Prostate-Specific Membrane Antigen Expression in Meningioma: A Promising Theranostic Target

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Abstract
Meningioma is the most common intracranial neoplasm, yet there is no effective therapy for recurrent/refractory meningiomas after surgery and radiation. Prostate-specific membrane antigen (PSMA) is an enzyme upregulated on endothelial cells of multiple neoplasms and is being investigated as a theranostic target. Until now, PSMA has not been studied in meningiomas. We aimed to verify PSMA endothelial expression in meningiomas, detect tumor grade variability, and investigate the relationship of PSMA signal with tumor recurrence. We analyzed 96 archival meningiomas including 58 de novo and 38 recurrent specimens. All specimens were stained routinely and immunostained for CD31 and PSMA. Slides were scanned and analyzed producing raw CD31 and PSMA images of PSMA, CD31, PSMA/CD31, and PSMA/vasculature for CD31 and PSMA. Slides were scanned and analyzed producing raw data for images of PSMA, CD31, PSMA/CD31, and PSMA/vasculature. PSMA expression was seen within 98.9% of meningioma samples. In the total cohort, higher-grade tumors had increased expression of raw PSMA and PSMA/CD31, and PSMA/vasculature ratios compared to grade 1 tumors. PSMA expression and PSMA/vasculature ratios (p = 0.0015) were higher in recurrent versus de novo tumors among paired samples. ROC curves demonstrated PSMA/CD31, PSMA/vasculature, and raw CD31 as indicators of tumor recurrence. Thus, PSMA is expressed within endothelial cells of meningiomas, is increased with tumor grade and recurrence, and persists with prior irradiation.

Key Words: CD31, Meningioma, Prostate-specific membrane antigen, PSMA, Recurrent, Tumor grade, Tumor marker.

INTRODUCTION
Meningioma is the most common central nervous system tumor, accounting for 39.0% of all intracranial neoplasms (1). Although the majority of meningiomas are benign (World Health Organization [WHO] grade 1), approximately 10%–15% have a more aggressive course and tend to display rapid tumor growth, recurrence, brain invasion, or rarely, metastatic disease (WHO grades 2 and 3) (2). Currently, surgery and radiation therapy are offered for WHO grade 2 and 3 tumors (3); however, there is no effective therapy for relapsed or refractory meningiomas other than stabilization of disease with systemic therapies. As meningiomas are highly vascularized, there may be a potential role for targeted vascular agents in therapy.

Prostate-specific membrane antigen (PSMA) is a 100-kDa transmembrane peptidase expressed in prostate epithelium that is upregulated in prostate adenocarcinoma and has a positive association with tumor aggressiveness markers such as the Gleason score (4, 5). Additionally, numerous studies have found PSMA to be upregulated in endothelial cells of solid tumors of the breast, lung, thyroid, pancreas, and urothelium, including sarcomas and primary glial tumors where the enzymatic activity may be involved in malignancy-associated angiogenesis (5–10).

Haemels et al (4) in 2020 reported the incidental findings of a relatively low PSMA-avid meningioma via fluorine-18 PSMA (F-PSMA)-1007 positron emission tomography-computed tomography (PET/CT) scan with CT contrast enhancement. Incidental PET/CT PSMA-avid meningiomas are recognized with greater frequency and are documented as known pitfalls of radiotracer analysis (4, 11–13). Thus, it is most likely that endothelial cells of meningiomas express PSMA (4).

As recent studies correlate tumor angiogenesis with PSMA staining and the ability to utilize molecular imaging to monitor PSMA-avid tumors and their response to therapy, targeting PSMA-expressing endothelial cells of brain tumors may prove to be therapeutically useful (14, 15). To our knowledge, PSMA expression in meningioma neovasculature has not been systematically analyzed.

By assessing PSMA endothelial staining in meningiomas grades 1–3, we aimed to demonstrate the variability of PSMA expression in meningiomas in the context of WHO-defined atypical features (including brain invasion, mitoses, presence of necrosis, hypercellularity, small-cell change, macronucleoli, and sheeting architecture), and the Ki-67 proliferation index. Importantly, we evaluated PSMA signal and tumor recurrence in paired patient samples.
MATERIALS AND METHODS

Patient Selection and Clinicopathologic Data Acquisition

After institutional board review and approval at Lifespan Hospital with the Warren Alpert Medical School of Brown University, Providence, Rhode Island, formalin-fixed, paraffin-embedded tissues from 69 individual patients who underwent surgical resection between 1996 and 2022 for meningioma, WHO grades 1–3 were obtained. We analyzed 96 archival meningioma cases, selecting 58 de novo (42 grade 1, 15 grade 2, 1 grade 3) and 38 recurrent (16 grade 1, 20 grade 2, and 2 grade 3) specimens. All cases were stained with hematoxylin and eosin (H&E), with subsequent examination by a neuropathologist who selected samples with the most viable tumor to perform immunohistochemical analysis. The selected tissue samples were stained with anti-CD31 to highlight tumor vasculature as a control. Immunohistochemical staining for PSMA was performed to assess PSMA expression within the tumor vasculature.

Antibodies/Reagents

The primary antibodies used were anti-CD31 Endothelial Cell (concentrate) Clone JC70A purchased from Cell Marque, (Rockland, California) and Prostate-Specific Membrane Antigen (concentrate) Clone 3E6, #M362001-2 purchased from DakoCytomation Inc. (Carpinteria, CA).

Immunohistochemistry

CD31

Four-micrometer-thick sections were cut and baked in an Isotemp oven for 30 minutes at 60°C. Slides were put onto the Dako Link48 for antigen retrieval, and the Flex kit was used for antigen detection. Antigen retrieval was performed for 45 minutes at pH 6 (Citrate). The primary antibody to CD31 was used at a dilution of 1:150 and was automatically titrated onto the slides and subsequently incubated for 30 minutes. The universal secondary antibody was automatically dispensed and incubated for 20 minutes. Slides were removed from the Link48 and put into deionized water mixture, counterstained with hematoxylin, 0.2% ammonia water for 1 minute, and then run under deionized water for 5 minutes. Slides were then dehydrated and coverslipped.

PSMA Primary Antibody

Four-micrometer-thick sections were cut and baked in an Isotemp oven for 30 minutes at 60°C. Slides were put onto the Dako Link48 for antigen retrieval, and the Flex kit with mouse linker was used for antigen detection. Antigen retrieval was performed for 45 minutes at pH 9 (EDTA). The primary antibody for PSMA was used at a dilution of 1:10 and was automatically titrated onto the slides and subsequently incubated for 10 minutes. The universal secondary antibody was automatically dispensed and incubated for 15 minutes. Slides were removed from the Link48 and put into a deionized water mixture, counterstained with hematoxylin, 0.2% ammonia water for 1 minute, and then run under deionized water for 5 minutes. Slides were then dehydrated and coverslipped.

Slide Scanning

Both H&E and immunostained sections were scanned on a Leica Aperio CS2 WSI slide scanner at 20× magnification objective lens (0.5 μm/pixel). All of the digital slides were then downloaded from the Aperio vendor agnostic whole slide image (WSI) viewer in the standard visualization system image format with variable degrees of JPEG image compression. WSIs were deidentified and labeled with a sticker and numbered 1–96. Data transfer and storage were undertaken in a Health Insurance Portability and Accountability Act of 1996 (HIPPA)-compliant manner.

Machine Learning

QuPath (version 0.2) (16) was utilized as an open access solution to machine learning for WSIs. All WSI files were imported and orientated appropriately. A supervised Random Forest machine learning model was developed and trained from annotated patches selected from a randomized training patient cohort. An average of 5 patches was selected from each WSI within the training set, and a total of 200 training patches were utilized for model development. The training set was selected to represent tissue diversity, including tumor histologic subtypes, grade, and other pathologic factors such as mitoses and atypia. The demographics of the training cohort can be found in the Supplementary Material.

Vascular density was calculated from H&E-stained slides, while PSMA and CD31 were calculated from IHC-stained slides. Ground truth for vasculature as defined on H&E-stained slides included 3 shared layers of the vessel wall for arteries and veins (tunica intima, media, and externa), with small arteries and arterioles usually containing 1 or 2 layers of smooth muscle cells, and often absence of the external elastic membrane in arterioles. Additionally, continuous capillaries are present in the central nervous system (17), ranging from 4 to 10 microns with a reduced vessel wall comprised endothelium surrounded by connective tissue. On H&E, a basement membrane that houses pericytes can also be visible. For PSMA and CD31, positive ground truth was defined as cytoplasmic and/or membranous unequivocal vascular staining of intensity above background. Negative staining was defined as the absence of any detectable IHC staining, characterized by a pale gray discoloration.

Assessment of Biomarker Expression

An overview biomarker quantification from WSIs is shown in Figure 1. Briefly, the models were developed under very high (downsample = 2.00) resolution. Features were set as: “Default multiscale features.” The live prediction parameter was set in default as: “Number of threads: –1. Maximum samples: 100,000. RNG seed: 100.” Preprocessing parameters were set to default as follows: “Feature normalization: None. Feature reduction: No feature reduction. PCA retained var-
FIGURE 1. Computational biomarker expression quantified by machine learning-based classification. (A) Hotspots demonstrating PSMA and CD31 immunohistochemical expression within meningioma endothelial cells, (B) annotated WSI with positive PSMA expression within tumor endothelium and adjacent negative tumor cells, (C) the process of image segmentation within the PSMA and CD31 stained WSI utilizing grouped pixels with common characteristics (superpixels) of the respective IHC, and (D) vasculature expression from H&E-stained slides and the corresponding superpixel. The end result differentiates PSMA and CD31 colocalization within meningioma endothelial cells, involved vascular walls, and tumor cells without expression of either PSMA or CD31.
sided, with p values < 0.05 considered statistically significant.

**RESULTS**

**Patient Cohort**

In total, 96 specimens (both de novo tumors and recurrent tumors) were included on the WSI open access solution to machine learning. These were derived from de novo (primary tumors) and recurrent (first, second, and third recurrences) from 69 patients, of which 37 cases comprised paired patient samples (both de novo and recurrent specimens). A total of 58 de novo (42 grade 1, 15 grade 2, and 1 grade 3) specimens and 38 recurrent (16 grade 1, 20 grade 2, and 2 grade 3) specimens were evaluated and are represented in Table 1. The mean age of the patient cohort was 58 years, of which 23 patients were under the age of 50 years, and 73 patients were 50 years of age or older. A total of 60 patients were female, and 36 were male. While 82 patients underwent craniotomy for resection of tumor, 14 patients underwent other procedures, including excision or biopsy. Neuropathologic reports indicated 55 patients had a tumor that was < 4 cm and 41 patients had a tumor that was ≥ 4 cm in greatest dimension on gross examination. A total of 58 specimens were grade 1 tumors, and 38 specimens were grades 2–3. Of note, 12/22 (54.5 %) of patients in the paired group had received radiotherapy. While higher-grade tumors revealed the presence or absence of necrosis, brain invasion, hypercellularity, sheeting architecture, macronucleoli, small-cell change, and elevated Ki-67 index of proliferation, there were no significant differences within this group either in the de novo specimens or entire patient cohort in any of these defining features. Staining for Ki-67, a marker of cellular proliferation, was estimated or quantified at < 4% in 33 specimens, and ≥ 4% in 52 specimens. Hypercellularity was reported present in 32 specimens and absent in 41 specimens. Sheeting architecture was reported present in 11 specimens and absent in 48 specimens. Small-cell change was reported present in 14 specimens and absent in 46 specimens. The presence of macronucleoli was reported in 25 specimens and absent in 38 specimens. Brain invasion was present in 11 specimens and absent in 60 specimens. Psammoma bodies were reported present in 35 specimens and absent in 28 specimens. Notably, specimens with < 4 mitoses per 10 high-power fields were seen in 66 cases and ≥ 4 mitoses per 10 high-power fields in 23 cases.

**Biomarker Expression in Total Cohort**

The entire cohort consists of 96 patient samples. Overall, patients ≥ 50 years old had increased raw PSMA (p = 0.010), PSMA/CD31 ratio (p = 0.010), and PSMA/vasculature ratio (p = 0.002) compared to patients < 50 years of age. Patients with tumor size ≥ 4 cm had increased raw CD31 (p = 0.006) and an increased PSMA/CD31 ratio (p = 0.038) compared to patients with tumor sizes < 4 cm in greatest dimension grossly. Higher-grade tumors (grades 2–3) had increased expression of raw PSMA (p = 0.039), PSMA/CD31 ratio (p = 0.034), and PSMA/vasculature ratio (p = 0.015) compared to grade 1 tumors. Biomarker expression among the total cohort with regard to increased raw PSMA and PSMA/CD31 ratios is shown in Figure 2. Additionally, patients with ≥ 4 mitoses per 10 high-power fields had increased PSMA/CD31 ratio (p = 0.024) and PSMA/vasculature ratio (p = 0.021) compared to patient samples with < 4 mitoses per 10 high-power fields.

**Biomarker Expression in De Novo Tumors**

Patients with de novo grade 1 meningiomas had no significant differences in raw PSMA expression (p = 0.821), raw CD31 (p = 0.754), PSMA/CD31 ratio (p = 0.958), or PSMA/vasculature ratio (p = 0.651) compared to de novo grade 2–3 tumors represented in Table 2. Of the de novo cohort, patient age ≥ 50 years old correlated with higher PSMA/CD31 ratio (p = 0.005) and PSMA/vasculature ratio (p = 0.013) compared to patients < 50 years of age. Males had a higher raw CD31 expression (p = 0.014) compared to females.

**Biomarker Expression in Relation to Localization**

The tumors in this cohort localize as follows: lobar (frontal, parietal, temporal, occipital), intraventricular, sphenoid, intraorbital/optic nerve, parasagittal, midline, posterior fossa, temporal fossa, frontal skull base, spine, and unspecified. According to the 2021 WHO brain tumor classification scheme (18), convexity and the majority of spinal meningiomas have similar molecular findings (loss of chromosome 22q and/or NF2 mutations), while skull base meningiomas demonstrate different mutations (AKT1, TRAF7, SMO, and PIK3CA). Accordingly, we analyzed 2 groups: one combining lobar, parasagittal, and spine into one group (57 cases), and sphenoid, optic nerve/intraorbital, posterior fossa, temporal fossa, and frontal skull base together in a second (“skull base”) group (16
cases). (“midline,” “intraventricular,” and “unspecified” were omitted from this analysis.) There were no significant differences in raw PSMA expression ($p = 0.286$), raw CD31 expression ($p = 0.714$), PSMA/CD31 ratio ($p = 0.191$), and PSMA/vasculature ratio ($p = 0.739$).

**TABLE 1. Computational Biomarkers in Relation to Clinical/Pathologic Features and Site**

| Biomarker                 | CD31 p  | PSMA p  | PSMA/CD31 p | PSMA/V p  |
|---------------------------|---------|---------|--------------|-----------|
| Age (96)                  | 0.263   | 0.010   | 0.010        | 0.002     |
| <50 (23)                  | 2.81    | 0.176   | 0.0855       | 0.0133    |
| ≥50 (73)                  | 1.66    | 0.972   | 0.373        | 0.0378    |
| Sex (96)                  | 0.222   | 0.442   | 0.607        | 0.418     |
| Male (36)                 | 2.67    | 0.741   | 0.391        | 0.0267    |
| Female (60)               | 1.59    | 0.596   | 0.202        | 0.0206    |
| Procedure (96)            | 0.876   | 0.135   | 0.262        | 0.217     |
| Craniotomy (82)           | 1.71    | 0.557   | 0.236        | 0.206     |
| Other (14)                | 1.99    | 1.83    | 0.953        | 0.0511    |
| Size (96)                 | 0.006   | 0.793   | 0.038        | 0.909     |
| <4 cm (55)                | 0.104   | 0.630   | 0.400        | 0.0225    |
| ≥4 cm (41)                | 2.67    | 0.603   | 0.162        | 0.0218    |
| Grade (96)                | 0.584   | 0.039   | 0.034        | 0.015     |
| Grade 1 (58)              | 1.93    | 0.483   | 0.219        | 0.0164    |
| Grade 2, 3 (38)           | 1.71    | 0.810   | 0.442        | 0.0361    |
| Ki-67 (85)                | 0.892   | 0.118   | 0.121        | 0.085     |
| <4% (33)                  | 1.64    | 0.314   | 0.139        | 0.0148    |
| ≥4% (52)                  | 1.94    | 0.697   | 0.319        | 0.0298    |
| Hypercellularity (73)     | 0.828   | 0.221   | 0.0165       | 0.362     |
| Present (32)              | 1.65    | 0.715   | 0.442        | 0.0234    |
| Absent (41)               | 1.94    | 0.386   | 0.201        | 0.0156    |
| Sheeting (59)             | 0.124   | 0.508   | 0.711        | 0.287     |
| Present (11)              | 3.06    | 0.386   | 0.201        | 0.0344    |
| Absent (48)               | 1.62    | 0.463   | 0.161        | 0.0179    |
| Small cell change (60)    | 0.827   | 0.733   | 0.916        | 1.000     |
| Present (14)              | 1.68    | 0.310   | 0.167        | 0.0150    |
| Absent (46)               | 1.94    | 0.581   | 0.208        | 0.0252    |
| Macronucleoli (62)        | 0.690   | 0.226   | 0.305        | 0.226     |
| Present (25)              | 1.66    | 0.559   | 0.278        | 0.0368    |
| Absent (38)               | 2.04    | 0.547   | 0.161        | 0.0213    |
| Necrosis (82)             | 0.963   | 0.304   | 0.197        | 0.274     |
| Present (36)              | 1.91    | 0.624   | 0.372        | 0.0435    |
| Absent (45)               | 1.94    | 0.575   | 0.208        | 0.0222    |
| Mitoses (89)              | 0.199   | 0.157   | 0.024        | 0.021     |
| <4 mitoses/10 HPF (66)    | 1.93    | 0.557   | 0.219        | 0.0200    |
| ≥4 mitoses/10 HPF (23)    | 1.29    | 0.972   | 1.65         | 0.0500    |
| Brain invasion (71)       | 0.639   | 0.436   | 0.332        | 0.668     |
| Present (11)              | 1.66    | 0.840   | 0.19         | 0.0225    |
| Absent (60)               | 1.93    | 0.463   |              | 0.0210    |
| Psammoma bodies (64)      | 0.605   | 0.172   | 0.354        | 0.066     |
| Present (36)              | 1.60    | 0.529   | 0.187        | 0.0209    |
| Absent (28)               | 2.66    | 0.801   | 0.283        | 0.0588    |
| Radiation (91)            | 0.228   | 0.369   | 0.095        | 0.390     |
| Yes (19)                  | 1.58    | 0.73    | 0.868        | 0.0214    |
| No (72)                   | 1.92    | 0.557   | 0.224        | 0.0213    |
| Site (73)                 | 0.714   | 0.286   | 0.191        | 0.739     |
| Convexity (57)            | 2.61    | 0.630   | 0.214        | 0.0253    |
| Skull base (16)           | 1.61    | 0.885   | 0.684        | 0.0211    

**Biomarker Expression in Relation to Recurrence**

Based on initial tumor biomarker expression, the following ROC curves were found: Raw CD31 had an AUC of 0.7278 with a SE of 0.05965 and 95% confidence interval between 0.6108 to 0.8447 ($p < 0.001$). Raw PSMA had an
AUC of 0.5642 with a SE of 0.0734 and a 95% confidence interval of 0.4202 to 0.7082 (p = 0.394). PSMA/CD31 AUC of 0.7843 with a SE of 0.05651 and a 95% confidence interval of 0.6736 to 0.8951 (p < 0.001). PSMA/vasculature had an AUC of 0.6456 with a SE of 0.06483 with a 95% confidence interval of 0.5185 to 0.7726 (p = 0.033).

The median raw CD31 expression was 1.675 in the initial and 1.40 in the paired recurrence specimens with a median difference of 0.1500 (p = 0.999). The median raw PSMA expression was 0.66 in the initial and 1.17 in the paired recurrence specimens with a median difference of 0.2215 (p = 0.2099). The median PSMA/CD31 ratio expression was 0.734 in the initial and 1.048 in the paired recurrence specimens with a median difference of 0.03019 (p = 0.7024). The raw PSMA and CD31 colocalization in an individual paired patient sample is shown in Figure 3. The median PSMA/vasculature ratio expression was 0.0387 in the initial and 0.0389 in the paired recurrence specimens with a median difference of 0.00042 (p = 0.999).

### TABLE 2. Computational Biomarkers in Relation to Clinical and Pathologic Features in De Novo Tumors

| Biomarker                          | CD31 p  | PSMA p  | PSMA/CD31 p | PSMA/V p  |
|-----------------------------------|---------|---------|-------------|-----------|
| Age (58)                          | 0.323   | 0.20    | 0.005       | 0.013     |
| <50 (12)                          | 3.56    | 0.121   | 0.0290      | 0.00526   |
| >50 (46)                          | 1.90    | 0.600   | 0.219       | 0.0238    |
| Sex (58)                          | 0.014   | 0.768   | 0.442       | 0.545     |
| Male (20)                         | 3.53    | 0.492   | 0.162       | 0.0220    |
| Female (38)                       | 1.59    | 0.411   | 0.186       | 0.0184    |
| Procedure (58)                    | 0.537   | 0.497   | 0.241       | 0.484     |
| Cranietomy (48)                   | 2.21    | 0.409   | 0.150       | 0.0185    |
| Other (10)                        | 1.89    | 1.01    | 0.391       | 0.0292    |
| Prior radiation (58)              | 0.921   | 0.260   | 0.360       | 0.087     |
| Yes (3)                           | 1.92    | 0.603   | 0.289       | 0.111     |
| No (55)                           | 1.94    | 0.417   | 0.184       | 0.0173    |
| Size (58)                         | 0.125   | 0.744   | 0.090       | 0.950     |
| <4 cm (30)                        | 1.61    | 0.493   | 0.236       | 0.0200    |
| ≥4 cm (28)                        | 2.74    | 0.411   | 0.106       | 0.0200    |
| Grade (58)                        | 0.754   | 0.821   | 0.958       | 0.651     |
| Grade 1 (42)                      | 2.23    | 0.434   | 0.186       | 0.0160    |
| Grade 2, 3 (16)                   | 1.81    | 0.495   | 0.182       | 0.0217    |
| Ki-67 (51)                        | 0.650   | 0.850   | 0.835       | 0.880     |
| <4% (23)                          | 2.91    | 0.436   | 0.138       | 0.0203    |
| ≥4% (28)                          | 2.59    | 0.330   | 0.133       | 0.0203    |
| Hypercellularity (49)             | 0.555   | 0.349   | 0.238       | 0.583     |
| Present (29)                      | 1.65    | 0.608   | 0.240       | 0.0228    |
| Absent (20)                       | 2.91    | 0.257   | 0.110       | 0.0133    |
| Sheet (41)                        | 0.176   | 0.322   | 0.711       | 0.487     |
| Present (8)                       | 4.23    | 0.583   | 0.194       | 0.0407    |
| Absent (33)                       | 2.51    | 0.257   | 0.103       | 0.0132    |
| Small cell change (41)            | 1.00    | 0.601   | 0.777       | 0.777     |
| Present (10)                      | 2.23    | 0.185   | 0.0872      | 0.00912   |
| Absent (31)                       | 2.91    | 0.559   | 0.138       | 0.0208    |
| Macromucleoloi (44)               | 0.379   | 0.448   | 0.656       | 0.443     |
| Present (17)                      | 3.50    | 0.436   | 0.132       | 0.0148    |
| Absent (27)                       | 2.86    | 0.411   | 0.108       | 0.0206    |
| Necrosis (51)                     | 0.469   | 0.512   | 0.500       | 0.512     |
| Present (20)                      | 2.74    | 0.493   | 0.213       | 0.0361    |
| Absent (31)                       | 2.67    | 0.436   | 0.138       | 0.0148    |
| Mitoses (57)                      | 0.531   | 0.876   | 0.639       | 0.557     |
| <4 mitoses/10 HPF (45)            | 2.51    | 0.436   | 0.205       | 0.0200    |
| ≥4 mitoses/10 HPF (12)            | 1.47    | 0.517   | 0.205       | 0.0350    |
| Brain invasion (48)               | 0.773   | 0.637   | 0.727       | 0.773     |
| Present (6)                       | 1.63    | 0.0821  | 0.174       | 0.0217    |
| Absent (42)                       | 2.66    | 0.411   | 0.161       | 0.0175    |
| Psammoma bodies (48)              | 0.788   | 0.703   | 0.543       | 0.439     |
| Present (25)                      | 1.94    | 0.559   | 0.138       | 0.0209    |
| Absent (23)                       | 2.81    | 0.436   | 0.214       | 0.0203    |
of 0.004605 (p = 0.4628). AUC for biomarkers and the Wilcoxon matched pairs signed rank test are shown in Figure 4.

**DISCUSSION**

The present study measured the expression of PSMA in meningiomas to explore the feasibility of targeting PSMA in initial diagnostic, subsequent PET/CT monitoring, and in the selective delivery of PSMA-targeted therapeutic agents. We found that 98.9% of meningioma specimens expressed PSMA within their endothelial cells. Additionally, across the total cohort PSMA expression was higher in tumors grades 2–3 when compared to grade 1 and in recurrent tumors compared to de novo tumors. As the vast majority of meningiomas are benign, the mainstay of therapy for grade 1 tumors is gross total resection. In tumors grades 2–3, patients are additionally treated with radiation therapy subsequent to surgical resection. Unfortunately, higher-grade tumors have increased aggressiveness, rates of recurrence, and refractoriness to therapy, including the presence of atypical features and a Ki-67 index of proliferation ≥4% (19). In tumors that recur, the standard of care relies on stabilization of disease by systemic therapies as there are no currently documented effective therapies for relapsed or recurrent tumors. Therefore, once confirming PSMA expression within the neovasculature of meningiomas, we sought to identify the variability of expression of PSMA within the neovasculature of recurrent tumors in paired patient samples. Our findings suggest that PSMA expression is not only limited to the tumor endothelial cells but extends within the vascular walls as highlighted by PSMA/vasculature ratios in recurrent patient cases, and that PSMA expression remains upregulated in recurrent tumors that have had prior radiation therapy.

Diagnostic imaging has made great changes to the early diagnosis and management of patients with PSMA-expressing tumors, namely those with a history of prostatic adenocarcinoma. 68GA-PSMA-11 was one of the first positron emitting radionuclides to be used in diagnostic imaging (15). Currently, the utility of 68GA PSMA-11 lies in the ability to localize within tumors that are somatostatin receptor (SSTR2)-positive (15). Thus, SSTR2-positive tumors, through labeling with PSMA ligands, can be targeted, resulting in an increased uptake of 68GA PSMA-11 (15). As the majority of meningiomas express SSTR2, it is expected that meningiomas also have uptake by 68GA PSMA-11 (15). Galldiks et al (20) showed a correlation between 68GA-DOTATATE-uptake and WHO grade in meningiomas but a major limitation to monitoring was a lack of tumor specificity and complete loss in anaplastic meningiomas. Additional studies by Sasikumar et al (15) concluded that 68GA PSMA-11 can be thought of as a potentially
useful imaging tool in the evaluation of brain lesions, as radiotracers targeting the enzyme PSMA could potentially target the neovasculature of brain tumors. These findings were confirmed by multiple studies that reported expression of PSMA in many solid tumors, primary gliomas, and brain metastases (6–10). In addition, case reports are becoming more frequent in which maintenance imaging of patients with a known history of prostate cancer with 68Ga PSMA-11 has led to the incidental finding of PSMA-avid meningiomas (4, 21).

Numerous studies have shown that PSMA has not been reported in normal vasculature and is the only specific neovascular target currently known (22). Additionally, PSMA has been reportedly expressed by tumor endothelium in nearly all solid tumor types without expression in adjacent tumor cells or the normal vasculature (22–24). Multiple phase I clinical trials utilizing combinations of antibody with JS91, which targets the extracellular domain of PSMA, have revealed selective targeting, good tumor localization, and tumor toxicity (22–24). These results suggest that PSMA may be a key regulatory agent of angiogenic activity, as preclinical data has demonstrated severely impaired angiogenesis in PSMA-null animals (23).

Our findings confirm that PSMA is expressed within the neovasculature of meningiomas. While de novo meningiomas had no significant expression of raw PSMA when comparing de novo grade 1 versus grades 2–3 tumors, the raw PSMA expression relative to grade was increased in recurrent tumors. This finding suggests that the angiogenesis of tumor neovascularization may increase with recurrence and may function as a driver of increased angiogenesis. As PSMA expression is increased in recurrent tumors, irrespective of prior radiation therapy, it is likely that PSMA is a driver of the neovascularization rather than as a tumor response to radiation therapy. Our findings show that in tumors that are recurrent, of higher grade, and have undergone radiation therapy, PSMA expression remains intact, and the raw expression is increased. Thus, as PSMA is unregulated in patients with recurrent tumor, the neovasculature is potentially targetable by PSMA directed therapies.

Our observed variability of PSMA staining between grade 1 tumors of differing subtypes needs to be more comprehensively studied. Of the de novo grade 1 tumors investigated, there were extremes of PSMA staining in relation to the variability of tumor neovasculature; namely, angiomatous samples...
were at the higher end of raw PSMA expression, while fibrous subtypes were at the lower end of the spectrum. Additionally, heterogeneity of tumor staining in the tissue sample should be studied more comprehensively. As the majority of de novo grades 1–3 tumors were acquired before the patients underwent radiation therapy, the intratumoral heterogeneity is most likely due to a combination of the proliferation of angiogenic vessels per subtype, and in cases of recurrence, to grade. As identified in prostate cancer, expression of PSMA is highest in higher-grade tumors (7).

The cutoff for patient age of 50 was instituted as the mean age of our entire cohort was 58 years old. Other variables, including the cutoff of <4 and ≥4 for both mitoses per 10 high-power fields and the rate of proliferation as determined by estimation or quantification of immunolabeling with Ki-67, were based on the 2021 CNS WHO criteria for evaluation of Ki-67 as a marker of tumor aggressiveness and recurrence and the number of mitoses as diagnostic criteria involved in the grading of meningiomas (18). Additional histological criteria, including brain invasion and the presence or absence of atypical features, were also evaluated as they are known grading criteria (18).

Our study has a few limitations. Although studies have correlated mitotic index with the mitosis marker phosphohistone H3 (pHH3) (25, 26), we do not utilize pHH3 immunohistochemistry at our institution. We use the Ki-67 labeling index. Neither pH3 nor Ki-67 IHC are official WHO histologic criteria for grading meningiomas (18). A recent meta-analysis has shown a good correlation between Ki-67 labeling index and prognosis (27). Molecular testing is also not part of our routine workup of meningiomas. Molecular analysis is becoming increasingly important in meningioma diagnostics, particularly for higher-grade tumors (18). Correlating PSMA expression with pHH3 immunohistochemistry and molecular testing in meningiomas are avenues for future research.

PSMA was a de novo tumor with poor preservation for immunohistochemical analysis.

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Machine learning was utilized in this study to completely objectify the results and eliminate human variance in light microscopic interpretation. The goal is to perform reproducible scoring of PSMA in meningioma along with other biomarkers from WSI. However, further research and validation of computational biomarkers is required before coming up with such a system for routine clinical pathologic use. One such project would be to correlate molecular imaging for PSMA with tissue expression to see if the PSMA positivity has relevance to the patient, particularly regarding anti-PSMA therapy. Determining cutoffs for PSMA expression could be coupled with more advanced machine learning and multi-omics approaches to predict treatment response to PSMA radioligand therapy.

PSMA radioligand therapy has emerged as a significant therapeutic in prostate cancer (28), where small-molecule PSMA inhibitors are labeled with both beta- and alpha-emitting radioisotopes, with different energy levels and path
lengths. Beta-emitting radioisotopes include lutetium-177 (177Lu), favored for use in prostate cancer secondary to minimal tissue penetration and a long half-life, which facilitates a higher degree of radiation. Radioligand therapy with 177Lu-PSMA-617 has been shown to prolong imaging-based survival when used in conjunction with standard of care for patients with PSMA-positive, advanced metastatic castration-resistant prostate cancer (29).

Lastly, since the current study focused mainly on banked tumor tissue, we did not look into the relationship between PSMA expression in meningioma and neuroimaging features such as peritumoral edema. Quantitative and qualitative information derived from perfusion MRI analyzing peritumoral edema may suggest the histological grade of meningioma preoperatively and potentially predict likely patient outcomes (30). This important correlation between imaging features and histopathological characteristics in meningioma will be studied in future clinical trials incorporating advanced neuroimaging techniques using PET/MRI hybrid and radiomics.

In conclusion, the present study suggests that PSMA expression in endothelial cells of meningiomas increases with recurrence and grade. These results provide a rationale for meningioma treatment in accordance with PSMA expression and call for clinical trial testing utilizing PSMA-targeted therapies as alternative diagnostic, therapeutic, and maintenance approaches for recurrent/refractory meningiomas.

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REFERENCES
1. Ostrom QT, Patil N, CiofﬁG, et al. CBTRUS statistical report: Primary brain and other central nervous system tumours diagnosed in the United States in 2013–2017. Neuro Oncol 2020;22:v1–96
2. Alruwaili AA, De Jesus O. Meningioma. Treasure Island, FL: StatPears Publishing LLC, 2022
3. Goldbrunner R, Stavrinou P, Jenkinson MD, et al. EANO guideline on the diagnosis and management of meningiomas. Neuro Oncol 2021;23:1821–34
4. Haemels M, Jentjens S, Cleeren F, et al. All that glitters is not prostate cancer: Incidental ﬁnding of PSMA-avid meningioma. Neuroradiol J 2020;23:79–80
5. Stock K, Steineust K, Wiesch R, et al. Neovascular prostate-speciﬁc membrane antigen expression is associated with improved overall survival under palliative chemotherapy in patients with pancreatic ductal adenocarcinoma. Biomed Res Int 2017;2017:1–8
6. Wernicke AG, Edgar MA, Lavi E, et al. Prostate-speciﬁc membrane antigen as a potential novel vascular target for treatment of glioblastoma multiforme. Arch Pathol Lab Med 2011;135:1486–9
7. Noma K, Nastorino S, Jiang P, et al. Prostate speciﬁc membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. Cancer Cell Int 2014;14:26
8. Tanjore Ramanathan J, Lehtipuro S, Sihvo H, et al. Prostate-speciﬁc membrane antigen expression in the vasculature of primary lung carcinomas associates with faster metastatic dissemination to the brain. J Cell Mol Med 2020;24:6916–27
9. Chang SS, Reuter VE, Heston WD, et al. Five different anti-prostate-speciﬁc membrane antigen (PSMA) antibodies conﬁrm PSMA expression in tumor-associated neovascularaure. Cancer Res 1999;59:3192–8
10. Heitkötter B, Trautmann M, Grünewald I, et al. Expression of PSMA in tumor neovasculature of high grade sarcomas including synovial sarcoma, rhabdomyosarcoma, undifferentiated sarcoma and MPNST. Oncotarget 2017;8:4268–76
11. Jain TK, Jouis AG, Kumar VS, et al. Incidental detection of tracer avidity in meningioma in (68)Ga-PSMA PET/CT during initial staging for prostate cancer. Rev Esp Med Nucl Imagen Mol 2017;36:133–4
12. Calabria F, Gangemi V, Gillà D, et al. (64)Cu-PSMA uptake in meningioma: A potential pitfall of a promising radiotracier. Rev Esp Med Nucl Imagen Mol 2017;36:335–6
13. Bilgin R, Ergül N, Čermik TF. Incidental meningioma mimicking metastasis of prostate adenocarcinoma in 68Ga-labeled PSMA ligand PET/CT. Clin Nucl Med 2016;41:956–8
14. Ristau BT, O’Reeke DS, Bacich DJ. The prostate-speciﬁc membrane antigen: Lessons and current clinical implications from 20 years of research. Urol Oncol 2014;32:272–9
15. Sasikumar A, Joy A, Pillar MR, et al. Diagnostic value of 68Ga PSMA-11 PET/CT imaging of brain tumors-preliminary analysis. Clin Nucl Med 2017;42:e41–8
16. Bankhead P, Loughrey MB, Fernández JA, et al. QuPath: Open source software for digital pathology image analysis. Sci Rep 2017;7:16878
17. Shnaya A, Momota R, Shiratsuchi E, et al. Architecture of the subendothelial elastic ﬁbers of small blood vessels and variations in vascular type and size. Microsc Microanal 2013;19:406–14
18. Sahm F, BP CE, Mawrin C, et al. Meningioma. In: Louis DN, ed. WHO Classiﬁcation of Tumours: Central Nervous System Tumours, 5th Edition. Lyon, France: IARC Press, 2021:283–97
19. Perry A, Stafford SL, Scheithauer BW, et al. The prognostic signiﬁcance of MIB-1, p53, and DNA ﬂow cytometry in completely resected primary meningiomas. Cancer 1998;82:2262–9
20. Galldiks N, Albert NL, Sommerauer M, et al. PET imaging in patients with meningioma-report of the RANO/PET Group. Neuro Oncol 2017;19:1576–87
21. Courtney M, Johnston C, Nasoodi A. Meningioma uptake of (68)Galium-PSMA-11 as a pitfall on positron emission tomography/computer tomography. Acta Radiol Open 2021;10:2058460120981001
22. Milowsky MI, Nanus DM, Kostakoglu L, et al. Vascular targeted therapy with anti-prostate-speciﬁc membrane antigen monoclonal antibody J591 in advanced solid tumors. J Clin Oncol 2007;25:540–7
23. Morris MJ, Pandit-Taskar N, Divgi CR, et al. Phase I evaluation of J591 as a vascular targeting agent in progressive solid tumors. Clin Cancer Res 2007;13:2707–13
24. Milowsky MI, Nanus DM, Kostakoglu L, et al. Phase I trial of yttrium-90-labeled anti-prostate-speciﬁc membrane antigen monoclonal antibody J591 for androgen-independent prostate cancer. J Clin Oncol 2004;22:2522–31
25. Ribalta T, McCutcheon IE, Aldape KD, et al. The mitosis-speciﬁc antibody anti-phosphohistone-H3 (PHH3) facilitates rapid reliable grading of meningiomas according to WHO 2000 criteria. Am J Surg Pathol 2004;28:1532–6
26. Kim YJ, Jeong HS, Chung T, et al. The value of phosphohistone H3 as a proliferation marker for evaluating invasive breast cancers: A comparative study with Ki67. Oncotarget 2017;8:65064–76
27. Liu N, Song SY, Jiang JB, et al. The prognostic role of Ki-67/MIB-1 in meningioma: A systematic review with meta-analysis. Medicine (Baltimore) 2020;99:e18644
28. Jones W, Griffiths K, Barata PC, et al. PSMA theranostics: Review of the current status of PSMA-targeted imaging and radioligand therapy. Cancers (Basel) 2020;12:1367
29. Sartor O, de Bonj D, Chi KN, et al. Lutetium-177–PSMA-617 for metastatic castration-resistant prostate cancer. N Engl J Med 2021;385:1091–103
30. Yan PF, Yan L, Hu TT, et al. The potential value of preoperative MRI texture and shape analysis in grading meningiomas: A preliminary investigation. Transl Oncol 2017;10:570–7