

# STAT3, VEGF, and PSMA Expression Patterns in Malignant Peripheral Nerve Sheath Tumors, Malignant Melanomas, and Glioblastomas: Does Staining Percentage and Intensity Have an Effect on Survival?

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**ABSTRACT:** Malignant peripheral nerve sheath tumors (MPNSTs), glioblastomas (GBMs), and malignant melanomas (MMs) are neural crest–originating aggressive tumors with a poor prognosis. Signal transducer and transcription activator 3 (STAT3) plays a role in many biological processes, including cell life and proliferation, the acute phase response, chronic inflammation, autoimmunity, metabolism, and cancer progression. It is also known to be a proto-oncogenic transcription factor. Vascular endothelial growth factor (VEGF) is one of the most potent proangiogenic stimuli ever identified. It mediates tumor neovascularization, and is associated with angiogenesis and lymphangiogenesis. The prostate-specific membrane antigen (PSMA) folate hydrolase I, despite its name, has been found in tissues other than the prostate. It is overexpressed in prostate cancer cells and several other cancers, and has the potential to be a target for radioligand therapy. We investigated the value of STAT3, VEGF and PSMA immunohistochemical expression patterns and their effects on survival in MPNSTs, GBMs, and MMs. Their expression patterns were evaluated in 25 MPNSTs, 27 GBMs, and 25 MM cases. All GBM cases stained positively for STAT3 and VEGF. In the other groups, the staining patterns were heterogeneous. None of the cases showed positive staining with PSMA. There was no statistically significant difference in survival between cases with differing VEGF and STAT3 staining patterns in the MPNST and MM groups, but there was an increase in mortality as the VEGF score increased in the GBM group. The suppression of VEGF and STAT3 may be a promising avenue for treatment of MPNSTs, GBMs, and MMs, although further research is needed.

**KEY WORDS:** VEGF, STAT3, PSMA, malignant peripheral nerve sheath tumor, malignant melanoma, glioblastoma

## I. INTRODUCTION

Malignant peripheral nerve sheath tumors (MPNSTs) are rare; their incidence is 0.0001%, and they constitute 5%–10% of all soft tissue sarcomas.<sup>1</sup> They have no specific histological criteria or immunohistochemical or molecular diagnostic markers. Malignant melanoma (MM) is an aggressive type of skin cancer. Although it constitutes less than 5% of all skin cancers, it causes the majority of deaths.<sup>2</sup> Glioblastomas (GBMs, World Health Organization Grade IV) are one of the most common and deadliest brain cancers in adults, and their diffuse infiltrative nature makes them difficult to treat.<sup>3</sup> MPNSTs, GBMs, and MMs are neural crest–originating tumors. MPNSTs and MMs in particular

share common morphological and immunohistochemical markers, creating a challenge for differential diagnosis.

The STAT family of transcription factors consists of seven members: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6.<sup>4</sup> Signal transducer and transcription activator 3 (STAT3) plays a role in many biological processes, including cell life and proliferation, acute phase response, chronic inflammation, autoimmunity, metabolism, and cancer progression; it is also known to be proto-oncogenic.<sup>5,6</sup> STAT3 activation was first identified in plasma cell myeloma and later shown to be present in various human cancer cell lines and tumors.<sup>7</sup>

VEGFs are recognized as endothelial cell-specific mitogens and mediators of tumor

neovascularization, and they are associated with angiogenesis and lymphangiogenesis.<sup>8</sup> VEGF is not limited to tumor cells; it is also produced by platelets, macrophages, renal mesangial cells, and keratinocytes.<sup>9</sup> The VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, placenta growth factor (PlGF), and endocrine gland-derived vascular endothelial growth factor (EG-VEGF).<sup>9</sup>

The growth and spread of sarcomas depends on angiogenesis, and markers that may be related to tumor angiogenesis in patients with sarcoma have been previously studied. Tumoral VEGF expression is associated with stage, grade, and prognosis in patients with soft tissue sarcoma.<sup>10</sup> It has been shown that VEGF blockade may be involved in the treatment of many cancers.<sup>8</sup>

Prostate-specific membrane antigen (PSMA), folate hydrolase I, is also known as glutamate carboxypeptidase II, a type II membrane protein. Despite its name, it has been found in tissues other than the prostate, such as brain, small bowel, some neuroendocrine cells, salivary glands, and proximal renal tubular cells.<sup>11</sup> Since it is overexpressed in prostate cancer cells, it is thought that it may be a target for radioligand therapy. Additionally, PSMA is expressed in renal carcinoma, colon carcinoma, soft tissue sarcomas among others. It has a role in peri- and endotumoral endothelial cells in neovascularization.<sup>12</sup>

We investigated the value of these markers' immunohistochemical expression patterns and their effects on survival in aggressive tumors with poor prognoses, such as MPNSTs, GBMs, and MM.

## II. MATERIALS AND METHODS

The study was performed according to the tenets of the Helsinki Declaration and obtained approval by the Ethics Committee of the Duzce University Medical School (prot. no./EC of 2018/178, October 15, 2018). It was supported financially by the BAP unit (Scientific Research Projects of Duzce University).

A total of 25 MPNST, 27 GBM, and 25 MM cases were included in the study. The inclusion criteria were (1) diagnosis of MPNST, GBM, or

MM; (2) sufficient clinical history; and (3) sufficient pathology material for analysis. All cases were recruited from the archives of the pathology departments of the Duzce and Uludag University Schools of Medicine. Demographic data such as age, gender, and survival time after diagnosis were obtained from pathology reports and patient files.

Immunohistochemistry was performed to determine STAT3, VEGF, and PSMA expression, using the same formalin-fixed, paraffin-embedded tissue blocks. To ensure the presence of sufficient tumor content on every histological section, the samples were reviewed by a pathologist. All specimens were cut into 3–4- $\mu$ m-thick sections for immunohistochemical staining using a fully automated assay according to the manufacturer's instructions. The antibodies used were Phospho-STAT3 (Tyr705) [RM261] Conc. 0.1 mL (1:1000–10000); VEGF [VG1] Conc. 0.5 mL (1:100–200); and PSMA [EP192] Conc. 0.1 mL (1:100–200). The assay was performed using the Ventana Benchmark XT (Ventana-Roche Diagnostics, Meylan, France).

PSMA immunoreactivity was evaluated as negative or positive. STAT3 and VEGF were scored between 0 and 3 points according to staining percentage and intensity. No staining was scored 0; light staining, 1; moderate staining, 2; and strong staining, 3. According to a semiquantitative grading score total absence was scored 0; focal expression up to 20% IHC staining of the core area was scored 1+; intermediate expression in 20%–50% of the core area was scored 2+; and diffuse expression in > 50% of the core area was scored 3+.

MM cases were evaluated according to the CAP protocol (pTNM, AJCC, 8th Edition), considering Breslow thickness, and divided into four groups (< 1, > 1–2 mm, > 2–4 mm, and > 4 mm).<sup>13</sup>

In the statistical analysis of the data, categorical variables were analyzed using the Pearson chi-square, Fisher's exact, or Fisher-Freeman-Halton test as appropriate, according to the expected count rule. When significant differences were observed between groups, subgroup comparisons were performed using the Bonferroni method. Statistical analyses were performed using the IBM SPSS v.22

statistical package and  $p$  values of  $< 0.05$  were considered statistically significant.

### III. RESULTS

Groups consisted of 27 GBMs, 25 MPNSTs, and 25 MMs. Male-to-female ratios were 15:12, 11:14, and 13:12, respectively. Median ages were 68 (41–85), 57 (7–87), and 56 (27–85) years, respectively.

All GBM cases were positive for STAT3; 5 showed focal expression up to 20%, 15 were positively stained between 20%–50%, and 7 showed diffuse expression over 50%. The majority of the GBM cases (20/27) showed strong staining for STAT3, 6 showed moderate staining, and 1 showed light staining. GBM cases showed highly intense staining for VEGF consistently with STAT3. All GBM cases were stained for VEGF, and 23/27 were diffusely stained. Only 1 case was lightly stained, with the remaining cases showing 2+ (10/27) or 3+ (16/27) staining intensity.

Staining patterns for STAT3 in the MM group were heterogeneous: there was no staining in 3 cases, focal positivity in 8, intermediate positivity in 8, and diffuse positivity in 6 cases. Ten cases (40%) showed moderate staining for STAT3 in the MM group, 6 showed strong expression, and 6 were stained lightly. VEGF staining was more dominant, with 88% (22/25) of MMs showing diffuse expression and 12% (3/25) showing staining between 20% and 50%. Staining intensity was also stronger for VEGF, with 16/25 cases showing strong staining and 6/25 of cases showing moderate staining.

In the MPNST group, 9/25 (36%) were negative for STAT3, 5/25 (20%) were focally stained, 10/25 (40%) showed intermediate positivity, and only 1 case (4%) was stained diffusely. Six, 4, and 6 cases showed light, intermediate, and strong expression of STAT3, respectively. For VEGF, there was no staining in 1 case, focal positivity in 2 cases, intermediate positivity in 8 cases; 14 cases showed more than 50% immune reactivity. Staining intensity was lower in comparison with other groups, 15 cases showed weak staining, 8 cases were moderately stained, and only 1 case showed strong staining.

PSMA was negative in all cases in the MPNST, GBM, and MM groups. Although there was no

significant difference in survival in terms of STAT3 staining percentage and score in the MPNST and GBM groups, there was a statistically significant difference in the MM group ( $p = 0.002$ ). Considering that the Breslow thickness may have affected survival in MM cases, we re-evaluated STAT3 immunoexpression and did not find a statistically significant relationship between the differences in staining intensity and percentage and survival. There was no statistically significant difference in survival in terms of VEGF score and percentage of staining in the MPNST or MM group. While the percentage of staining with VEGF did not impact survival in the GBM group ( $p = 0.211$ ), a statistically significant difference was found between the staining score and the survival time ( $p = 0.040$ ). An increase in mortality rates was seen as the VEGF score increased in the GBM group. The relevant data are shown in Tables 1 and 2 and Figs. 1 and 2.

### IV. DISCUSSION

STAT3 plays a role in many biological processes, including cell survival and proliferation, and is known as a protooncogenic transcription factor.<sup>5</sup> Pharmacological inhibition of this pathway may contribute favorably to prognosis.<sup>14</sup> STAT3 activation is found in approximately 50%–90% of tumors.<sup>7</sup> There are conflicting findings in the literature regarding the effects of STAT3 expression on the prognosis of various tumors. Some researchers have found high nuclear expression of STAT3 to be associated with poor prognosis in diffuse large B-cell lymphoma, and others have found it to be associated with better prognosis in colorectal carcinomas.<sup>15,16</sup>

Although the ability of cancer cells to proliferate uncontrollably and resist apoptosis is essential in tumor development, progressive development is not possible without blood vessels that provide oxygen and nutrients to rapidly growing tumor tissue. VEGF is the most potent proangiogenic stimulus ever identified.<sup>7</sup> Previous research has reported that STAT3 participates directly in the regulation of VEGF gene transcription.<sup>17,18</sup> This association between VEGF and STAT3 may explain the similar statistical results for the immunostaining of these markers in our study.

**TABLE 1:** STAT3 and VEGF expression patterns in MPNSTs, GBMs, and MMs

|                   | MPNST (n = 25) | GBM (n = 27)  | MM (n = 25) |
|-------------------|----------------|---------------|-------------|
| STAT3 %           |                |               |             |
| 0                 | 9 (36%)        | 0 (0%)        | 3 (12%)     |
| 1                 | 5 (20%)        | 5 (18.5%)     | 8 (32%)     |
| 2                 | 10 (40%)       | 15 (55.6%)    | 8 (32%)     |
| 3                 | 1 (4%)         | 7 (25.9%)     | 6 (24%)     |
| STAT3 score       |                |               |             |
| 0                 | 9 (36%)        | 0 (0%)        | 3 (12%)     |
| 1                 | 6 (24%)        | 1 (3.7%)      | 6 (24%)     |
| 2                 | 4 (16%)        | 6 (22.2%)     | 10 (40%)    |
| 3                 | 6 (24%)        | 20 (74.1%)    | 6 (24%)     |
| VEGF %            |                |               |             |
| 0                 | 1 (4%)         | 0 (0%)        | 0 (0%)      |
| 1                 | 2 (8%)         | 1 (3.7%)      | 0 (0%)      |
| 2                 | 8 (32%)        | 3 (11.1%)     | 3 (12%)     |
| 3                 | 14 (56%)       | 23 (85.2%)    | 22 (88%)    |
| VEGF score        |                |               |             |
| 0                 | 1 (4%)         | 0 (0%)        | 0 (0%)      |
| 1                 | 15 (60%)       | 1 (3.7%)      | 2 (8%)      |
| 2                 | 8 (32%)        | 10 (37%)      | 7 (28%)     |
| 3                 | 1 (4%)         | 16 (59.3%)    | 16 (64%)    |
| Ex (n = 24/27/24) | 19/24 (79.2%)  | 21/27 (77.8%) | 12/24 (50%) |

MPNSTs are associated with poor prognosis and limited treatment options due to their aggressive biological behavior and insensitivity to chemoradiotherapies.<sup>19</sup> Previous studies have suggested that the EGFR-STAT3 pathway plays a role in malignant transformation in MPNSTs.<sup>20</sup> Rad et al. found that cell migration, invasion, and tumor formation are suppressed in MPNST cell cultures with inhibition of STAT3, and concluded that STAT3 may be an important trigger of tumorigenesis in NF-1-related MPNSTs.<sup>21</sup> Wu et al. reported that a specific JAK2/STAT3 inhibitor delayed the development of MPNST in rats and concluded that therapies targeting the JAK/STAT3 pathway may be beneficial.<sup>10</sup> In our series, 9 of 25 (36%) MPNSTs showed no expression of STAT3. Moreover, there was no significant difference in survival in terms of STAT3 staining percentage and score in the MPNST group ( $p = 0.233$ ). While STAT3 may be associated with tumorigenesis and be a potential treatment target, we did not observe any effects of its expression on MPNST prognosis.

The growth and spread of sarcomas depends on angiogenesis.<sup>22</sup> A number of markers that may be related to tumor angiogenesis in patients with sarcoma have been studied. Tumoral VEGF expression is associated with stage, grade, and prognosis in patients with soft tissue sarcoma.<sup>10</sup> Gesundheit et al. suggested that malignant transformation and tumorigenesis is angiogenesis-dependent in NF-1-related MPNSTs, and that VEGF may play a role as an angiogenic switch.<sup>23</sup> Anti-angiogenic therapies would seem to be essential and logical options for all cancer types; however, pharmacological inhibition of VEGF has not shown miraculous outcomes, despite some promising combination therapies.<sup>22</sup> Friedrich et al. reported no staining in 2 tumors (22.2%), poor staining in 3 (33.3%), and medium staining in 4 (44.4%) of 9 MPNSTs, and found an increased density of vessels and VEGF expression compared with benign cases.<sup>24</sup> Similarly, we found increased VEGF expression in more than 50% of MPNST cases. However, VEGF staining patterns were not associated with statistically significant changes in

**TABLE 2:** Analysis of prognostic factors due to STAT3 staining patterns in melanoma cases

| STAT3 %                 | None<br>(n = 3) | < 20%<br>(n = 8) | 20%–50%<br>(n = 8) | > 50%<br>(n = 6) | p            |
|-------------------------|-----------------|------------------|--------------------|------------------|--------------|
| Ex*                     | 2 (100)         | 5 (62.5)         | 3 (37.5)           | 2 (33.3)         | <b>0.603</b> |
| Breslow thickness       |                 |                  |                    |                  | <b>0.159</b> |
| < 1 mm                  | 0 (0.0)         | 1 (12.5)         | 3 (37.5)           | 1 (16.7)         |              |
| 1–2 mm                  | 0 (0.0)         | 3 (37.5)         | 0 (0.0)            | 2 (33.3)         |              |
| 2–4 mm                  | 0 (0.0)         | 3 (37.5)         | 2 (25.0)           | 2 (33.3)         |              |
| 4 mm                    | 3 (100)         | 1 (12.5)         | 3 (37.5)           | 1 (16.7)         |              |
| Lymphovascular invasion | 1 (33.3)        | 1 (12.5)         | 2 (25.0)           | 0 (0.0)          | <b>0.621</b> |
| TIL**                   |                 |                  |                    |                  | <b>0.361</b> |
| None                    | 0 (0.0)         | 0 (0.0)          | 2 (25.0)           | 2 (33.3)         |              |
| Nonbrisk                | 3 (100)         | 7 (87.5)         | 4 (50.0)           | 4 (66.7)         |              |
| Brisk                   | 0 (0.0)         | 1 (12.5)         | 2 (25.0)           | 0 (0.0)          |              |
| Growth phase            |                 |                  |                    |                  | <b>0.660</b> |
| Vertical                | 1 (33.3)        | 5 (62.5)         | 2 (25.0)           | 3 (50.0)         |              |
| Radial                  | 0 (0.0)         | 0 (0.0)          | 2 (25.0)           | 1 (16.7)         |              |
| Vertical + radial       | 2 (66.7)        | 3 (37.5)         | 4 (50.0)           | 2 (33.3)         |              |
| Clark level             |                 |                  |                    |                  | <b>0.663</b> |
| 2                       | 0 (0.0)         | 1 (12.5)         | 2 (25.0)           | 1 (16.7)         |              |
| 3                       | 0 (0.0)         | 2 (25.0)         | 2 (25.0)           | 1 (16.7)         |              |
| 4                       | 2 (66.7)        | 5 (62.5)         | 2 (25.0)           | 4 (66.7)         |              |
| 5                       | 1 (33.3)        | 0 (0.0)          | 2 (25.0)           | 0 (0.0)          |              |

\*Due to 24 patients' survival, data are available, n of comparison for exitus 2-8-8-6.

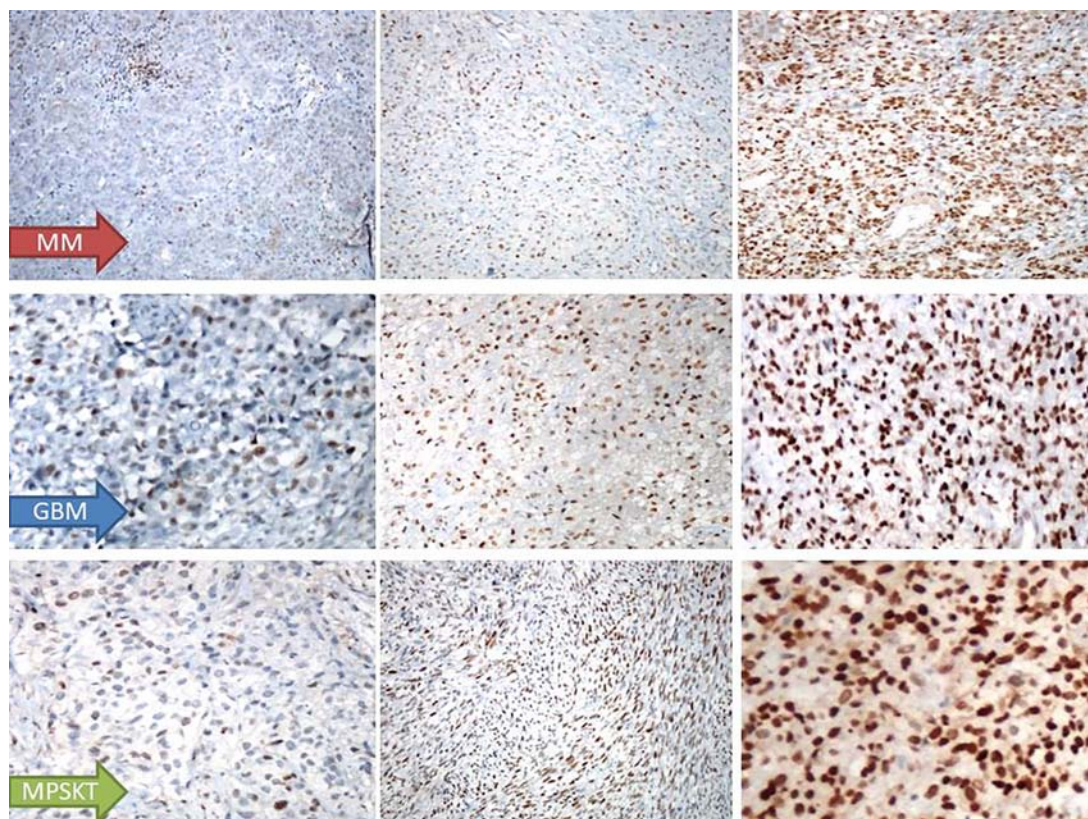
\*\*Tumor-infiltrating lymphocytes.

survival. This result may have been related to the small number of MPNST cases.

Although melanomas constitute less than 5% of all skin cancers worldwide, they cause the majority of deaths.<sup>2</sup> Swoboda et al. suggested that STAT3 has a role in melanoma fate, promoting invasion and metastatic spread.<sup>25</sup> STAT3 can also play an important role in both malignant cell growth and immune function.<sup>14</sup> In our series, 26 of 29 melanoma cases showed STAT3 immunoexpression to various degrees. We found a statistically significant difference in survival in terms of STAT3 staining score and percentage in the MM group, but when re-evaluated by Breslow thickness, no statistically significant effect of STAT3 expression on survival was detected. Targeting STAT3 in melanoma models seems effective at inducing tumor regression, inhibiting angiogenesis, preventing metastasis, and stimulating the immune system to act against the tumor.<sup>7</sup> Lee et al. concluded that STAT3 expression does not affect overall survival in tumor

samples from stage IV melanoma patients.<sup>6</sup> Despite these results, selectively targeting STAT3 still seems a promising avenue for melanoma treatment, especially for patients who are resistant to immunotherapies.<sup>26–28</sup>

Although VEGF expression is not seen in normal melanocytes, upregulation is present in malignant melanoma cells.<sup>29</sup> However, the size of the role that angiogenic factors play in the clinical course and prognosis of MM is still unclear.<sup>30</sup> The correlation of VEGF expression patterns with angiogenesis in MM tissues is also controversial.<sup>31</sup> In our series, all of the MMs showed diffuse expression of VEGF, supporting the upregulation of VEGF in MM cells. The staining intensity of VEGF was stronger in MM cells in comparison to that in MPNSTs and GBMs, but there was no statistically significant difference in survival in terms of VEGF staining intensity. Nevertheless, increased VEGF staining percentage and intensity is suggestive of the possible benefits of anti-VEGF therapies.



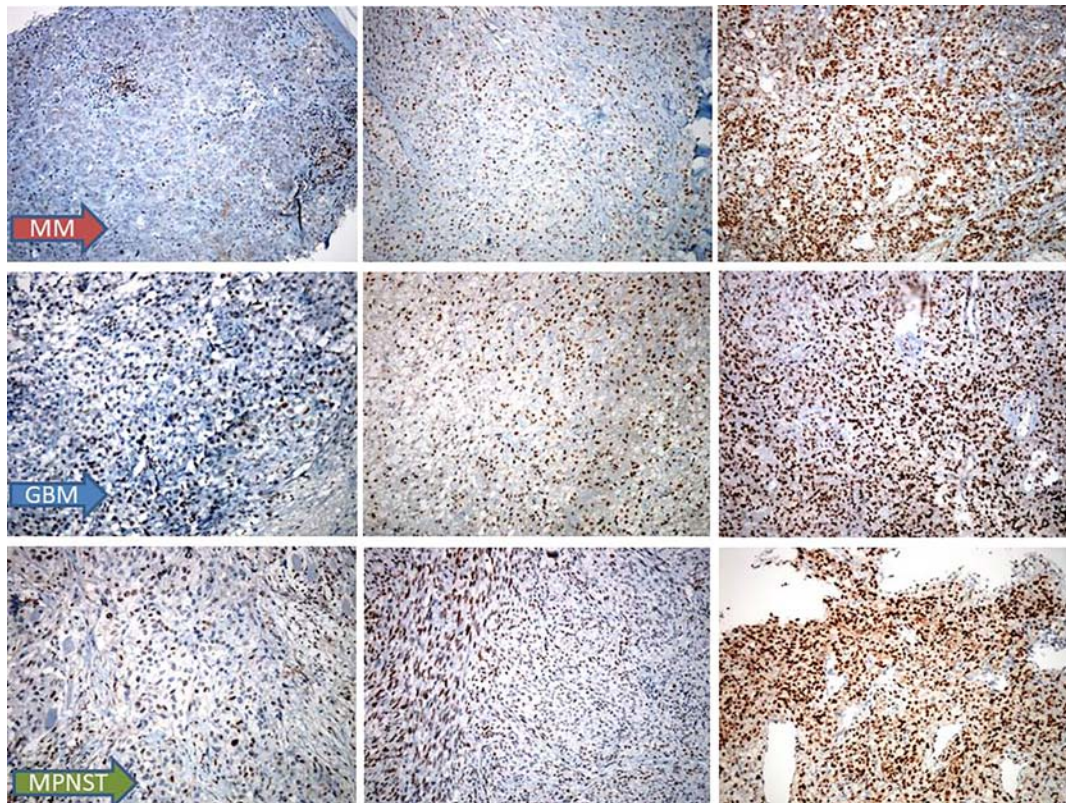
**FIG. 1:** Immunohistochemical staining patterns of STAT3 (left to right, score 1-2-3)

GBM is one of the most aggressive tumors in the central nervous system, and its intratumoral heterogeneity has a negative influence on treatment.<sup>32,33</sup> Activation of STAT3 contributes to tumor progression by weakening antitumor immunity.<sup>32</sup> Numerous studies in the literature have put forth clinical and experimental evidence of the effects of STAT3 as a potential target in GBM treatment.<sup>3,32-36</sup> All GBM cases in our series showed immunoreactivity with STAT3 and 20/27 (74.1%) showed strong positivity, but there was no significant difference in survival in terms of STAT3 staining percentage and score.

Turkowski et al. reported that VEGF shows suppressive effects on the innate immune response and that high levels of VEGF reduce infiltration of microglia and macrophages by up to 50%.<sup>37</sup> Soubéran et al. investigated whether it is possible to reverse this effect by depleting VEGF in mice, and showed that blockade of VEGF induces an adaptive immune response.<sup>38</sup> Loureiro et al. found consistent VEGF

overexpression in the majority of GBMs in their study, but expression patterns did not correlate with patients' overall survival, similar to our results.<sup>39</sup> Nevertheless, we observed an increase in mortality rates as the VEGF score increased in the GBM group. Several antiangiogenic therapy options have been tested in clinical trials, aiming to find a new or complementary therapy in addition to existing cancer treatments. Chemotherapies combined with antiangiogenic therapies have shown favorable results, but resistance and toxicity still constitute an obstacle.

PSMA is physiologically expressed in normal prostate tissue. Since it is overexpressed in prostate cancer cells, it is thought that it may be a target for radioligand therapy. Heitkötter et al. showed that PSMA expression plays a role in tumor-associated neovascularization in soft tissue/bone tumors. Strong neovascular PSMA expression was found in 19.05% of MPSCTs.<sup>40</sup> Previous studies have shown



**FIG. 2:** Immunohistochemical staining patterns of VEGF (left to right, score 1-2-3)

that PSMA expression is found in many malignancies, such as non-small-cell lung carcinomas, pancreatic ductal adenocarcinomas, and malignant thyroid neoplasms.<sup>40–42</sup> PSMA expression was not found in any of our cases.

The major limitation of this study is the lack of information about therapies targeting STAT3 and VEGF. Although we evaluated the association between staining patterns and survival, the relationship between immunoexpression and response to the targeted therapies remains unclear.

## V. CONCLUSION

Individual therapy planning is crucial, especially for high-grade tumors with poor prognosis such as GBMs, MMs, and MPNSTs. The search for new molecules that can act as a beacon of hope in treatment continues. Overall, VEGF and STAT3 play a significant role in tumorigenesis and angiogenesis

in several cancers, and therapies suppressing these molecules are still promising despite further research being required. We think that future work on this subject in larger series will be worthwhile.

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