly and focally positive. Phyllodes tumors of the breast were positive in 15% of cases and the staining was limited to the neoplastic mesenchymal compartment.

Soft tissue leiomyosarcomas (n=35) were PRAME-negative with one exception. However, 19% (6/32) of uterine leiomyosarcomas revealed positive staining with diffuse pattern in 4 cases. All PRAME-positive uterine tumors showed at least focal estrogen receptor expression compared with only 14 of the remaining 26 PRAME-negative leiomyosarcomas.

Seventy-six percent (19/25) of myxoid liposarcomas expressed PRAME with diffuse staining pattern in 68% of positive cases. (Supplemental figure 4C) In contrast only 8% (4/51) of dedifferentiated liposarcomas revealed PRAME positivity including two diffusely stained tumors.

Positivity for PRAME was relatively frequent among neuroblastomas (61%), Wilms tumors (46%) and desmoplastic small round cell tumors (43%) and while less common in rhabdomyosarcomas (ca. 30%). (Figure 4A–D) In Wilms tumors, the staining was observed in either blastemal, stromal or epithelial component, mostly in the latter. PRAME expression was seen in 19% (3/16) of Ewing sarcomas (all cases confirmed to harbor EWSR1 rearrengements and EWSR1:FLI1 fusion transcripts), but only in a small fraction (<25%) of tumor cells.

Majority of angiosarcomas (20/23) lacked PRAME expression. However, three poorly differentiated tumors showed strong and diffuse PRAME staining. (Supplemental figure 4D) Other vascular lesions including epithelioid hemangioendotheliomas, hemangiomas and glomus tumors were all negative.

Occasional cases of undifferentiated pleomorphic sarcoma, plexiform fibromyxoma, ovarian fibrothecoma, paraganglioma, osteosarcoma, glioblastoma and chordoma demonstrated PRAME staining, often weak and limited to <50% of cells.

Among lymphoid tumors tested, PRAME staining was infrequent in large B-cell lymphomas, but present in over 40% of Hodgkin lymphomas, where it was limited to Hodgkin/Reed-Sternberg cells. (Supplemental figure 5)

DISCUSSION

Diagnosis of melanocytic tumors has been the main application for PRAME immunohistochemistry. Several studies reported PRAME as a primary and metastatic melanoma marker with high sensitivity (>90%) and specificity in the context of melanocytic lesions. [22–25] However, the reported frequency of tumors with a diffuse staining pattern has varied from 41% to over 80%. [22,24,25] In this study, 87% of metastatic melanomas were positive. Among diffusely PRAME-positive tumors were 4 clinically and molecularly confirmed dedifferentiated melanomas (no expression of S100, SOX10, Melan-A, HMB45). Dedifferentiated melanomas are rare and can be easily misdiagnosed as undifferentiated pleomorphic sarcomas depriving patients from potentially effective melanoma-oriented therapies. [26] In this study, only 6% of undifferentiated pleomorphic sarcomas demonstrated PRAME expression, usually focal. Thus, placed in appropriate clinicopathological context, PRAME positivity might indicate dedifferentiated melanoma. However, the diagnosis must be supported by molecular genetic studies including DNA/RNA NGS for driver mutations and fusion genes and DNA methylation analysis. [27]

Almost all (99%) mucosal melanomas expressed PRAME and diffuse staining was more common than in metastatic cases. A recent study reported 17% of 24 mucosal melanomas negative or weakly positive for PRAME although by a different scoring method. [28]

In the current study, other melanocytic lesions including congenital and cellular blue nevi were negative in agreement with previous observations. [22] Also, few melanotic schwannian tumors, potential mimics of malignant melanoma, were negative. Previously only a single case of this entity with focal PRAME expression was reported. [29]

Clear cell sarcoma shares immunophenotypic and clinical characteristics of malignant melanoma. There are no morphological or immunohistochemical features that would reliably distinguish these entities and confirmation of *EWSR1* rearrangement or/and detection EWSR1-ATF1/CREB1 fusion gene transcripts remains the gold standard for diagnosis of clear cell sarcoma. Recent studies suggested that lack or barely perceptible PRAME expression (≤5% of tumor cells) might be useful in this distinction of clear cell sarcoma from diffuse labelling melanoma. [30] In the current study, 1 of 24 clear cell sarcomas revealed PRAME immunopositivity in ca. 10% of cells with variable intensity ranging from weak to strong. Focal staining of clear cell sarcomas was also reported by another study, but it is unknown whether the diagnosis had molecular confirmation. [29] PRAME undoubtedly has outstanding sensitivity for malignant melanomas, but caution should be applied in the differential between melanoma and clear cell sarcoma, and EWSR1-FISH or fusion studies may be necessary in problem cases.

PRAME expression in basal and Merkel cell carcinomas, as seen in this and a previous study [31] constitutes a potential pitfall that might lead to erroneous diagnosis of malignant

melanoma, especially in small biopsies and when melanoma is suspected clinically. Therefore, pathologists need to be aware of possible PRAME immunopositivity in non-melanoma skin cancers.

Recently, *PRAME* was shown to be one of the most upregulated genes in thymic squamous cell carcinomas compared with normal thymus by RNA profiling. [32] The same study demonstrated strong/diffuse PRAME immunostaining in thymic carcinomas and weak/focal in thymomas and concluded that PRAME immunohistochemistry may be used to differentiate between the two entities. [32] Although the pattern of staining was not specified, only cytoplasmic reaction is apparent from included microphotographs. [32] In contrast, this study documented nuclear expression of PRAME in 75% of thymic carcinomas and almost 40% of thymomas, 38% of which were stained diffusely. Of note, type B3 thymoma (which could be challenging to differentiate from thymic carcinomas) was strongly and diffusely positive. Overall, a diagnosis of thymic carcinoma cannot be reliably confirmed by PRAME positivity, nor can it be precluded by a negative staining result.

PRAME expression was seen in most seminomas while absent in embryonal carcinomas. This is also supported by previous mRNA and proteomic data. [19,29] Some studies suggest low *PRAME* mRNA levels in teratomas, yolk sac tumors and choriocarcinomas. [19,33] However, the latter two entities often showed PRAME immunoreactivity. Moreover, diffuse PRAME expression was documented in spermatocytic tumors for the first time.

Salivary gland tumors have not been extensively evaluated for PRAME expression. In this study, immunohistochemistry of >100 salivary gland tumors of different types revealed, for the first time, prominent PRAME positivity in adenoid cystic carcinoma, making it a potential immunotarget for anti-PRAME therapies. Lesser PRAME immunoreactivity was noted in salivary duct carcinomas, where it was usually focal. Although a previous study noted much greater frequency of staining (83%), most PRAME-expressing tumors displayed only cytoplasmic reaction, which is of uncertain significance. [18]

More than 300 endometrial and ovarian cancers were analyzed in this study. Frequent PRAME immunopositivity was seen in endometrioid and serous carcinomas. This observation remains in agreement with The Cancer Genome Atlas mRNA expression data showing upregulation of *PRAME* mRNA in endometrial and serous tumors, as well as with previous data on the protein level. [11,13,29,34] Although comparable levels of *PRAME* mRNA were previously found in endometrial carcinomas and uterine carcinosarcomas, the latter were characterized by less frequent overall and more typically focal PRAME immunoreactivity. Moreover, nearly consistent (90%) PRAME positivity was noticed in ovarian clear cell carcinomas. This observation could be useful in determining the origin of metastatic clear cell carcinomas, although occasional positivity was also seen in high-grade renal clear cell carcinomas.

PRAME expression in mesenchymal and neuroectodermal tumors was relatively uncommon. However, a high frequency (70%) of PRAME positivity in synovial sarcomas, although lower than previously reported on mRNA and protein levels, substantiates targeting these tumors by anti-PRAME immunotherapies. [11,35,36] Solitary fibrous tumors, potential

mimickers of monophasic synovial sarcoma, were all negative. This contrasts a previous immunohistochemical study that reported >90% PRAME positivity in SFTs, but the staining was cytoplasmic. [37]

Myxoid liposarcoma was another soft tissue tumor with high frequency (76%) of PRAME positive cases. Two earlier investigations on myxoid/round cell liposarcomas revealed even greater positivity rates (90% and 100%), but the use of polyclonal antibodies may account for this difference. [38,39] In contrast, dedifferentiated liposarcomas were rarely positive. Preexisting literature concerning PRAME expression in these tumors is discordant with frequency ranging from 4% to 43%. [10,38]

Upregulation of *PRAME* transcription was previously demonstrated in neuroblastomas and medulloblastomas, [6,7] but we did not find any protein level-based studies on PRAME expression in these or related entities. In this study, PRAME immunolabelling was noted in all tested small blue round cell tumors. Positivity ranged from 19% to 61% in solid malignancies and was 6% in large B-cell lymphomas. Therefore, specificity and sensitivity of PRAME IHC limits its diagnostic use in this context, but there is a rationale for potential predictive application. Recently, a transcriptomic study of solid pediatric malignancies identified two high-affinity MHC class I peptides derived from PRAME protein and confirmed that one of them elicits a significant cytotoxic response of co-cultured T cells *in vitro*. [40] Moreover, adoptive transfer of PRAME-targeting T cells in a mouse model of Ewing sarcoma resulted in a significant regression of tumors and prolonged survival. [40]

In summary, PRAME is expressed in a wide variety of epithelial and non-epithelial tumors. PRAME immunohistochemistry may be useful in selected diagnostic contexts, but its routine application is greatly limited by low specificity. Despite its misleading and unfortunate full name, PRAME should not be regarded as a melanoma-restricted marker. Instead, pathologists need to be aware of potential pitfalls, e.g. frequent PRAME staining in non-melanoma skin cancers and possible immunolabelling of miscellaneous poorly differentiated tumors. Moreover, PRAME always needs to be interpreted together with other IHC results and placed in the clinicopathological context. Rather than a novel diagnostic marker, immunohistochemical evaluation of PRAME might become a predictive parameter for emerging anti-PRAME immunotherapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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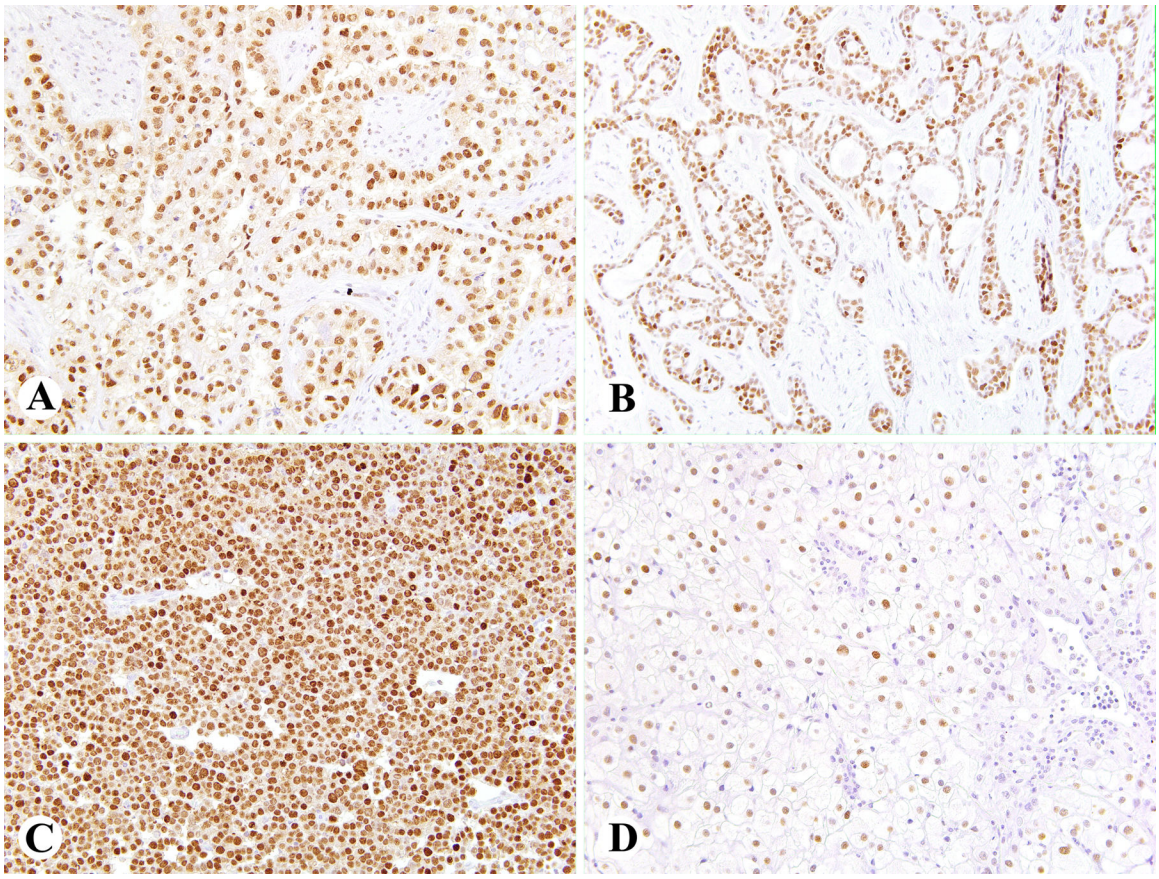

Figure 1.

PRAME expression in selected epithelial tumors. A. Strong expression in clear cell ovarian carcinoma. B. Staining in salivary gland adenoid cystic carcinoma present in either epithelial and myoepithelial cells. C. Diffuse immunolabelling in a spermatocytic tumor. D. Clear cell renal cell carcinoma, G3 with weak to moderate intensity of PRAME expression.

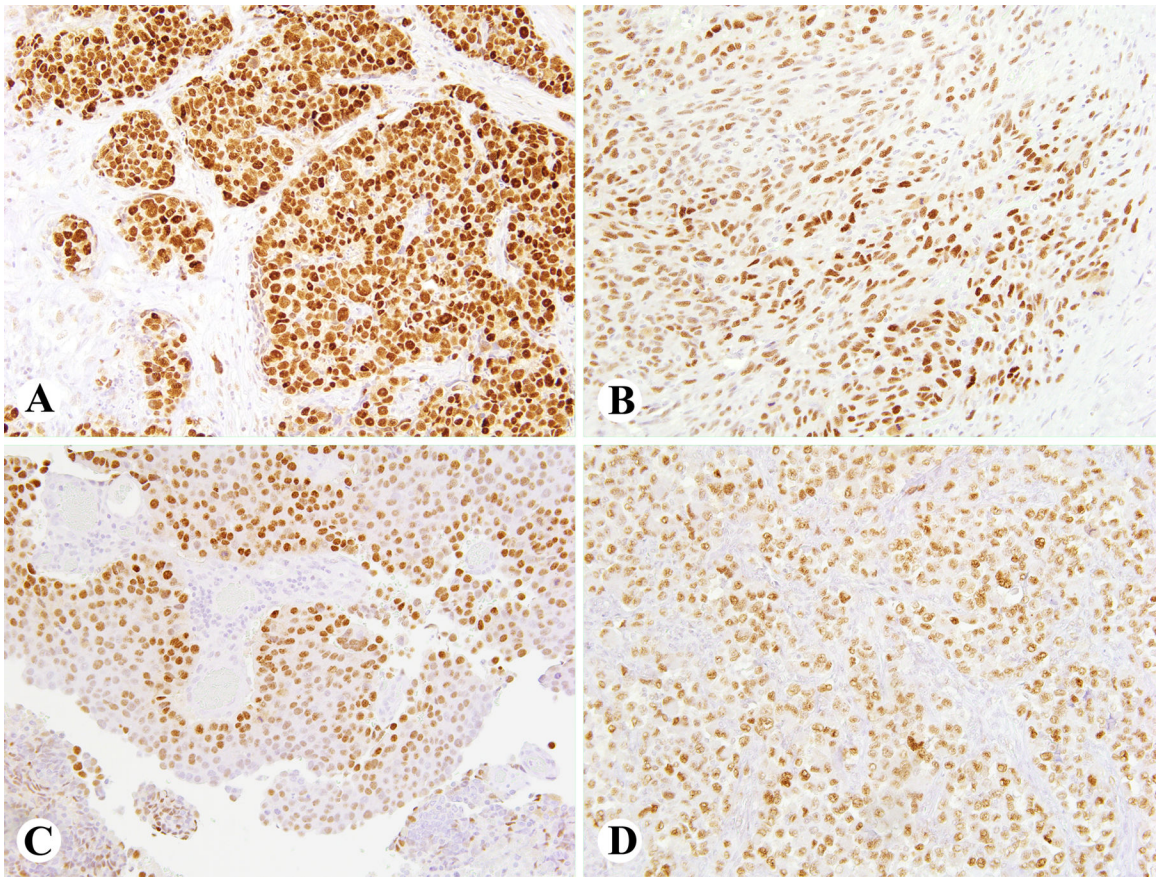

Figure 2.

PRAME expression in poorly differentiated malignancies. A. Poorly differentiated thymic carcinoma. B. Anaplastic thyroid carcinoma with sarcomatoid morphology. C. Poorly differentiated colorectal cancer with unusual morphology metastatic to the brain. D. Dedifferentiated melanoma verified by an NRAS Q61L mutation. The tumor was negative for all melanoma markers, including S100 protein and SOX10.

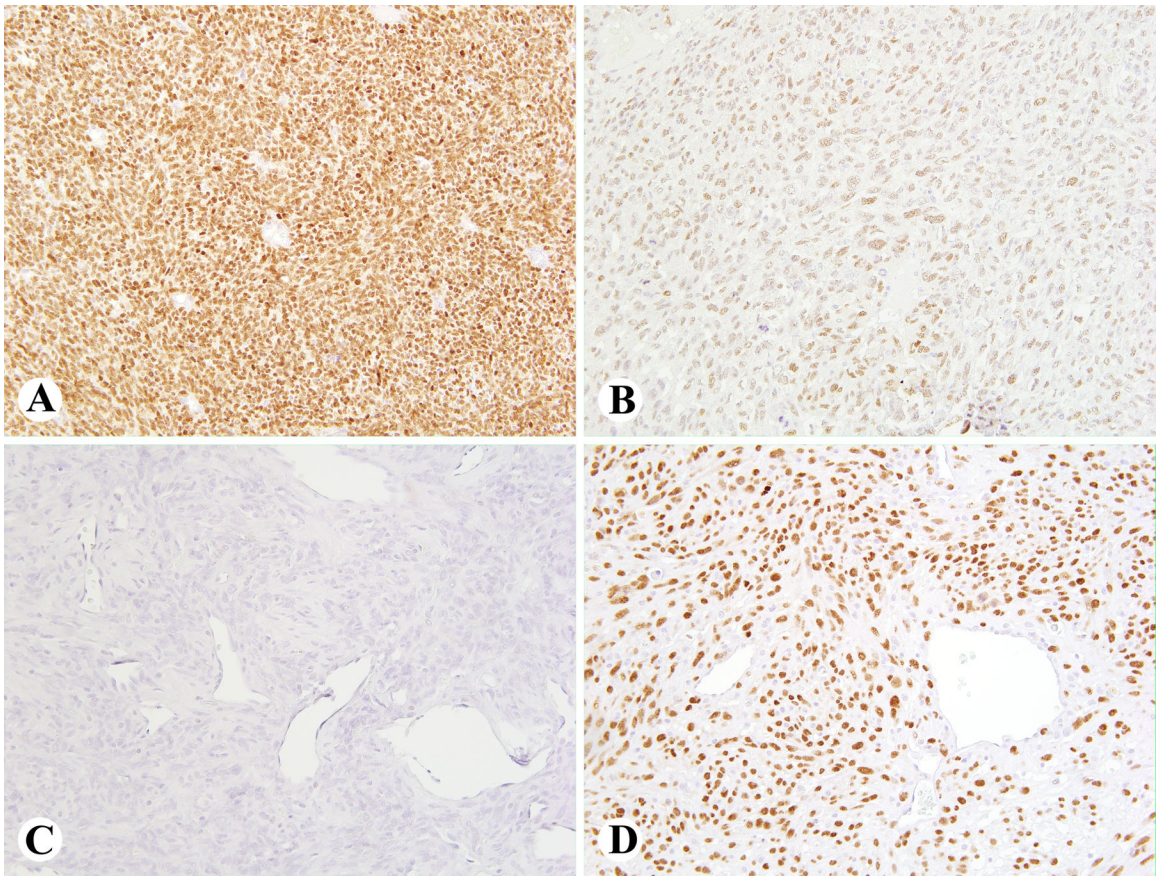

Figure 3.

PRAME expression in synovial sarcoma and histologic mimics. A. Diffuse and strong staining was relatively frequent among synovial sarcomas. B. Malignant peripheral nerve sheath tumors were less frequently PRAME-positive and the staining had usually weak or moderate intensity. C. Solitary fibrous tumor is negative. D. One of several solitary fibrous tumor-like tumors that exhibited PRAME expression; these cases were negative for STAT6 and CD34.

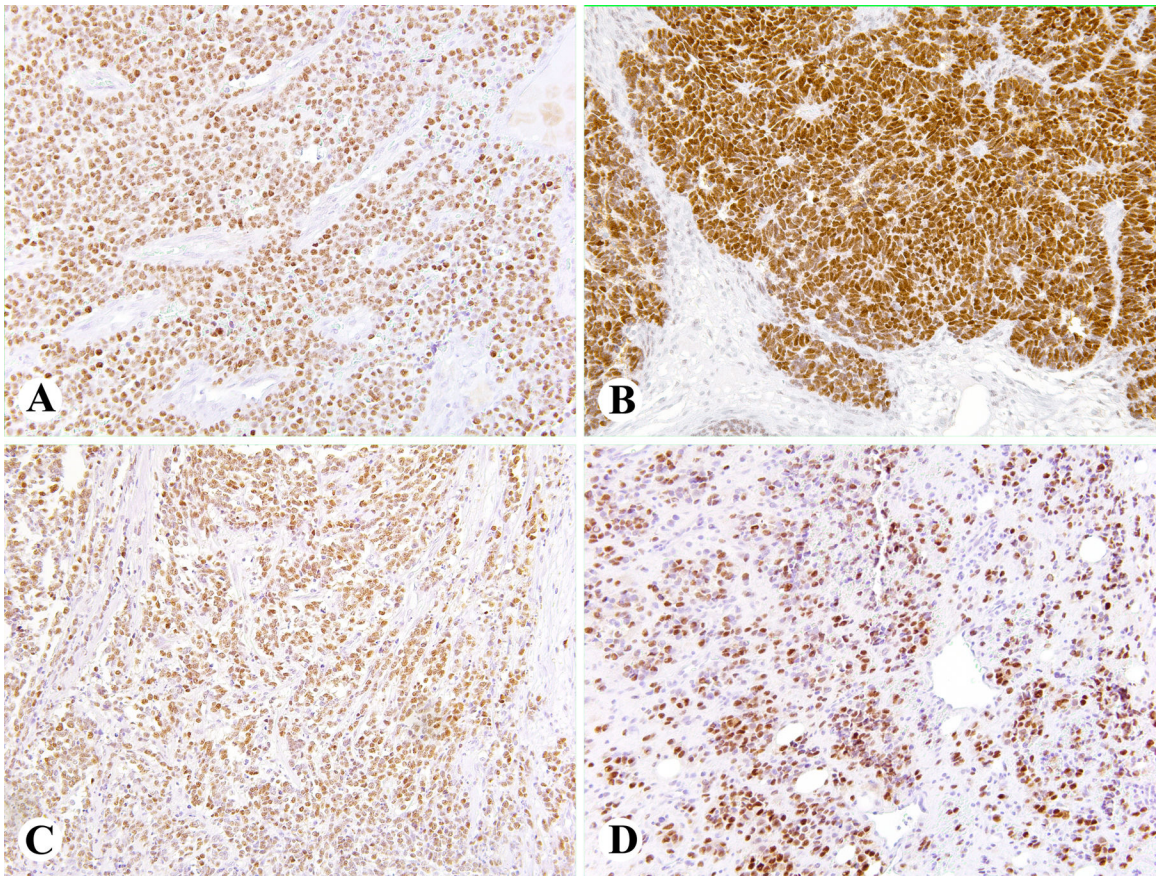

Figure 4.

PRAME expression in small blue round cell tumors. A. Neuroblastoma; diffuse staining was observed in half of positive cases. B. Wilms tumor; diffuse positivity in epithelial structures. C. Desmoplastic small round cell tumor; diffuse, moderately intense immunolabelling. D. Alveolar rhabdomyosarcoma; variable intensity of PRAME expression.

Table 1.

Expression of PRAME in 3627 epithelial neoplasms.

Tumor type	Positive/total cases	% positive/total	% diffusely positive/positive
Adrenocortical carcinoma	18/54	33%	78%
Breast, ductal carcinoma	32/223	14%	38%
Breast, lobular carcinoma	5/53	9%	80%
Colorectal adenocarcinoma *	10/951	1%	40%
Duodenum, adenocarcinoma	4/38	11%	25%
Germ cell tumor, choriocarcinoma	3/3	100%	33%
Germ cell tumor, embryonal carcinoma	1/68	1%	0%
Germ cell tumor, seminoma	73/94	78%	44%
Germ cell tumor, spermatocytic tumor	3/3	100%	100%
Germ cell tumor, teratoma	2/38	5%	0%
Germ cell tumor, yolk sac tumor	12/34	35%	33%
Head and neck SCC, oral cavity	7/39	18%	71%
Head and neck SCC, tongue	8/38	21%	63%
Head and neck SCC, tonsil	8/40	20%	25%
Kidney, clear cell RCC G1-G2	9/125	7%	0%
Kidney, clear cell RCC G3-G4	11/50	22%	27%
Kidney, oncocytoma	0/44		
Kidney, other tumors **	0/20		
Kidney, papillary RCC	1/60	2%	0%
Liver, cholangiocarcinoma	15/117	13%	40%
Liver, hepatocellular carcinoma	3/86	3%	0%
Lung, adenocarcinoma	20/100	20%	50%
Lung, small cell carcinoma	11/38	29%	73%
Lung, squamous cell carcinoma	15/38	39%	60%
Malignant mesothelioma	1/63	2%	0%
Myoepithelioma/mixed tumor, skin and soft tissue	2/40	5%	0%
Neuroendocrine carcinoma, large cell	9/25	36%	78%
Neuroendocrine tumor, pancreas	2/83	2%	0%
Ovary, clear cell carcinoma	43/48	90%	51%
Ovary, serous carcinoma	15/24	63%	53%
Ovary, undifferentiated carcinoma	12/24	50%	42%
Pancreas, adenocarcinoma	3/57	5%	0%
Prostate, adenocarcinoma	0/87		
Salivary gland, acinic cell carcinoma	0/14		
Salivary gland, adenoid cystic carcinoma	26/32	81%	50%
Salivary gland, oncocytoma	0/12		
Salivary gland, salivary duct carcinoma	7/32	22%	29%
Salivary gland, Warthin tumor	0/19		
Skin, basal cell carcinoma	26/42	62%	58%

Tumor type	Positive/total cases	% positive/total	% diffusely positive/positive
Skin, Merkel cell carcinoma	8/27	30%	63%
Stomach, adenocarcinoma	0/56		
Thymic carcinoma	9/12	75%	56%
Thymoma	16/41	39%	38%
Thyroid, anaplastic carcinoma	3/30	10%	67%
Thyroid, follicular carcinoma	0/50		
Thyroid, medullary carcinoma	0/47		
Thyroid, papillary carcinoma	1/57	2%	0%
Thyroid, poorly differentiated carcinoma	1/15	7%	0%
Urothelial carcinoma	7/96	7%	29%
Uterus, carcinosarcoma	31/52	60%	16%
Uterus, choriocarcinoma	1/4	25%	0%
Uterus, clear cell carcinoma	1/2	50%	0%
Uterus, endometrioid carcinoma	117/143	82%	48%
Uterus, serous carcinoma	32/39	82%	56%

*over 95% of colon cancers

**13 FH-deficient RCCs, 2 clear cell papillary RCCs, 2 hybrid oncocytic-chromophobe tumors, 1 chromophobe RCC, 1 mixed epithelial and stromal tumor, 1 mucinous tubular and spindle cell RCC

Table 2.

Expression of PRAME in 2213 mesenchymal and neuroectodermal neoplasms.

Tumor type	Positive/total cases	% positive/total	% diffusely positive/ positive
Angiosarcoma	3/23	13%	100%
Benign fibrous histiocytoma	0/91		
Breast, phyllodes tumor	8/52	15%	25%
Chondrosarcoma	0/46		
Chordoma	2/41	5%	0%
Clear cell sarcoma	1/24	4%	0%
Clear cell sarcoma-like tumor of the gastrointestinal tract	0/1		
Dermatofibrosarcoma protuberans	0/42		
Desmoid fibromatosis	0/39		
Desmoplastic small round cell tumor	6/14	43%	33%
Epithelioid hemangioendothelioma	0/3		
Epithelioid sarcoma	0/42		
Ewing sarcoma	3/16	19%	0%
Gastrointestinal stromal tumor, conventional	0/20		
Gastrointestinal stromal tumor, SDH deficient	0/14		
Giant cell tumor of bone	0/32		
Glioblastoma	5/42	12%	0%
Glomus tumor	0/64		
Granular cell tumor	0/33		
Hemangiomas	0/32		
Leiomyoma, uterine	0/89		
Leiomyosarcoma, non-uterine	1/35	3%	0%
Leiomyosarcoma, uterine	6/32	19%	67%
Liposarcoma, dedifferentiated	4/51	8%	50%
Liposarcoma, myxoid	19/25	76%	68%
Lymphoma, DLBCL	3/49	6%	67%
Lymphoma, Hodgkin	18/44	41%	0%
Malignant peripheral nerve sheath tumor	17/63	27%	47%
Melanocytic nevus, cellular blue nevus	0/11		
Melanocytic nevus, congenital	0/25		
Melanocytoma, meningeal	0/1		
Melanoma, metastatic *	216/248	87%	75%
Melanoma, mucosal	85/86	99%	85%
Melanotic schwannian tumor	0/4		
Meningioma	0/89		
Neuroblastoma	17/28	61%	47%
Neurofibroma	0/35		
Osteosarcoma	5/33	15%	40%

Tumor type	Positive/total cases	% positive/total	% diffusely positive/ positive
Ovary, fibrothecoma	1/30	3%	100%
Paraganglioma	4/77	5%	0%
Perineurioma	0/30		
Plexiform fibromyxoma	1/1	100%	0%
Rhabdomyosarcoma, alveolar	11/43	26%	82%
Rhabdomyosarcoma, embryonal	18/56	32%	33%
Schwannoma	0/81		
Solitary fibrous tumor	0/66		
Solitary fibrous tumor-like tumors STAT6 (-)	3/7	43%	33%
Synovial sarcoma, confirmed by SS18-SSX IHC	72/102	71%	60%
Synovial sarcoma, morphological diagnosis	80/114	70%	56%
Unfifferentiated pleomorphic sarcoma/malignant fibrous histiocytoma	4/63	6%	0%
Wilms tumor	12/26	46%	42%

* predominantly metastases of cutaneous melanomas