The Incidence, Correlation with Tumor-Infiltrating Inflammation, and Prognosis of Phosphorylated STAT3 Expression in Human Gliomas

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Abstract

Purpose: The signal transducer and activator of transcription 3 (STAT3) is frequently overexpressed in most cancers, propagates tumorigenesis, and is a key regulator of immune suppression in cancer patients. We sought to determine the incidence of phosphorylated STAT3 (p-STAT3) expression in malignant gliomas of different pathologic types, whether p-STAT3 expression is a negative prognostic factor, and whether p-STAT3 expression influences the inflammatory response within gliomas.

Methods: Using immunohistochemical analysis, we measured the incidence of p-STAT3 expression in 129 patients with gliomas of various pathologic types in a glioma tissue microarray. We categorized our results according to the total number of p-STAT3–expressing cells within the gliomas and correlated this number with the number of infiltrating T cells and T regulatory cells. We then evaluated the association between p-STAT3 expression and median survival time using univariate and multivariate analyses.

Results: We did not detect p-STAT3 expression in normal brain tissues or low-grade astrocytomas. We observed significant differences in the incidence of p-STAT3 expression between the different grades of astrocytomas and different pathologic glioma types. p-STAT3 expression was associated with the population of tumor-infiltrating immune cells but not with that of T regulatory cells. On univariate analysis, we found that p-STAT3 expression within anaplastic astrocytomas was a negative prognostic factor.

Conclusions: p-STAT3 expression is common within gliomas of both the astrocytic and oligodendroglial lineages and portends poor survival in patients with anaplastic astrocytomas. p-STAT3 expression differs significantly between gliomas of different pathologic types and grades and correlated with the degree of immune infiltration.

A key transcription factor, signal transducer and activator of transcription (STAT3), has been shown to drive the fundamental components of tumorigenesis and metastasis by preventing apoptosis (by increasing survivin, BCL-XL, and MCL1 expression) and enhancing proliferation (by increasing c-Myc and cyclin D1/D2 expression; ref. 1), angiogenesis (by increasing vascular endothelial growth factor and hypoxia-inducible factor-1α expression), invasion (by increasing matrix metalloproteinase-2 and matrix metalloproteinase-9 expression), and metastasis (2, 3). Growth factors and cytokines, including interleukin (IL)-6, can activate Janus kinase 2, which subsequently activates STAT3 by phosphorylating the tyrosine residue in the STAT3 transactivation domain (4). Phosphorylated STAT3 (p-STAT3) then translocates into the nucleus and induces the expression of a variety of target genes. IL-6, which is expressed in the central nervous system (CNS) under a variety of conditions, such as hypoxia (5), traumatic and metabolic injury (6), and inflammation (7), has been shown to attract T cells to the CNS (8). Specifically, IL-6 signaling by means of STAT3 is tightly linked to the homing and migrational capacity of T cells (8).

STAT3 is constitutively overexpressed in a variety of cancers; for instance, in melanoma, 81% of CNS metastases express activated STAT3 (9). Preclinical studies using decoy antisense STAT3 oligonucleotides, dominant-negative vectors, and small-molecule inhibitors have provided convincing evidence that STAT3 is highly relevant to the growth and survival of many tumor types (10–20), including gliomas (14), *in vitro* and *in vivo*.

STAT3 is also a key regulator of immune suppression; it is believed to regulate anti-inflammatory responses by suppressing macrophage activation (21–23) and limiting inflammatory responses (24). The tumor microenvironment induces STAT3 activity in tumor-associated immune cells (25, 26). STAT3 activity within natural killer cells and neutrophils directly

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Translational Relevance

Signal transducer and activator of transcription (STAT3) has been found to play a significant and varied role within the tumor microenvironment, involving not only tumor cells, but also the immune system through dendritic cells, T regulatory cells (Tregs), and others as well. Our hypothesis was that phosphorylated STAT3 (p-STAT3) expression in human gliomas influences inflammatory responses and is an independent negative prognostic indicator for patient survival. This article details that p-STAT-3 expression differs between tumor pathology, is greatest in astrocytic tumors, and is predictive of shortened overall survival (OS) in patients with anaplastic astrocytoma. In addition, p-STAT-3 expression closely correlates with the degree of intratumoral T-cell infiltration. In conclusion, p-STAT-3 is frequently observed in gliomas and represents a viable therapeutic target for a variety of small-molecule inhibitors of p-STAT-3 that are in preclinical development.

reduces their cytotoxicity, whereas STAT3 activity in dendritic cells reduces the expression of MHC II, CD80, CD86, and IL-12, rendering the dendritic cells unable to stimulate T cells and generate antitumor immunity (27). By ablating STAT3 in hematopoietic cells in tumor-bearing mice, Kortylewski et al. (27) showed that there was marked enhancement of activated and functional T cells, natural killer cells, and dendritic cells. STAT3 ablation in only the hematopoietic cells resulted in marked antitumor effects *in vivo*, indicating that STAT3 expression in immune cells restrains the immune cell eradication of the tumor. Furthermore, IL-2 has been shown to regulate FoxP3 expression in human CD4+CD25+ Tregs by inducing STAT3 binding of the first intron of the *FoxP3* gene (28). We have previously shown that STAT3 blockade within immune cells restores immune responses (29) and inhibits Treg induction (30).

We previously examined whether the glioma-infiltrating Treg population acted as a prognostic marker in patients with gliomas (31). However, we did not see a significant relationship for glioma cell infiltration, which was likely due to the presence of multiple redundant immunosuppressive mechanisms, including Tregs. Recent data have emerged that p-STAT3 may be a key component to the overall immune suppression seen in cancer patients. Thus, the purpose of this study was to determine the incidence of p-STAT3 expression among patients with gliomas of various pathologic types and grades and to determine if p-STAT3 expression was a prognostic marker, especially because p-STAT3 confers properties of enhanced tumorgenesis. We hypothesized that the presence of p-STAT3 would be associated with a poor prognosis within specific glioma pathologies. Finally, we sought to determine whether p-STAT3 expression was correlated with enhanced infiltration of glioma by T cells and, specifically, Tregs.

Materials and Methods

Glioma tissue microarray slides. We used a previously described (32) glioma tissue microarray (TMA) that included tissue sections from 53 patients with WHO grade IV glioblastomas (GBM), 17 with WHO grade III anaplastic astrocytomas (AA), 3 with WHO grade II low-grade astrocytomas, 16 with WHO grade II oligodendrogliomas, 15 with WHO grade III anaplastic oligodendrogliomas (AO), 6 with WHO grade II mixed oligoastrocytomas (MOA), 12 with WHO grade III anaplastic MOAs (AMOA), and 7 with WHO grade IV gliosarcomas. The study neuropathologist (GNF) gathered the tissue sections and confirmed the tumor pathology based on the archived paraffin-blocked tissue sections. The TMA also contained normal brain tissues (white matter, cortex, and cerebellum), which were obtained from surgical specimens, excluding autopsy specimens, containing parenchyma that overlaid deep metastases but that were not themselves involved by the neoplasm. We conducted our study under the institutional review board-approved protocol LAB03-0228 at The University of Texas M. D. Anderson Cancer Center.

Immunohistochemical analysis of p-STAT3 expression, CD3+ T cells, CD8+ T cells, and Tregs. Formalin-fixed, paraffin-embedded sections of the glioma TMA were first deparaffinized in xylene and rehydrated in ethanol. We blocked the endogenous peroxidase with 0.3% hydrogen peroxide/methanol for 10 min at room temperature before beginning antigen retrieval. We did antigen retrieval for p-STAT3 by immersing the sections in a citrate-buffered solution (pH 6.0) and heating the sections in a microwave for 20 min. The sections were then cooled to room temperature for 40 min. For antigen retrieval of FoxP3 staining, we autoclaved the sections in 10 mmol/L citrate buffer (pH 6.0) for 10 min at 121°C. We did antigen retrieval for CD3 and CD8 expression by placing the sections in an electric kitchen pot filled with ~800 mL of 0.05% citraconic anhydride solution (pH 7.4; Immunosaver; Nisshin EM Co.) for 35 min at 100°C. The sections were then cooled to room temperature for 20 min and washed 6 times in PBS solution. After blocking with a protein block serum-free solution (DAKO), we added diluted anti–p-STAT3 (tyrosine^{705}) antibody (1:50; Cell Signaling Technology), a primary antibody to CD3 (clone SK7 8-11, 1:100; DAKO) or CD8 (clone 144B, 1:20; DAKO), or an antibody to FoxP3 (1:20; provided by Dr. Nobuyoshi Hiraoka, Pathology Division, National Cancer Research Institute, Tokyo, Japan; ref. 33) to the tissue arrays and incubated the specimens overnight in a humidified box at 4°C. We subjected the slides to biotin-labeled secondary antibody staining (biotinylated link universal solution; DAKO) for 60 min at room temperature. Finally, we added streptavidin-horseradish peroxidase (DAKO) and incubated the slides for 30 min at room temperature. We used diaminobenzidine (DAKO) as the chromogen, and we stopped color development by gently dipping slides in distilled water. The nuclei were then counterstained with hematoxylin. We used a human melanoma TMA (9) as a positive control for p-STAT3 staining, and we used MDACC banked human lymph nodes and tonsils as a positive control for CD3, CD8, and FoxP3 staining. Omitting the primary antibody from the immunohistochemical analysis and replacing it with protein block serum-free solution (DAKO) acted as the negative control for CD3 and CD8 staining. For p-STAT3 staining, we used normal goat serum (Santa Cruz Technology) as a negative isotype control.

Three independent observers (MA-G, DSY, GNF) quantitatively evaluated p-STAT3 expression and lymphocyte infiltration by analyzing the cores using high-power fields (max, ×40 objective and ×10 eyepiece) of each specimen. The observers examined each sample in duplicate from different areas of the same tumor in a blinded fashion. Each observer recorded the absolute number of cells with positive staining for p-STAT3 seen per 1-mm diameter core. The duplicate numbers were then averaged for the final number of p-STAT3–expressing cells and lymphocytes per surgical specimen. If there were discrepancies between the recorded numbers, the observers recounted the number of cells with positive staining in each specimen, and the neuropathologist (GNF) conducted the final arbitration. We minimized potential mismatching of the data by staining an intact microarray with H&E and identifying the correct location of each tissue core by visually matching the tumors based on their unique histologic elements.

Table 1. Demographic characteristics of patients with glioma stratified according to pathology

Pathology	Age (y)			KPS			Median survival time (mo)*
	Median	Minimum	Maximum	Median	Minimum	Maximum	
O	38.5	7.0	55.0	100	80	100	99.8
MOA	42.5	24.0	52.0	100	70	100	—†
AO	40.0	25.0	59.0	90	70	100	—†
AMOA	35.5	22.0	47.0	100	80	100	89.2
LGA	33.0	4.0	44.0	95	90	100	166.7
AA	49.0	24.0	91.0	90	90	100	27.7
GS	51.0	23.0	68.0	80	60	100	4.4
GBM	56.0	17.0	77.0	90	50	100	13.8

Abbreviations: GS, gliosarcoma; KPS, Karnofsky performance score; LGA, low-grade astrocytoma.
*Based on Kaplan-Meier estimates.
† Not analyzed due to rarity.

Statistical analysis. We conducted an equal proportion examination of tumor grade, pathologic tumor type, and glial lineage (astrocytic versus oligodendroglial; ref. 34). We calculated the Kaplan-Meier product-limit OS probability estimates (35) and did log-rank tests (36) to determine whether there was an association between OS and p-STAT3 expression (versus none), tumor grade, astrocytic and oligodendroglial lineage, and age. For each fitted OS regression model, we eliminated the nonsignificant variables in a step-down fashion using a P value cutoff of 0.10. A P value of <0.05 was considered significant.

Results

Study population. Tissue sections from 129 patients with gliomas were included in the TMA. The median age of the patients was 44 years (range, 4-91 years). Most patients (97%) had a Karnofsky performance score of ≥70, with a median Karnofsky performance score of 90 at the time of diagnosis (range, 50-100). Table 1 summarizes the patients' demographic characteristics and is stratified according to the patients' pathologic diagnoses, age, Karnofsky performance score, and OS time. The patients' demographic characteristics in our study did not differ significantly from the demographic characteristics of patients with gliomas in previous studies examining prognostic markers (32, 37, 38). Of the GBM and AA cases, 19 (36%) and 6 (35%) were recurrent, respectively. Of the patients with GBM, 9 (17%) had received prior chemotherapy and 11 (21%) had received prior radiation therapy. Table 2 summarizes the overall composition of the glioma TMA, and Fig. 1 shows the immunohistochemical staining of p-STAT3 and CD3+ T-cell infiltration.

Incidence of p-STAT3 expression varies according to tumor grade in astrocytomas. To determine whether oligodendrogliomas and astrocytomas expressed p-STAT3, we stained the tumor specimens with an anti–p-STAT3 antibody and determined the number of cells with positive nuclear staining. We did not observe p-STAT3 expression in the normal brain tissue specimens (n = 5) or in the patients with WHO grade II low-grade astrocytomas (n = 3; Table 3; Fig. 1). In patients with WHO grade III AAs (n = 17), 9 (53%) expressed p-STAT3, and in patients with WHO grade IV GBMs or gliosarcomas (n = 60), 32 (53%) expressed p-STAT3.

We did not observe an increase in p-STAT3 expression corresponding with an increase in tumor grade in either the oligodendrogliomas or MOAs. Specifically, 38% of the patients with WHO grade II oligodendrogliomas (n = 16) had p-STAT3 expression, and 40% of the patients with WHO grade III AOs (n = 15) had p-STAT3 expression, indicating the incidence of p-STAT3 expression did not increase with increasing tumor grade in oligodendrogliomas. This trend is further supported by our finding that 100% of the patients with WHO grade II MOAs (n = 6) had p-STAT3 expression, whereas only 58% of the patients with WHO grade III AMOAs (n = 12) had p-STAT3 expression.

Incidence of p-STAT 3 expression varies according to tumor pathology. We observed significant differences in p-STAT3 expression according to the pathologic type of the tumor. Specifically, the presence of any p-STAT3 staining was most often observed in MOAs (100%), followed by GBMs (53%) and AAs (53%; Table 3; Fig. 1). The pathologic types with the lowest incidence of p-STAT3 staining were low-grade astrocytomas (0%) followed by oligodendrogliomas (38%; Table 3).

Number of p-STAT3–expressing cells varies according to tumor grade in astrocytomas and oligodendrogliomas. Although the incidence of p-STAT3 expression was not significantly different between patients with WHO grade III AAs and patients with WHO grade IV GBMs, the number of cells within the gliomas that expressed p-STAT3 increased. Specifically, in patients with WHO grade II low-grade astrocytomas, there were no p-STAT3–expressing cells per core, which increased to a mean of 5.6 p-STAT3–expressing cells per core (SD, 7.6; range, 0-23.0) for patients with WHO grade III AAs, and 11.7 cells per core (SD, 24.4; range, 0-133.5) for patients with WHO grade IV GBMs. Similarly, among patients with WHO grade II oligodendrogliomas, there was a mean of 4.3 cells per core (SD, 7.7;

Table 2. Composition of the glioma TMA

Lineage	Pathology	No. of patients (%)
Oligodendroglial (n = 49)	O	16 (12.4%)
	MOA	6 (4.7%)
	AO	15 (11.6%)
	AMOA	12 (9.3%)
Astrocytic (n = 80)	LGA	3 (2.3%)
	AA	17 (13.2%)
	GS	7 (5.4%)
	GBM	53 (41.1%)

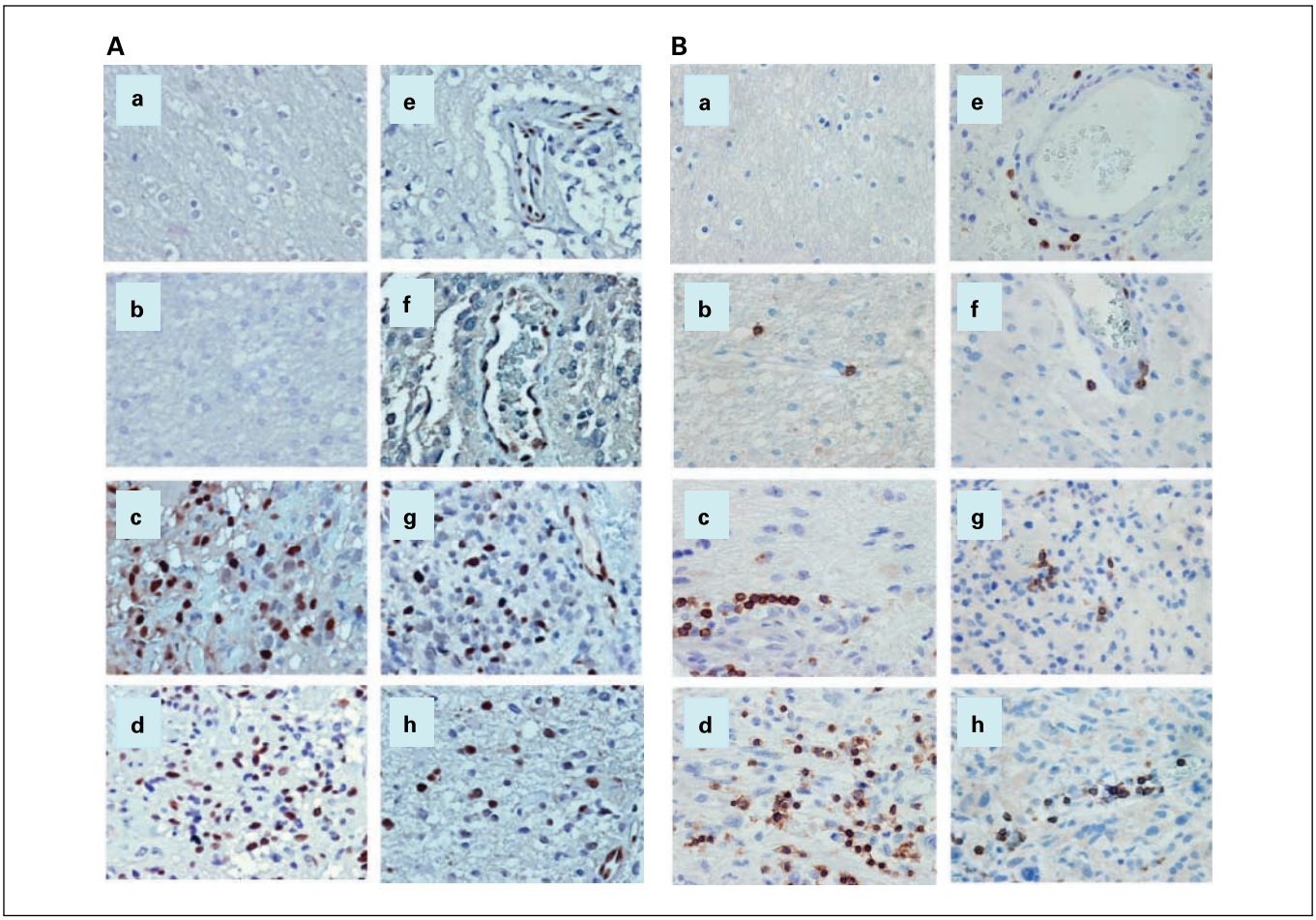

Fig. 1. Immunohistochemical staining of human glioma tissue sections demonstrating p-STAT3 and CD3+ lymphoid cells. The p-STAT3 staining was confined to the nucleus, whereas CD3 staining was noted on the cell surface. *A*, the number of p-STAT3 – expressing cells was more evident in astrocytic, higher-grade gliomas. *B*, CD3 staining showed high numbers of infiltrative CD3+ T cells, also within higher-glioma grades. All images were taken at a magnification of ×400. Normal brain (*a*), low-grade astrocytoma (*b*), AA (*c*), GBM multiforme (*d*), oligodendroglioma (*e*), MOA (*f*), AO (*g*), and AMOA (*h*).

range, 0-23.5), which increased to 17.5 cells per core (SD, 41.0; range, 0-153.0) for patients with WHO grade III AOs. This propensity did not hold true for the patients with WHO grade II MOAs, in whom the mean was 67.9 cells per core (SD, 55.2; range, 9.0-136.0), when compared with patients with WHO grade III AMOAs, in whom the mean was 13.3 cells per core (SD, 27.0; range, 0-85.5).

Correlation of p-STAT3 expression and T-cell infiltration. Across all tumor pathologies and tumor grades, increased p-STAT3 expression was associated with an increase in glioma-infiltrating T cells. In the pair-wise scatter plots with Loess smooth curves examining the relationship between p-STAT3 expression and CD3+ and CD8+ T-cell infiltration, an almost straight trend indicated that there was a strong linear corre-

Table 3. Proportion of p-STAT3 – positive cases according to pathology and WHO tumor grade

Pathology	p-STAT3 >0/total (%)	Mean (SD)*	(Minimum, maximum)
O	6/16 (38%)	4.3 (7.7)	(0.0, 23.5)
MOA	6/6 (100%)	67.9 (55.2)	(9.0, 136.0)
AO	6/15 (40%)	17.5 (41.0)	(0.0,153.0)
AMOA	7/12 (58%)	13.3 (27.0)	(0.0, 85.5)
LGA	0/3 (0%)	0	0
AA	9/17 (53%)	5.6 (7.6)	(0.0, 23.0)
GS	5/7 (71%)	23.1 (34.1)	(0.0, 91.0)
GBM	27/53 (51%)	11.7 (24.4)	(0.0, 133.5)

*The mean calculation was based on the mean p-STAT3 value.

lation between CD3+ and CD8+ T-cell infiltration and p-STAT3 expression ($P < 0.001$ for both; Fig. 2).

Lack of correlation between p-STAT3 expression and Treg infiltration. To determine if there was an association between p-STAT3 expression and the presence of Tregs, we stained the glioma TMA with FoxP3 (31). Within the GBM specimens that expressed p-STAT3, 59% (16 of 27) had an infiltrating Treg population. However, the level of p-STAT3 expression was not associated with the number of infiltrating Tregs. In the MOA, AMOA, and AO specimens, in which there was a high incidence of p-STAT3–expressing cells, there was no correlating Treg glioma-infiltrating population. Thus, the presence of p-STAT3 within the tumor does not seem to be a key regulator of the presence or degree of the tumor infiltrating Treg population.

p-STAT-3 expression level is a prognostic marker for survival times. Among the patients with gliomas with no p-STAT3 expression, regardless of the specific tumor pathology, the median survival time was 34.6 months [95% confidence interval (CI), 19.2 months to not estimable]. In contrast, for patients with gliomas with p-STAT3 expression, the median survival time was 20.1 months (95% CI, 13.8-43.0 months); however, this was not statistically significant (Fig. 3A).

For patients with GBMs and p-STAT3 expression, the median survival time was 10.7 months (95% CI, 7.5-15.0 months), whereas the median survival time was 18.1 months (95% CI, 6.6-41.8 months) for patients with GBMs without p-STAT3 expression; however, this was not statistically significant (Fig. 3B). In patients with AAs expressing p-STAT3, the median survival time was 12.2 months (95% CI, 6.2 months to NA), whereas the median survival time was 34.6 months (95% CI, 33.9 months to NA) for patients with AAs that lacked p-STAT3 expression ($P = 0.02$; Fig. 3C). On univariate analysis, the presence of p-STAT3 ($P = 0.04$; hazard ratio, 5.94) in patients with AAs was a prognostic factor. However, after adjusting for age in the multivariate analysis, the presence of p-STAT3 was only marginally significant ($P = 0.06$; hazard ratio 5.32), which could be due to our limited sample size. All AA patients underwent combinational radiation and chemotherapy. The absolute number of p-STAT3–expressing cells did not have a prognostic effect for patients with AAs on either univariate or multivariate analyses. In addition, p-STAT3 expression did not seem to be a prognostic factor in patients with either AOs or oligodendrogliomas. Because there was no p-STAT3 expression in low-grade gliomas and all MOAs expressed p-STAT3, no prognostic significance for survival can be ascertained within these pathologic subtypes. Removing GBMs and AAs with prior radiotherapy or chemotherapy (i.e., recurrent) from our analysis did not have a statistically significant effect on our results.

Discussion

In this study, we found that p-STAT3 is commonly expressed in a variety of gliomas. Furthermore, as astrocytomas become more malignant, the number of p-STAT3–expressing cells increases. We frequently observed p-STAT3–expressing cells in GBMs, gliosarcomas, and AAs, but we did not see significant p-STAT3 expression in normal brain tissues or low-grade gliomas. Because we used a glioma TMA that contains only a small proportion of the overall tumor, we cannot completely exclude the possibility that p-STAT3–expressing cells were present in the low-grade tumors and normal tissue. However, in an attempt to negate this as a possibility, we used duplicate tissue cores from different areas of the tumors and normal tissue from various CNS anatomic positions in the TMA.

Investigators have examined p-STAT3 expression in other malignancies, such as gastric (39), renal (40), ovarian (41), squamous (42, 43), and hepatocellular carcinomas (44) and anaplastic large-cell lymphoma (45, 46), and have determined that p-STAT3 expression was associated with poor prognosis. Other studies have also shown that p-STAT3 expression correlated with lymph node spread of colorectal cancer (47) and the depth of tumor invasion (48). However, some studies found no relationship between p-STAT3 expression and prognosis in patients with non–small cell lung cancer (49, 50). Although these studies addressed p-STAT3 expression at the tyrosine^{705} location, similar to our current study, another group found that p-STAT3 at the serine^{727} location correlated with the degree of cervical intraepithelial neoplasia (51). Similar to most studies examining the prognostic significance of p-STAT3 expression, we found on univariate analysis that in AAs, p-STAT3 expression was a negative prognostic factor.

Our study is in stark contrast, however, to a previous study in which a similar glioma microarray was used, which found that <10% of p-STAT3 expression at tyrosine^{705} occurred in gliomas

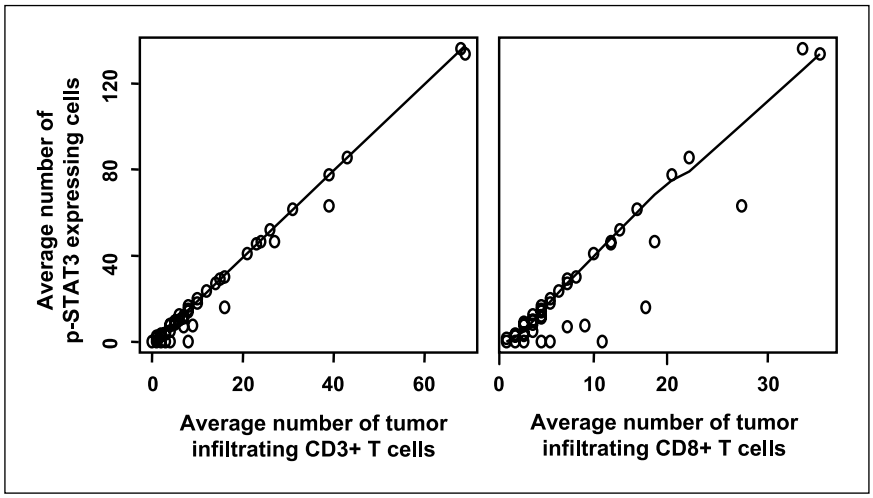

Fig. 2. Pair-wise scatter plots between p-STAT3, CD3, and CD8 across all tumor pathologies and tumor grades with Loess smooth curves added. The almost straight trend of the Loess curves indicates that both CD3 and CD8 had a strong linear correlation with p-STAT3 expression. Specifically, the correlation of the number of p-STAT3 expressing cells with the number of tumor infiltrating CD3+ Tcells was 0.99 ($P < 0.001$) and with the number of tumor infiltrating CD8+ Tcells was 0.94 ($P < 0.001$).

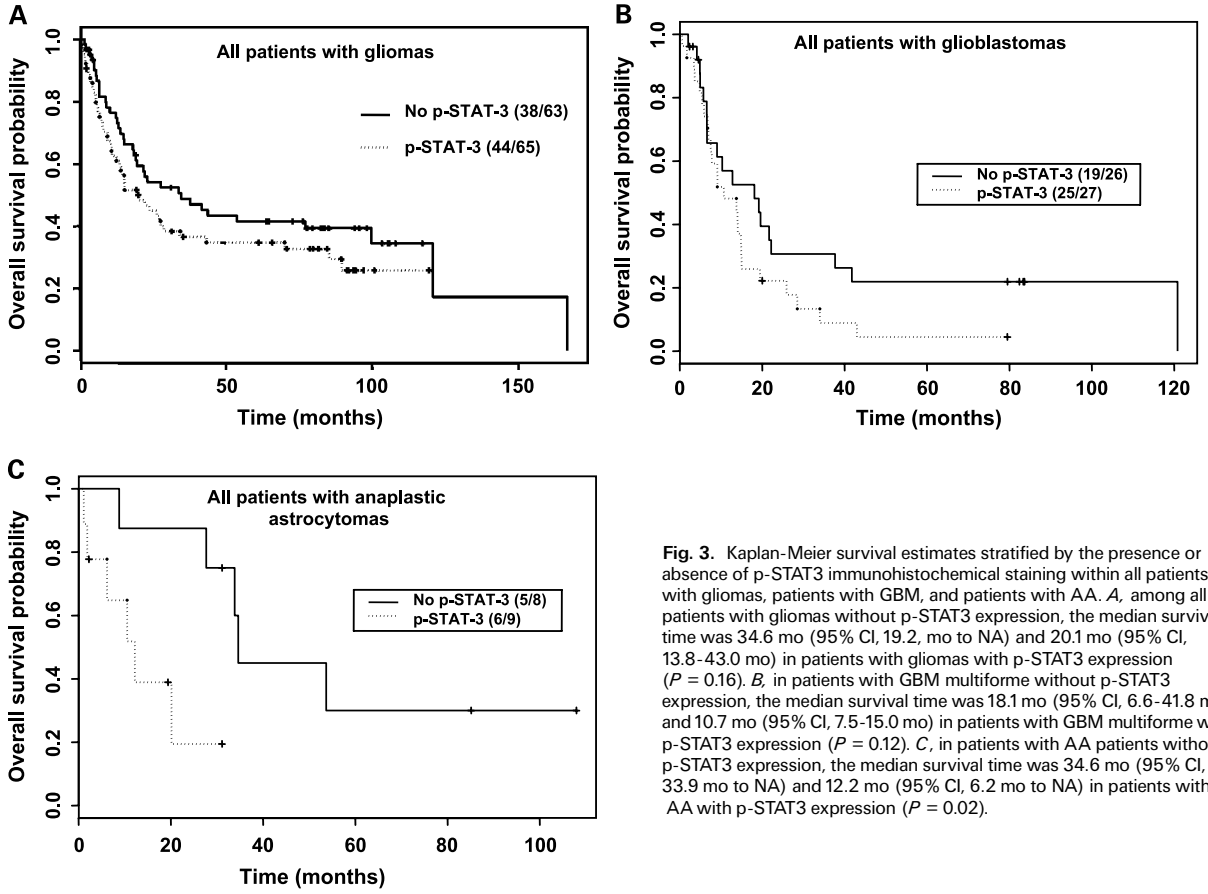

Fig. 3. Kaplan-Meier survival estimates stratified by the presence or absence of p-STAT3 immunohistochemical staining within all patients with gliomas, patients with GBM, and patients with AA. *A,* among all patients with gliomas without p-STAT3 expression, the median survival time was 34.6 mo (95% CI, 19.2, mo to NA) and 20.1 mo (95% CI, 13.8-43.0 mo) in patients with gliomas with p-STAT3 expression ($P = 0.16$). *B,* in patients with GBM multiforme without p-STAT3 expression, the median survival time was 18.1 mo (95% CI, 6.6-41.8 mo) and 10.7 mo (95% CI, 7.5-15.0 mo) in patients with GBM multiforme with p-STAT3 expression ($P = 0.12$). *C*, in patients with AA patients without p-STAT3 expression, the median survival time was 34.6 mo (95% CI, 33.9 mo to NA) and 12.2 mo (95% CI, 6.2 mo to NA) in patients with AA with p-STAT3 expression ($P = 0.02$).

of all grades and pathologic types (52). The potential reasons for the difference between this previous study and our current study may include our process for antigen retrieval and the titration of the primary antibody. Because very little p-STAT3 expression was seen in the previous study, the authors did not attempt to correlate p-STAT3 expression with prognosis. However, another immunohistochemical study of p-STAT3 expression in just GBMs and AAs found an almost identical incidence of p-STAT3 expression (i.e., 55.6% in AAs and 56.4% in GBMs; ref. 53) as in our current study. In this latter study, the authors saw a trend that activated STAT3 conferred a survival advantage, but this was not statistically significant. The difference between the results of our current study and the results of the previous study may be due to the sample set but, most likely, it is attributed to the analysis. In the study by Mizoguchi et al. (53), the association for prognosis was with activation of STAT3 alone and not activation of STAT3 in general. More specifically, they compared cases having only STAT3 activation against those cases containing combinations of STAT3/MAPK/AKT activation. Since they observed a correlation of AKT activation with a worse prognosis, it was not surprising that the cases with worse survival would fall into the combined activation group in their analyses and that STAT3 activation alone would not be a negative prognostic factor.[6] This study also showed a correlation between STAT3 activation and epidermal growth factor receptor status that was attributed exclusively to epidermal growth factor receptor vIII expression.

We also found that in gliomas with p-STAT3 expression, there was a statistically significant corresponding influx of CD3+ T cells. Because IL-6 is expressed in the CNS under a wide variety of conditions and has been shown to attract T cells to the CNS, it was not surprising that the amount of p-STAT3 expression directly correlated with the number of CD3+ glioma-infiltrating T cells. One needs to bear in mind, however, that the presence of infiltrating T cells does not correlate with their having functional activity (54). The tumor microenvironment induces STAT3 activity in tumor-associated immune cells (25, 27), and the induced p-STAT3 in the tumor-infiltrating immune cells resulted in their functional down modulation (21–24, 27, 29). Thus, although STAT3 expression in the tumor correlates with the degree of immune infiltration in the tumor microenvironment, it has also likely resulted in their immune suppression. This may partially resolve the divergent findings of various studies that have examined the prognostic effects of glioma infiltrating T-cell populations on prognosis (55–57).

Investigators have reported the presence FoxP3$^+$ Tregs in a variety of cancers, including hepatocellular carcinoma, colorectal cancer, ovarian cancer, and others (32, 58–62). Studies

[6] C.L. Nutt, personal correspondence, October 31, 2008.

have shown that the presence of FoxP3$^+$ Tregs in ovarian cancer is not only a predictor of poor prognosis but also an independent predictor of OS and progression-free survival times (58, 59). However, in anal squamous cell carcinoma, the presence of Tregs did not have a prognostic influence (63). We examined FoxP3 expression in gliomas and did not find it to be an independent negative prognostic factor for median survival time (31). Within some pathologies, such as oligodendrogliomas, we did not observe the presence of FoxP3 despite the presence of p-STAT3 expression. Conversely, we identified Treg infiltration in tumors without p-STAT3 expression. Thus, although p-STAT3 may be a transcriptional factor related to the induction of FoxP3 expression, it may not be the only factor that influences Treg generation.

Because p-STAT3 is frequently observed in gliomas, it represents a viable target for a variety of small-molecule inhibitors of p-STAT3, such as WP1066 (14, 29), JSI-124 (cucurbitacin I; ref. 64), and S3I-201 (65), which are in various stages of preclinical development. However, the variability of p-STAT3 expression within the various glioma pathologies suggests that not all patients may uniformly benefit from treatment with these types of anti–p-STAT3 approaches. Future studies will be directed at evaluating the *in vivo* effects of small molecule inhibitors of p-STAT3 in preclinical models of intracerebral gliomas with variable expression levels of p-STAT3. In conclusion, p-STAT3 is frequently expressed among patients with high grade gliomas, MOA and AMOA, has a prognostic influence within patients with AAs, and its expression correlates with enhanced T-cell infiltration within gliomas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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