

Prostate-Specific Membrane Antigen Expression in Neovasculature of Glioblastoma Multiforme

Abstract

Background: Glioblastoma (GBM) is the most malignant brain tumor with a poor prognosis that can be very difficult to cure, and the current treatment options have no optimal outcomes. Hence, it is essential to find new treatment modalities. Histologically, this tumor has high microvascular density that makes it desirable for vascular target agent drugs. Prostate-specific membrane antigen (PSMA) is a novel antigen with unique features that expresses in the vascular endothelium of some malignant tumors. **Materials and Methods:** Formalin-fixed paraffin-embedded tissues from sixty patients who underwent GBM tumor resection from 2012 to 2016 were evaluated for the expression of PSMA by immunohistochemistry. Sections were also assessed for the extent and intensity of endothelial staining in tumor microvessels and for clinicopathologic factor correlation. **Results:** A considerable PSMA expression level was detected in 66% of the cases, and the intensity was strong and moderate in 63%. There was no significant correlation neither between PSMA expression with tumor site, presence of necrosis, and endothelial proliferation nor with age and sex. **Conclusion:** The expression of PSMA in GBM, as observed in the current study, may suggest a new role of PSMA-targeted therapy and indicate more investigations focused on complementary treatment strategies that specifically target tumor vasculature.

Keywords: Glioblastoma, prostate-specific membrane antigen, tumor vasculature

Introduction

Glioblastoma (GBM) is an astrocytic WHO Grade IV tumor, with a median survival time of 14.6 months after diagnosis.^[1,2] It is the most common brain and central nervous system malignancy, which accounts for 45.2% of malignant primary brain tumors and diagnosed in the median age of 64 years, 1.6 times higher in men than women.^[3]

Surgical resection followed by radiation therapy and chemotherapy is the current standard therapy. However, because of their infiltrating nature, the complete neurosurgical resection of these tumors is impossible,^[4] and current GBM treatments have not improved overall patient survival rates. Therefore, new modalities for treatment are required.^[5]

GBM in nature is a solid brain tumor with high vascular density, and patients with high tumor microvascular densities (MVDs) have shorter postoperative survival time than the patients with low MVDs, suggesting that

the tumor vasculature is an important factor of tumor progression.^[6]

This feature makes a favorable field for vascular targeting agents (VTAs) as a complementary therapy. The VTAs all lead to rapid reductions in tumor blood flow and extensive necrosis in tumors.^[7]

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with foliate hydrolase I and neurocarboxypeptidase activities.^[8] Originally, PSMA expression was identified in prostate cancer cells,^[9] and it has been shown to be expressed in the endothelial cells of neovasculature from a variety of tumors, including breast and renal cell carcinoma but not normal endothelium.^[10,11]

Therefore, it could be beneficial to use PSMA-targeted agents for tumor treatment with PSMA expressing vasculature.

PSMA is qualitatively distinct from other neovascular targets, such as vascular endothelial growth factor, endoglin, or the integrins involved in the general process of angiogenesis, that is not specific to tumor

Parvin Mahzouni,
Mitra Shavakhi

From the Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Address for correspondence:

Dr. Mitra Shavakhi,
Department of Pathology,
School of Medicine, Isfahan
University of Medical Sciences,
Hezar Jarib Avenue,
Isfahan, Iran.
E-mail: mitra.shv@gmail.com

Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_209_18

Quick Response Code:



How to cite this article: Mahzouni P, Shavakhi M. Prostate-Specific Membrane Antigen Expression in Neovasculature of Glioblastoma Multiforme. *Adv Biomed Res* 2019;8:18.

Received: October, 2018. **Accepted:** January, 2019.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

vasculature. PSMA expression has not been reported in normal vasculature and represents the specific currently known neovascular target. This specificity makes PSMA an ideal target for delivery of a cytotoxic agent designed to tumor vasculature destruction.^[12] Some studies have revealed the effectiveness of anti-PSMA monoclonal antibody (mAb) J591 in the treatment of tumors expressing PSMA.^[12-14]

Therefore, in this study, we aimed to show the PSMA expression and its intensity in GBM tumors in patients with GBM referred to Alzahra Hospital from 2012 to 2016.

Materials and Methods

In this study, formalin-fixed paraffin-embedded tissues of sixty patients who underwent tumor resection from 2012 to 2016 for GBM were retrieved from the archive of Alzahra Hospital, Isfahan, Iran. The Medical Ethics Committee of the Isfahan University of Medical Sciences approved the study protocol (research project number: 395820). The inclusion criteria were the adequate material available in the paraffin blocks for immunohistochemistry (IHC) and in the case of improperly fixed specimens, the questionable cases or if the relevant clinical history was not available, the samples were excluded. Immunohistochemical staining was performed on the GBM specimens' vessels for PSMA expression in tumor endothelium. The tissue samples for CD31 were also stained to verify vessels. From each case, H & E-stained histologic sections were reexamined by a skilled neuropathologist, and also features such as presence of necrosis and endothelial proliferation were recorded.

The sequential sections showing the most viable tumor were selected for IHC.

Antibodies/reagents

Anti-PSMA mAb clone SP29, peroxidase block, target retrieval solution, peroxidase-labeled polymer conjugated to anti-rabbit immunoglobulin, and DAB+ chromogen were purchased from Biogenex company, CA, USA

The mAb to human CD31 was obtained from Dako Company (DAKO, CA, USA).

Staining procedure

For PSMA staining, paraffin sections were deparaffinized, and rehydrated sections were placed in target retrieval solution (pH 9.0) and heated in a water bath from 95°C to 99°C for 30 min prior to the immunostaining. For CD31 staining, the deparaffinized and rehydrated sections were placed in 0.01 M citrate retrieval solution (pH 6.0) and heated in a pressure cooker for 1 min prior to the immunostaining. The sections were washed in Tris-buffered saline-Tween 20. Peroxidase block was incubated for 5 min. After washing in Tris-buffered saline-Tween 20, mAb SP29 was incubated on the sections for 1 h at room temperature. Antibody binding was detected using peroxidase-labeled

polymer conjugated to anti-rabbit immunoglobulins and DAB+ chromogen. The sections were counterstained with 10% hematoxylin.

Prostate adenocarcinoma tissue was considered as a positive control and normal brain tissue as a negative control.

Assessment

Immunostained sections were assessed for the extent and intensity of endothelial staining in tumor microvessels. PSMA extent of vascular endothelial staining (percent staining) was evaluated and scored by an expert neuropathologist as follows:

- <5% staining of vessels (none)
- 6%–25% staining of vessels (minimal)
- 26%–50% staining of vessels (moderate)
- 51%–75% staining of vessels (strong)
- 76%–100% staining of vessels (very strong).

The staining intensity was scored as follows:

- 1+: faint, barely perceptible staining
- 2+: moderately intense staining that was readily apparent at low-power magnification [Figure 1]
- 3+: maximum intensity staining [Figure 2].

When there was heterogeneity in the intensity of staining, the score was considered based on the predominant pattern.

Statistical analysis

After data collection, the analysis of quantitative and qualitative data was performed using the Statistical Package for the Social Sciences software version 22 (IBM corporation, New York, USA).

The relationship of variables was investigated using Student's *t*-test and for categorical variables Pearson's test or Fisher's exact test used. *P* < 0.05 was considered statistically significant.

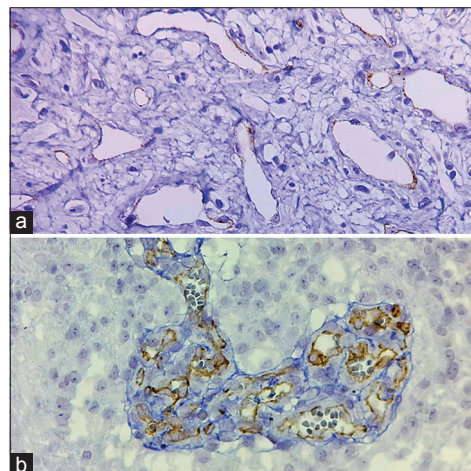


Figure 1: Prostate-specific membrane antigen expression in glioblastoma was scored 1+ (a) and 2+ (b) (×400)

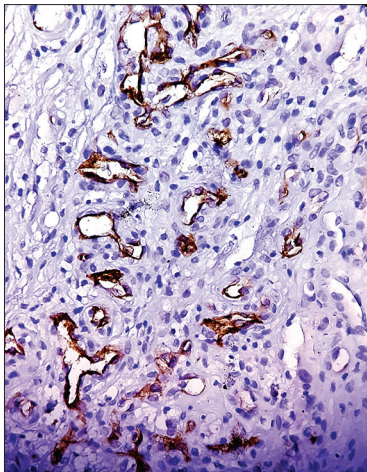


Figure 2: Prostate-specific membrane antigen expression in glioblastoma was scored 3+ (×400)

Results

In this study, sixty patients in the age range of 19–80 years and a median age of 53 years were enrolled, of which 40 patients were male (66.7%) and 20 were female (33.3%), with a male:female ratio of 2:1.

The frontal and parietal lobes were the most commonly affected sites, representing 30% and 28.3%, respectively.

Moreover, tumor ischemic necrosis was detected in 80% of cases and endothelial proliferation in 86.7% of cases.

PSMA staining was positive in the endothelium of 66.7% of tumor cells, and the intensity was strong and moderate in 63% of cases [Tables 1 and 2]. There was no staining in the vessels of any normal brain specimen.

No significant correlation was seen between PSMA expression with tumor site, neither with age nor sex [Tables 3 and 4].

Statistical analysis revealed $P = 0.55$ and 0.56 for the correlation between PSMA status with necrosis and endothelial proliferation, respectively. These values were considered to be statistically insignificant. Hence, there was no correlation between the PSMA status with necrosis [Table 5] and endothelial proliferation.

Discussion

The present study demonstrated remarkable PSMA expression in sixty surgical tissue samples of GBM. The PSMA immunostaining was distinct in tumor vascular endothelium but barely positive in glomeruloid microvascular proliferation. This finding is in concordance with the result of Bychkov *et al.* which claimed that PSMA expression is not directly related to endothelial cell proliferation in thyroid tumors.^[15] Further investigations are needed to evaluate the causes of such findings.

Table 1: Extent of vascular staining

Variable	n (%)
Extent of vascular staining	
<5%	20 (33.3)
6%-25%	12 (20)
26%-50%	9 (15)
75%-51%	16 (26.7)
76%-100%	3 (5)

Table 2: Intensity of vascular staining

Variable	n (%)
Intensity of vascular staining	
0	15 (25)
Mild	7 (11.7)
Moderate	23 (38.3)
Severe	15 (25)

Table 3: Gender distribution in glioblastoma

Gender	PSMA negative, n (%)	PSMA positive, n (%)	P
Male	14 (35)	26 (65)	0.7
Female	6 (30)	14 (70)	

PSMA: Prostate-specific membrane antigen

Table 4: Age distribution in glioblastoma

Variable	PSMA negative		PSMA positive		P
	Mean	SD	Mean	SD	
Age	53.6	11.6	53.2	16.5	0.93

PSMA: Prostate-specific membrane antigen, SD: Standard deviation

Table 5: Presence of necrosis in glioblastoma

Variable	PSMA negative, n (%)	PSMA positive, n (%)	P
No necrosis	4 (36.4)	7 (63.6)	0.55
Necrosis	16 (33.3)	32 (66.7)	

PSMA: Prostate-specific membrane antigen

Comparison of the demographic features following observations showed that the number of males exceeded females. Furthermore, frontal and parietal lobes were the most common sites of occurrence. These findings are consistent with the literature.^[16-18]

In the present study, ischemic necrosis and/or pseudo palisading necrosis was detected in 80% of patients, which is less than the results reported by Homma *et al.* (87%).^[19]

There is a wide variation in the PSMA expression in GBMs, which varies from 6% to 100%.

We detected PSMA expression in 60% of the investigated cases, and the intensity was strong and moderate in 63% of cases.

Wernicke *et al.* evaluated the expression of PSMA among 32 GBM specimens and revealed that all specimens exhibited staining for PSMA to a variable extent. Of these, 69% had more than 51% vascular staining for

PSMA, and the intensity of staining was 2+ (moderate) to 3+ (maximum) in most of the specimens.^[20]

In another study, Nomura *et al.*^[21] had evaluated the PSMA expression in blood vessels of gliomas and breast cancer metastases to brain and concluded that highly angiogenic IV graded gliomas show intense PSMA staining.

Mhaweche-Fauceglia *et al.*, in a study on GBM samples investigated by IHC using microarray, revealed 49 of 52 (94%) negative results of PSMA. Two samples had weakly and one had moderately positive results with no strong staining.^[20]

Recently, Saffar *et al.* compared PSMA expression in different grades of glioma and reported positive staining in 40.7% of high-grade glial tumor.^[22]

Controversial results in different studies might be due to the use of different types of monoclonal antibodies and different IHC methods or the lack of standardization in scoring system.

This study had some limitations; the criteria for scoring PSMA staining were not well defined in the literature and hence, the results of the study might differ from those of various other studies.

Conclusion

Expression of PSMA in GBM, as observed in the current study, may suggest a new role for PSMA-targeted therapy and indicate more investigations focusing on complementary treatment strategies that specifically target tumor vasculature.

Financial support and sponsorship

This article is derived from proposal no. 395820, Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, *et al.* The 2016 World Health Organization classification of tumors of the central nervous system: A summary. *Acta Neuropathol* 2016;131:803-20.
2. Purow B, Schiff D. Advances in the genetics of glioblastoma: Are we reaching critical mass? *Nat Rev Neurol* 2009;5:419-26.
3. Ostrom QT, Gittleman H, Farah P, Ondracek A, Chen Y, Wolinsky Y, *et al.* CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro Oncol* 2013;15 Suppl 2:ii1-56.
4. Juratli TA, Schackert G, Krex D. Current status of local therapy in malignant gliomas – A clinical review of three selected approaches. *Pharmacol Ther* 2013;139:341-58.
5. Carlsson SK, Brothers SP, Wahlestedt C. Emerging treatment strategies for glioblastoma multiforme. *EMBO Mol Med* 2014;6:1359-70.
6. Kim WY, Lee HY. Brain angiogenesis in developmental and

- pathological processes: Mechanism and therapeutic intervention in brain tumors. *FEBS J* 2009;276:4653-64.
7. Thorpe PE. Vascular targeting agents as cancer therapeutics. *Clin Cancer Res* 2004;10:415-27.
8. Carter RE, Feldman AR, Coyle JT. Prostate-specific membrane antigen is a hydrolase with substrate and pharmacologic characteristics of a neuropeptidase. *Proc Natl Acad Sci U S A* 1996;93:749-53.
9. Horoszewicz JS, Kawinski E, Murphy GP. Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. *Anticancer Res* 1987;7:927-35.
10. Chang SS, Reuter VE, Heston WD, Bander NH, Grauer LS, Gaudin PB, *et al.* Five different anti-prostate-specific membrane antigen (PSMA) antibodies confirm PSMA expression in tumor-associated neovasculature. *Cancer Res* 1999;59:3192-8.
11. Liu H, Moy P, Kim S, Xia Y, Rajasekaran A, Navarro V, *et al.* Monoclonal antibodies to the extracellular domain of prostate-specific membrane antigen also react with tumor vascular endothelium. *Cancer Res* 1997;57:3629-34.
12. Milowsky ML, Nanus DM, Kostakoglu L, Sheehan CE, Vallabhajosula S, Goldsmith SJ, *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.
13. Morris MJ, Pandit-Taskar N, Divgi CR, Bender S, O'Donoghue JA, Nacca A, *et al.* Phase I evaluation of J591 as a vascular targeting agent in progressive solid tumors. *Clin Cancer Res* 2007;13:2707-13.
14. Fung EK, Cheal SM, Fareedy SB, Punzalan B, Beylergil V, Amir J, *et al.* Targeting of radiolabeled J591 antibody to PSMA-expressing tumors: Optimization of imaging and therapy based on non-linear compartmental modeling. *EJNMMI Res* 2016;6:7.
15. Bychkov A, Vutrapongwatana U, Tepmongkol S, Keelawat S. PSMA expression by microvasculature of thyroid tumors – Potential implications for PSMA theranostics. *Sci Rep* 2017;7:5202.
16. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee S. Glioblastoma multiforme: A review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pac J Cancer Prev* 2017;18:3-9.
17. Philips A, Henshaw DL, Lamburn G, Carroll MJ. Brain tumours: Rise in glioblastoma multiforme incidence in England 1995 and 2013;2015 suggests an adverse environmental or lifestyle factor. *J Environ Public Health* 2018;2018:10.
18. Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, *et al.* Brain tumor epidemiology: Consensus from the brain tumor epidemiology consortium. *Cancer* 2008;113:1953-68.
19. Homma T, Fukushima T, Vaccarella S, Yonekawa Y, Di Patre PL, Franceschi S, *et al.* Correlation among pathology, genotype, and patient outcomes in glioblastoma. *J Neuropath Exp Neurol* 2006;65:846-54.
20. Wernicke AG, Edgar MA, Lavi E, Liu H, Salerno P, Bander NH, *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.
21. Nomura N, Pastorino S, Jiang P, Lambert G, Crawford JR, Gymnopoulos M, *et al.* Prostate specific membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. *Cancer Cell Int* 2014;14:26.
22. Saffar H, Noohi M, Tavangar SM, Saffar H, Azimi S. Expression of prostate-specific membrane antigen (PSMA) in brain glioma and its correlation with tumor grade. *Iran J Pathol* 2018;13:45-53.

To,
The Editor

Covering Letter

Submission of Manuscript for publication

Dear Sir,

We intend to publish an article entitled

in your journal.

On behalf of all the contributors I will act and guarantor and will correspond with the journal from this point onward.

Prior presentation of the data reported in this manuscript:

Organisation

Place

Date

We have done sufficient work in the field to justify authorship for this manuscript.

We hereby transfer, assign, or otherwise convey all copyright ownership, including any and all rights incidental thereto, exclusively to the journal, in the event that such work is published by the journal.

Thank you,

Yours' sincerely,

Name of corresponding contributor

Signature

Title of the manuscript:

Title Page

Type of manuscript:

Running title:

Contributors:

	First name	Middle name initial	Last name	Highest academic degree	Names of departments and institutions (including city and state)	Email addresses
1						
2						
3						
4						
5						
6						

Corresponding Author:

Name:

Address:

Phone numbers:

Facsimile numbers:

E-mail address:

Total number of pages:

Total number of tables:

Total number of figures:

Total number of supplementary files:

Word counts: For abstract:

For the text:

Acknowledgement:

Conflict of interest:

Financial Support:

Contribution details (to be ticked marked as applicable):

Contributors' form

	Contributor 1	Contributor 2	Contributor 3	Contributor 4	Contributor 5	Contributor 6
Concepts						
Design						
Definition of intellectual content						
Literature search						
Clinical studies						
Experimental studies						
Data acquisition						
Data analysis						
Statistical analysis						
Manuscript preparation						
Manuscript editing						
Manuscript review						
Guarantor						