Turk Neurosurg 35(1):22-33, 2025
DOI: 10.5137/1019-5149.JTN.43568-23.2

Original Investigation

Neuro-Oncology

Received: 05.02.2023
Accepted: 11.01.2024
Published Online: 30.12.2024

Clinicopathological and Prognostic Significance of Tim-3 and Rel-B Expressions in Grade 4 Diffuse Gliomas

Fatih YILMAZ[1], Evrim YILMAZ[1], Deniz ARIK[1], Funda CANAZ[1], Bulent YILDIZ[2], Melek AKCAY[3], Emre OZKARA[4], Cengiz BAL[5]

[1]Eskisehir Osmangazi University, Faculty of Medicine, Department of Pathology, Eskisehir, Türkiye
[2]Eskisehir Osmangazi University, Faculty of Medicine, Department of Medical Oncology Eskisehir, Türkiye
[3]Eskisehir Osmangazi University, Faculty of Medicine, Department of Radiation Oncology Eskisehir, Türkiye
[4]Eskisehir Osmangazi University, Faculty of Medicine, Department of Neurosurgery Eskisehir, Türkiye
[5]Eskisehir Osmangazi University, Faculty of Medicine, Department of Biostatistics, Eskisehir, Türkiye

Corresponding author: Fatih YILMAZ ✉ faatiihyiilmaaz@yahoo.com.tr

ABSTRACT

AIM: To assess the clinicopathological and prognostic significance of Tim-3, an immune checkpoint molecule, and Rel-B, an NF-κB subunit, in grade 4 diffuse glioma samples and their relationship with each other.

MATERIAL and METHODS: The demographic, radiologic, treatment, and prognostic data of patients diagnosed with grade 4 diffuse glioma between 2016 and 2019 were reviewed and recorded. Tim-3 and Rel-B were applied to the paraffin-embedded tissues by immunohistochemistry method. Tim-3 expression was grouped as immunoreactivity density score (IDS) (Low, High) and expression percentage (<12%, >12%), while Rel-B expression was divided into positive and negative groups.

RESULTS: Ninety-nine grade 4 diffuse glioma samples were detected, 8 of which were IDH-1 positive. Tim-3 was expressed only in immune cells around and inside the tumoral tissue, and expression was detected only in tumoral cells with Rel-B. Tim-3 IDS was found at lower levels (median 31.8) in IDH-1 positive cases and higher (median 158) in IDH-1 negative ones (p=0.020). A significant correlation was found between the Tim-3 IDS high group and Rel-B positivity (p=0.007).

In the IDH-1 negative cohort, the univariate analysis revealed higher Tim-3 expression percentage and higher IDS were associated with better overall survival (OS) (p=0.041 and p=0.042 respectively) and progression-free survival (PFS) (p=0.023 and p=0.029 respectively), while in the multivariate analysis higher Tim-3 expression percentage was found to be an independent predictor for better OS (p=0.008) and PFS (p=0.022). Rel-B positive cases exhibited longer OS and PFS but the result was not statistically significant (p>0.05).

CONCLUSION: Tim-3 can be a good prognostic predictor and treatment candidate, especially in patients with IDH-1 negative grade 4 diffuse gliomas however, further studies with more cases are needed for Rel-B. The significant relationship between Tim-3 and Rel-B expressions supported the interaction between NF-κB and immune checkpoint pathways.

KEYWORDS: Glioblastoma, Grade 4 diffuse glioma, Immune checkpoint molecule, T-Cell immunoglobulin and mucin domain-containing protein 3, Transcription factor Rel-B, Isocitrate dehydrogenase, Mutant/Wild

ABBREVIATIONS: Tim-3: T-Cell Immunoglobulin and Mucin Domain-Containing Protein 3, **Rel-B:** Transcription Factor Rel-B, **NF-κB:** Nuclear Factor kappa B, **IDS:** Immunoreactivity density score, **OS:** Overall survival, **PFS:** Progression-free survival, **TIIC:** Tumor-infiltrating immune cells, **WHO:** World Health Organization, **HGG:** High-Grade Glioma

Fatih YILMAZ : 0000-0001-8216-1753
Evrim YILMAZ : 0000-0003-1937-8313
Deniz ARIK : 0000-0003-0905-2731
Funda CANAZ : 0000-0002-5642-3876
Bülent YILDIZ : 0000-0002-9111-9132
Melek AKCAY : 0000-0002-9042-9489
Emre OZKARA : 0000-0001-5448-6446
Cengiz BAL : 0000-0002-1553-2902

INTRODUCTION

Grade 4 diffuse gliomas are highly malignant astrocytic neoplasms with limited resectability, aggressive progression, and poor prognosis, with a 1-year overall survival (OS) rate of 76.3%, even in those with IDH mutations [World Health Organization (WHO) grade 4 IDH-mutant astrocytomas] (16,21). In WHO grade 4 IDH wild-type glioblastomas, this rate drops to 53.7 (21). Several clinical trials have been conducted over the last few decades to investigate this issue and extend these short survival times, but only limited therapeutic success has been achieved (2). The immune microenvironment in tumors, which has recently received more attention, is also thought to play a critical role in the efficacy of glioblastoma immunotherapy (4). Immunotherapy can change the clinical course of glioblastoma patients who are most likely to benefit from immunotherapy and determine the best treatment regimens to improve outcomes (2).

T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) is a member of the Tim immunoregulatory protein family that was discovered in 2002 (17). Although it was initially identified as a molecule expressed by interferon-γ-producing CD4+ and CD8+ T cells, it has since been demonstrated to be represented by a variety of other cell types, including regulatory T cells (Treg), myeloid cells, natural killer cells, and mast cells (36). Tim-3 is an immune checkpoint molecule that, like other immune checkpoint molecules (PD-1, Lag-3, and CTLA-4), is involved in the pathogenesis of many autoimmune and viral diseases by depleting T cells and acting as a negative regulator of T-cell response (12). Although previous studies have focused on its role in the pathogenesis of viral and autoimmune diseases, it has recently received increased attention due to its clinical and prognostic significance in various solid and myeloid neoplasms (22,23,27,33). Furthermore, RT-qPCR analysis in clinical glioblastoma samples revealed that Tim-3 expression is significantly higher than that of other well-known immune checkpoint molecules (11). However, immunohistochemical (IHC) studies of human glial neoplasms are uncommon, and their prognostic significance, particularly in high-grade tumors, is unknown (31,40).

NF-κB is a dimeric DNA binding complex comprising various combinations of five family members, including p50, p52, Rel-A, Rel-B, and c-Rel, that play important roles in biological processes (32). By inducing epithelial–mesenchymal transition, NF-κB promotes cancer initiation, tumor cell proliferation, antiapoptotic effect, invasion, angiogenesis, treatment resistance, and distant metastasis (32,38). While the canonical NF-κB signaling pathway, which is mediated by Rel-A-containing dimers, has been shown to play an important role in regulating cancer invasion and progression, the noncanonical NF-κB signaling pathway, which is mediated by Rel-B-containing dimers, has only recently been investigated (28,39). Few studies have examined Rel-B's role and prognostic value in solid tumors (10,24,30,44), and there have been few reports of Rel-B expression in glial neoplasms (14,28,35,39). A recent study indicates that the interaction of Tim-3 and NF-κB is important in the pathogenesis of glioblastomas, and breaking this interaction via antibodies is promising in terms of potential treatment candidacy (11).

This study aimed to assess the prognostic values of Rel-B and Tim-3 IHC expressions in human grade 4 diffuse glioma samples and their correlation with IDH status, each other, and histopathological features.

MATERIAL and METHODS

Case Selection and Histopathological Assessment

Following approval from the institutional ethics committee (decision date: 14.07.2020, decision number: 40), the pathology archives for all cases of grade 4 diffuse glioma biopsied and resected at the Faculty of Medicine, Eskişehir Osmangazi University Hospital between 2016 and 2019 were reviewed. The study included patient files containing clinical information and sufficient formalin-fixed paraffin-embedded tissue for IHC. Histological findings, IDH status, and accompanying components, if any, were recorded on H&E slides were re-examined by two pathologists (EY, FY). Based on the WHO Classification of Tumors of the Central Nervous System revised in 2022, the histological findings, IDH status, and accompanying component (s), if any, were recorded.

IHC Staining and Evaluation

Immunohistochemical staining was performed on 4-m-thick sections of paraffin blocks. Tim-3 and Rel-B IHC staining was conducted using previously described standard techniques (25,40), with the staining procedures recommended in the datasets for each marker being considered. A DAKO Omnis automatic IHC stainer (DAKO Omnis Denmark) was used for IDH-1 (R132H [H09]), Tim-3 (anti-Tim-3 antibody [BLR033F]–BSA free, monoclonal, 1/300 dilution), and Rel-B (anti-Rel-B antibody [EP613Y], monoclonal, 1/200 dilution) staining. IDH-1 staining was used in cases that could not be evaluated due to technical reasons and those that were used before the period of routine use. In positive cases, the absence of combined whole-arm deletions of 1p and 19q was also genetically confirmed.

Cytoplasmic immunoreactivity in tumor cells was considered positive for IDH-1, and positive control was a tissue sample from an astrocytoma with previously confirmed mutation status. Tim-3 membranous and cytoplasmic immunoreactivity and Rel-B cytoplasmic immunoreactivity were considered positive. For both markers, tonsil tissue was used as a positive control. Tim-3 expression in the immune cells (tumor-infiltrating immune cells [TIICs]) was calculated by counting and averaging the five high power fields (HPF, x400, diameter: 0.55 mm) that were similar to those used in previous studies in hotspot and nonnecrotic areas of tumoral tissue (18). The intensity of staining was scored as 0 = *negative*, 1 = *mild*, 2 = *moderate*, and 3 = *strong* (Figure 1A–C). The immunoreactivity density score (IDS) was calculated by multiplying the mean values and staining intensity scores. Tim-3 IDS were classified as 0 = *absent*, 1 = ≥ *1 – 149*, 2 = ≥ *150 – 300*, and 3 = ≥ *300*. Then, "0, 1" was considered a low expression, and "2, 3" was considered a high expression. Furthermore, the extent of Tim-3 expression in tissue was scored as a percentage between 0 and 100. The cases were then classified as ≤12% or >12% based on the 12% value determined by receiver-operating characteristic (ROC) analysis.

Cytoplasmic (25) and ≥1% immunoreactivity in tumor cells were considered positive for Rel-B, with the degree of immunoreactivity specified in percentages. The staining intensity was graded as 0 = *negative*, 1 = *mild*, 2 = *moderate*, and 3 = *strong* (Figure 1D–F). The Rel-B immunoreactivity score (IRS) was calculated by multiplying the percentage by the staining intensity.

Statistical Analysis

The Shapiro–Wilk test of normality test was used to determine the distribution pattern of the variables. Distribution characteristics represented data as mean ± standard deviation or median (minimum–maximum or 95% confidence interval). The Chi-square (Pearson, continuity of correction, or Fisher's exact) test was used to analyze categorical variables. The Mann–Whitney *U* test was used to determine the difference between the two groups, and the Spearman's rank correlation *n* test was used to determine the correlation between Tim-3 and Rel-B expressions.

The OS was calculated using the time between the operation date and the date of death or the survival status determined at the most recent follow-up. Progression-free survival (PFS) time was determined based on the interval between the operation date and the date the progression was detected radiologically or the survival status at the last follow-up. Patients who died within the first month (n=9) were excluded from the survival analysis due to the effects of complications that could have occurred as a result of the operation. To determine the relationship between Tim-3 and Rel-B expression and survival, survival analyses were performed on the whole cohort (n=90) and the IDH-1 negative cohort (n=82) due to the potential effect of IDH mutation on immune expression. Kaplan–Meier survival analysis was used to determine the distribution of OS and PFS over time, and p values were calculated using the log-rank test. Univariate and multivariate analyses in the Cox proportional hazard model were used to demonstrate the effect of independent variables on survival. A value of $p<0.05$ was considered statistically significant in multivariate analysis. ROC analysis was used to determine the optimal cut-off predicting survival of the extent of Tim-3 expression, and the ≤12% was found to be the best predictor of survival (sensitivity: 46.1%, specificity: 100%, area under the curve [AUC-ROC]: 0.755, 0.648–0.844; p=0.0008).

Statistical analyses were performed in the Department of Biostatistics using IBM SPSS Statistics (Version 23.0; IBM Corp., New York, USA) software, and the ROC curve was obtained from Medcalc software (version 13.3.0.0, 2014).

RESULTS

Patient Characteristics

There were 99 patients in this study: 54 (54.5%) males and 45 (45.5%) females (male/female = 1.2). The patient's ages ranged from 15 to 83 (mean, 58.7 ± 12.6), with 7 (7.07%) having previously diagnosed low-graded glial neoplasia. Table I shows the other clinicopathological characteristics.

Immunohistochemical findings

IDH-1 was positive in eight (8.1%) patients (3 frontal, 3 temporal, 1 parietal, and 1 occipital). IDH-1 was found in six (85.7%) of 7 patients with a history of low-grade glial neoplasia. The two remaining patients (2.1%) had no history of low-grade glial neoplasia. Tumor cells and nonneoplastic glial tissue did not

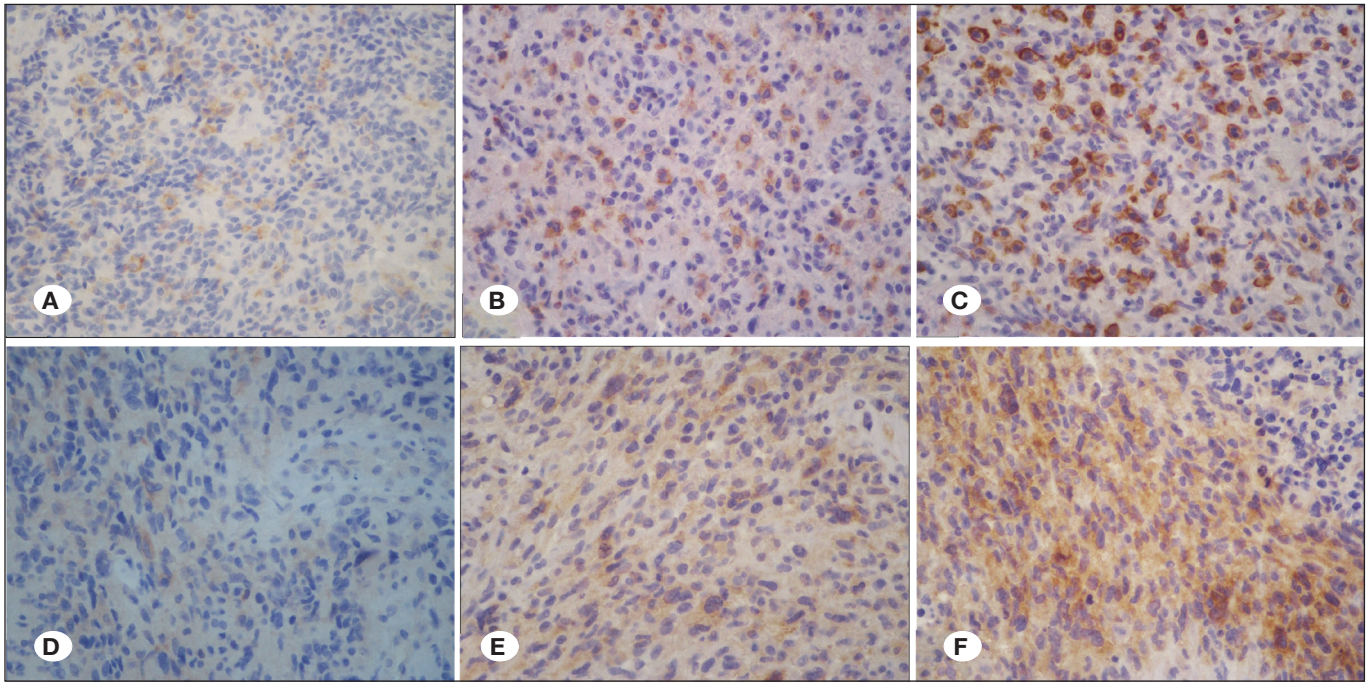

Figure 1: Weak (**A**, x200), moderate (**B**, x400), and strong (**C**, x400) expression of Tim-3 by TIICs. Weak (**D**, x400), moderate (**E**, x400), and strong (**F**, x400) expression of Rel-B by tumor cells.

Table I: Clinicopathological Parameters

Parameters	Number (%)
Gender	
Male	54 (54.5)
Female	45 (45.5)
Age, mean 58.7±12.6	
≤58	49 (49.5)
>58	50 (50.5)
Surgical procedure	
Total	25 (25.3)
Subtotal	59 (59.6)
Incisional	13 (13.1)
NK	2 (2.0)
Lesion diameter, mean 5.3±1.8	
<5 cm	41 (41.4)
≥5 cm	37 (37.4)
NK	21 (21.2)
Localization	
Frontal	23 (23.3)
Perietal	18 (18.2)
Temporal	33 (33.3)
Occipital	11 (11.1)
Basal ganglion	6 (6.1)
Corpus callosum	3 (3.0)
Cerebellum	2 (2.0)
Insular cortex	1 (1.0)
Pons	1 (1.0)
NK	1 (1.0)

Parameters	Number (%)
IDH-1 Mutation	
Positive	8 (8.1)
Negative	91 (91.9)
OG component	
Yes	18 (18.2)
No	81 (81.8)
Multiplicity	
Solitary	75 (75.7)
Multiple	8 (8.1)
NK	16 (16.2)
CT	
Yes	43 (43.4)
No	56 (56.6)
RT	
Yes	52 (52.5)
No	47 (47.5)
OS, median 5 (0-75) (months)	
≤5	54 (54.5)
5<	45 (45.5)
PFS, median 4 (0-47) (months)	
≤4	56 (56.6)
4<	43 (43.4)
Life status at last follow-up	
Death	91 (91.9)
Alive	8 (8.1)

NK: *Not known,* ***OG:*** *Oligodendroglioma,* ***CT:*** *Chemotherapy,* ***RT:*** *Radiotherapy,* ***OS:*** *Overall survival,* ***PFS:*** *Progression-free survival.*

express Tim-3, but the expression was observed in immune cells around and inside the tumoral tissue (TIIC). The Tim-3 IDS was found at lower levels (median, 31.8; range, 5.8–226)] in IDH-1-positive cases and higher levels (median, 158; range, 0.6–461.4)] in IDH-1 negative cases (p=0.020). There was no significant correlation between Tim-3 IDS (low–high) and other clinicopathological parameters (p>0.05). Tim-3 expression in tissue ranged from 1% to 90% (median, 15). Tim-3 expression was lower in IDH1-positive cases (median, 5) and higher in IDH1-negative cases (median, 15), but the difference was not statistically significant (p=0.082). There was no significant correlation (p>0.005) between the percentage of Tim-3 expression and clinicopathological parameters.

Rel-B was found to be positive in 26 (26.3%) of the patients, and there was no statistically significant correlation between clinicopathological parameters and Rel-B expression (p>0.05). Table II summarizes the relationship between Tim-3 and Rel-B expression and clinicopathological parameters. Chi-square analysis revealed a significant relationship (p=0.007) between Tim-3 IDS (low–high) and Rel-B positivity. The Spearman correlation test showed a positive relationship between Tim-3 IDS and Rel-B IRS (r=0.284, p=0.004) but no significant relationship with Tim-3 expression percentage (p=0.138).

Table II: The Relationship Between Tim-3 and Rel-B Expressions with Clinicopathological Parameters

	Tim-3 Percentage			Tim-3 IDS			Rel-B		
Parameters	**≤12%**	**>12%**	**p-value**	**Low**	**High**	**p-value**	**(-)**	**(+)**	**p-value**
Total (n=99) (%)	43 (43.4)	56 (56.6)		51 (51.5)	48 (48.5)		73 (73.7)	26 (26.3)	
Gender			1.000			0.594			0.440
Male	23 (42.6)	31 (57.4)		26 (48.1)	28 (51.9)		42 (77.8)	12 (22.2)	
Female	20 (44.4)	25 (55.6)		25 (55.6)	20 (44.4)		31 (68.9)	14 (31.1)	
Age (mean 58.7±12.6) (years)			0.930			0.613			1.000
≤58	22 (44.9	27 (55.1)		27 (55.1)	22 (44.9)		36 (73.5)	13 (26.5	
58	21 (42.0)	29 (58.0)		24 (48.0)	26 (52.0)		37 (74.0)	13 (26.0)	
Surgical procedure			0.684			0.587			0.781
Total	11 (44.0)	14 (56.0)		13 (52.0)	12 (48.0)		17 (68.0)	8 (32.0)	
Subtotal	24 (40.7)	35 (59.3)		32 (54.2)	27 (45.8)		44 (74.6)	15 (25.4)	
Incisional	7 (53.8)	6 (46.2)		5 (38.5)	8 (61.5		10 (76.9)	3 (23.1	
NK	1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)		2 (100.0)	0 (0.0)	
Lesion diameter (mean 5.3±1.8)			0.543			0.633			0.329
<5 cm	15 (36.6)	26 (63.4)		20 (48.8)	21 (51.2)		27 (65.9)	14 (34.1)	
≥5 cm	17 (45.9)	20 (54.1)		21 (56.8)	16 (43.2)		29 (78.4)	8 (21.6	
NK	11 (52.4)	10 (47.6)		10 (47.6)	11 (52.4)		17 (81.0)	4 (19.0)	
Localization			0.140			0.088			0.300
Frontal	14 (60.9)	9 (39.1)		15 (65.2)	8 (34.8)		18 (78.3)	5 (21.7)	
Parietal	8 (44.4)	10 (55.6)		6 (33.3)	12 (66.7)		13 (72.2)	5 (27.8)	
Temporal	12 (36.4)	21 (63.6)		15 (45.5)	18 (54.5)		23 (69.7)	10 (30.3)	
Occipital	2 (18.2)	9 (81.8)		5 (45.5)	6 (54.5)		6 (54.5)	5 (45.5)	
Others	7 (53.8)	6 (46.2)		10 (76.9)	3 (23.1)		12 (92.3	1 (7.7	
NK	0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)		1 (100.0)	0 (0.0)	
IDH-1 mutation			0.289			0.060			0.677
Positive	5 (62.5)	3 (37.5)		7 (87.5)	1 (12.5)		7 (87.5)	1 (12.5)	
Negative	38 (41.8)	53 (58.2)		44 (48.4)	47 (51.6)		66 (72.5)	25 (27.5)	
OG component			0.867			0.687			0.774
Yes	7 (38.9)	11 (61.1)		8 (44.4)	10 (55.6)		14 (77.8)	4 (27.2)	
No	36 (44.4)	45 (55.6)		43 (53.1)	38 (46.9)		59 (72.8)	22 (27.2)	
Multiplicity			0.458			0.714			0.1000
Solitary	33 (44.0)	42 (56.0)		38 (50.7)	37 (49.3)		54 (72.0)	21 (28.0)	
Multiple	2 (25.0)	6 (75.0)		5 (62.5)	3 (37.5)		6 (75.0	2 (25.0)	
NK	8 (50.0)	8 (50.0)		8 (50.0)	8 (50.0)		13 (81.3)	3 (18.8)	

IDS: *Immune reactivity dencity score,* ***NK:*** *Not known,* ***OG:*** *Oligodendroglioma.*

Survival Results

At the last follow-up, eight (8.1%) patients were alive, and 91 (91.9%) died, with OS ranging from 0 to 75 (median, 5) months and PFS ranging from 0 to 47 (median, 4) months. The average patient age at diagnosis was 58.78 ± 12.6 yr, ranging from 15 to 83 yr. Patients below ≤58 yr had a median survival time of 9 months, whereas those over the age of >58 yr had a survival time of 5 months. The age factor was strongly associated with OS (p=0.001) but not with PFS (p=0.184). IDH-1 positivity was observed in younger patients (median age of positive patients: 49 yr, median age of negative patients: 62 yr, p=0.008) and associated with longer OS and PFS (p=0.022 and p=0.033, respectively). Table III summarizes the clinical characteristics of the patients and the relationship between OS and PFS.

In the whole cohort (n=90), there was no correlation between survival and Tim-3 percentage, Tim-3 IDS, or Rel-B positivity (n=90, p>0.05). Longer OS and PFS were found in patients with a Tim-3 percentage >12% (p=0.028 and p=0.013, respectively; Figure 2A, B) and high Tim-3 IDS (p=0.029 and p=0.017, respectively; Figure 2C, D). Rel-B-positive patients had longer OS and PFS than negative patients, but the results were not statistically significant (p=0.338 and p=0.195, respectively; Figure 2E, F). The associations of Tim-3 and Rel-B expressions with OS and PFS in the whole cohort and the IDH-1-negative cohort are shown in Table IV.

Age (p=0.024), CT (p<0.001), multiplicity (p=0.003), and Tim-3 percentage (p=0.008) were found to be independent factors affecting OS in the IDH-1 negative cohort (n=82). PFS was affected by three independent factors: RT (p=0.036), multiplicity (p=0.020), and Tim-3 percentage (p=0.022; Table V).

DISCUSSION

High-grade gliomas (HGGs) are the most common malignant brain tumors (20), and treatment is not curative even today, with radiotherapy and adjuvant chemotherapy following surgical resection remaining the standard treatment protocols. However, despite their limited use and therapy success, several clinical trials and alternative treatment approaches have recently been investigated (1,2). Although various factors can contribute to this situation, the immunosuppressive tumor microenvironment is believed to be the primary reason for the failure of many immunotherapy clinical trials (26). Tumor-associated local and systemic immunosuppression is gaining attention due to recent findings that HGGs induce tumor-infiltrating lymphocyte (TIL) energy, activate immunosuppressive regulatory T cells (Treg), and activate immune checkpoints (13). Furthermore, immune checkpoint pathways allow many solid tumors, including HGGs, to evade the immune response (11,26).

Tim-3 is expressed more in many solid tumors, including gliomas, than in non-tumoral tissues (6,8,11,22,23,43). Furthermore, expression levels in gliomas increase with WHO glioma grade (15,29). However, no expression was found in nonneoplastic glial parenchyma, and no expression was found in neoplastic tissues in this study. Expression was found primarily in cells with microglia/macrophage morphology in TIIC and surrounding tumoral tissue. Furthermore, Tim-3 expression and

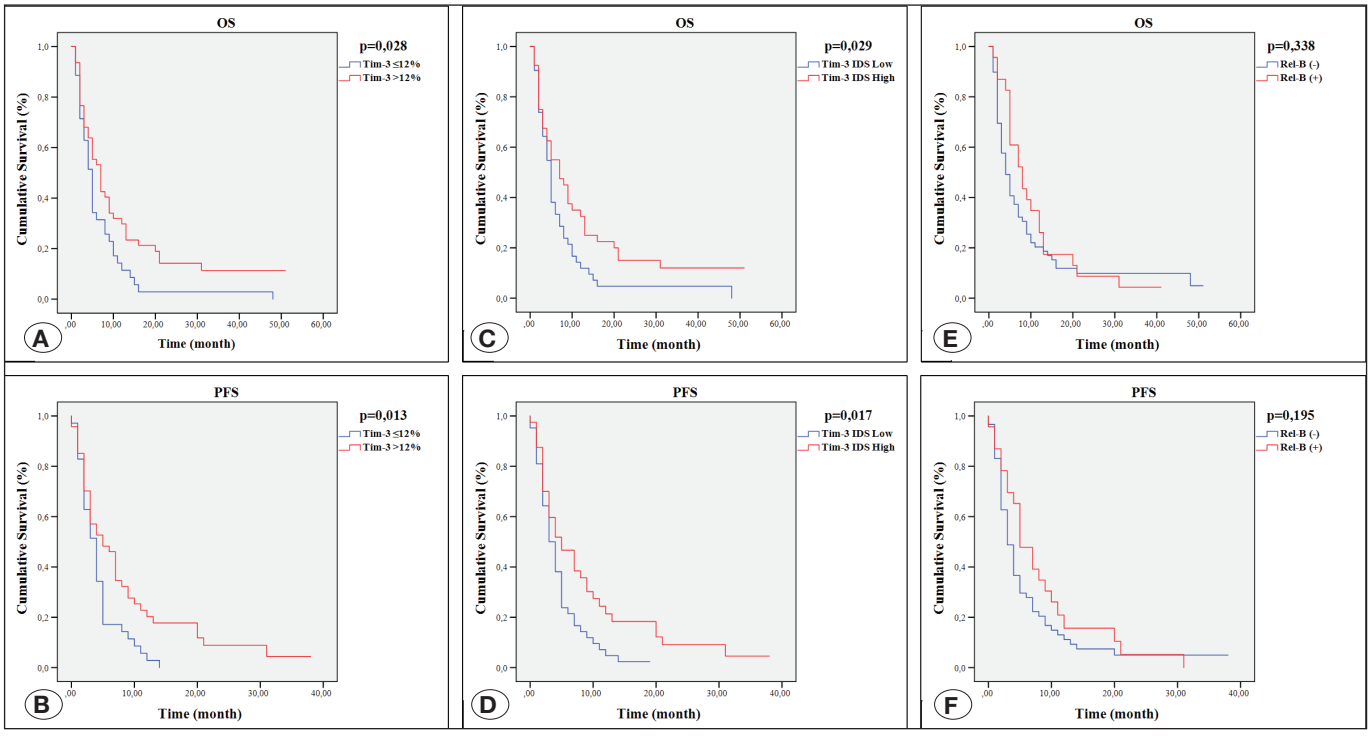

Figure 2: The relationship between **(A, B)** Tim-3 expression percentage (≤12% or >12%), **(C, D)** Tim-3 IDS (low or high), and **(E, F)** Rel-B expression (positive or negative) and OS and PFS in the IDH-1 negative cohort

Table III: Relationship Between OS and PFS with Clinicopathological Parameters

Parametres	Number (%)	Median OS (95% CI)	p-value	Median PFS (95% CI)	p-value
Total	90 (100.0)				
Gender			0.321		0.382
Male	49 (54.4)	6 (3.9- 8.0)		5 (3.3-6.6)	
Female	41 (45.6)	5 (2.9- 7.0)		3 (1.7-4.2)	
Age (mean 58.7±12.6) (years)			**0.001**		0.184
≤58	46 (51.1)	9 (6.3-11.6)		5 (1.1-8.8)	
>58	44 (48.9)	5 (3.9-6.0)		4 (2.9-5.0)	
Surgical procedure			0.681		0.922
Total	22 (24.5)	5 (1.3-8.6)		5 (2.2-7.7)	
Subtotal	55 (61.1)	5 (2.2-7.7)		4 (2.6-5.3)	
Incisional	11 (12.2)	6 (4.0-7.9)		5 (3.4-6.5)	
NK	2 (2.2)	2		2	
Lesion diameter (mean 5.3±1.8)			0.628		0.259
≤5 cm	38 (42.2)	5 (2.5-7.4)		3 (1.7-4.2)	
>5 cm	35 (38.9)	7 (5.1-8.8)		6 (3.7-8.2)	
NK	17 (18.9)	5 (2.3-7.6)		3 (0.5-5.4)	
Localization			0.559		0.431
Frontal	21 (23.3)	5 (2.0-7.9)		3 (1.2-4.7)	
Parietal	17 (18.9)	5 (2.3-7.6)		3 (0.5-5.4)	
Temporal	30 (33.4)	7 (2.9-11.0)		5 (2.9-7.0)	
Occipital	10 (11.1)	6 (1.3-10.6)		4 (0.0-8.1)	
Other	11 (12.2)	4 (0.7-7.2)		4 (1.9-6.0)	
NK	1 (1.1)	3		3	
IDH-1			**0.022**		**0.033**
Positive	8 (8.9)	10 (0.0-36.1)		7 (4.2-9.7)	
Negative	82 (91.1)	5 (3.5-6.4)		4 (3.0-4.9)	
OG component			0.785		0.949
Yes	18 (20.0)	7 (1.4-12.5)		5 (3.6-6.3)	
No	72 (80.0)	5 (3.3-6.6)		4 (2.6-5.3)	
Multiplicity			0.081		0.081
Solitary	70 (77.8)	6 (3.9-8.0)		5 (3.8-6.1)	
Multiple	7 (7.8)	6 (0.0-16.2)		2 (0.7-3.2)	
NK	13 (14.4)	4 (1.8-6.1)		2 (1.8-4.1)	
CT			**<0.001**		**0.037**
Yes	42 (46.7)	10 (7.2-12.7)		6 (3.4-8.5)	
No	48 (53.3)	4 (2.5-5.4)		3 (1.0-4.9)	
RT			**<0.001**		**0.016**
Yes	50 (55.6)	9 (7.0-10.9)		5 (2.4-7.5)	
No	40 (44.4)	3 (0.5-5.4)		2 (1.1-2.8)	

***OS:** Overall survival, **PFS:** Progression-free survival, **CI:** Confidence interval, **OG:** Oligodendroglioma, **CT:** Chemotherapy, **RT:** Radiotherapy.*

Table IV: Relationship Between Tim-3 and Rel-B Expressions and OS and PFS

Whole Cohort (n=90)									
OS	**Tim-3 Percentage**			**Tim-3 IDS**			**Rel-B**		
	≤12%	**>12%**	**p-value**	**Low**	**High**	**p-value**	**(-)**	**(+)**	**p-value**
Total	40	50		49	41		66	24	
OS (median, 95% CI)	5 (3.9-6.0)	7 (4.7-9.2)	0.427	5 (4.0-5.9)	7 (3.8-10.1)	0.281	5 (3.1-6.8)	8 (4.1-11.8)	0.326
PFS (median, 95% CI)	4 (2.9-5.0)	5 (2.5-7.4)	0.236	4 (2.6-5.3)	5 (2.0-7.9)	0.166	4 (2.9-5.0)	5 (2.9-7.0)	0.467
IDH-1 (-) Cohort (n=82)									
PFS	**Tim-3 Percentage**			**Tim-3 IDS**			**Rel-B**		
	≤12%	**>12%**	**p-value**	**Low**	**High**	**p-value**	**(-)**	**(+)**	**p-value**
Total	35	47		42	40		59	23	
OS (median, 95% CI)	5 (3.8-6.1)	7 (4.7-9.2)	**0.028**	5 (3.8-6.1)	7 (2.3-11.6)	**0.029**	4 (2.4-5.5)	8 (4.5-11.4)	0.338
PFS (median, 95% CI)	4 (2.8-5.1)	5 (2.4-7.5)	**0.013**	3 (1.8-4.1)	5 (2.0-7.9)	**0.017**	3 (2.0-3.9)	5 (2.6-7.3)	0.195

IDS: *Immune reactivity density score,* ***OS:*** *Overall survival,* ***PFS:*** *Progression-free survival,* ***CI:*** *Confidence interval.*

Table V: Univariate and Multivariate Analyses of Clinicopathologic Parameters for OS and PFS in the IDH-1 Negative Cohort

OS	**Univariate Cox Analysis**				**Backward Stepwise (Wald) Multivariate Cox Analysis**			
Parameters	**B**	**HR**	**95% CI**	**p-value**	**B**	**HR**	**95% CI**	**p-value**
Age (≤58 vs >58)	-0.597	0.551	0.345-0.879	**0.012**	-0.610	0.543	0.320-0.922	**0.024**
CT (no vs yes)	-0.934	0.393	0.245-0.631	**<0.001**	1.006	2.736	1.596-4.690	**<0.001**
RT (no vs yes)	-0.969	0.380	0.237-0.608	**<0.001**				
Multiplicity (solitary vs multiple)	0.571	1.770	0.792-3.955	0.164	-1.304	0.271	0.113-0.651	**0.003**
Tim-3 percentage (≤12% vs >12%)	-0.480	0.619	0.390-0.981	**0.041**	0.722	2.059	1.205-3.517	**0.008**
Tim-3 IDS (low vs high)	-0.482	0.617	0.388-0.982	**0.042**				
Rel- B (negative vs positive)	-0.229	0.795	0.483-1.310	0.368				
PFS	**Univariate Cox Analysis**				**Backward Stepwise (Wald) Multivariate Cox Analysis**			
Parameters	**B**	**HR**	**95% CI**	**p-value**	**B**	**HR**	**95% CI**	**p-value**
Age (≤58 vs >58) (years)	-0.136	0.873	0.554-1.375	0.558				
CT (no vs yes)	0.468	1.596	1.007-2.531	**0.047**				
RT (no vs yes)	0.559	1.749	1.106-2.765	**0.017**	0,556	1.744	1.036-2.935	**0.036**
Multiplicity (solitary vs multiple)	-0.575	0.563	0.253-1.254	0.160	-1,011	0.364	0.155-0.852	**0.020**
Tim-3 percentage (≤12% vs >12%)	0.549	1.731	1.080-2.775	**0.023**	0,634	1.885	1.096-3.241	**0.022**
Tim-3 IDS (low vs high)	0.527	1.694	1.055-2.720	**0.029**				
Rel- B (negative vs positive)	0.306	1.357	0.823-2.238	0.231				

IDS: *Immune reactivity density score,* ***OS:*** *Overall survival,* ***PFS:*** *Progression-free survival,* ***CT:*** *Chemotherapy,* ***RT:*** *Radiotherapy,* ***CI:*** *Confidence interval,* ***HR:*** *Hazard ratio.*

IDS were higher in IDH-1-negative samples and lower in positive samples (p=0.082 and p=0.020, respectively). According to a recent study, Tim-3 expression is mainly detected in cells with microglia/macrophage morphology, and Tim-3-positive tumor cells account for 0.10% of the total cell population (31).

Several studies have also shown that Tim-3 checkpoint-related immune responses are more common in IDH-wild-type gliomas, and the Tim-3-positive cell population is significantly lower in IDH-mutant tumors than in IDH-wild-type tumors (15, 31). This case could refer to the 2-hydroxyglutarate molecule formed in IDH-mutant tumors, which suppresses gene expression by increasing DNA methylation (19,31). These characteristics also manifested in masking the association between high expression and a favorable prognosis, which is one of our study's most important findings.

There are conflicting findings regarding the impact of Tim-3 expression on prognosis in tumor cells or immune cells. Tim-3 expression levels in tumor cells that are positive or high have been linked to a better to a poor prognosis (22,23,33). Alternatively, it has been linked to a better prognosis, possibly due to the activation of immune responses against tumor cells (34). Furthermore, Tim-3-positive TILs in esophageal squamous cell carcinoma are an independent risk factor for RFS and OS (42); conversely, Tim-3+ intraepithelial TILs in early breast cancer patients were associated with better cancer-specific survival (5).

A growing body of research has found that high expression of Tim-3 in glial neoplasms is associated with poor prognosis (11,15,29), and drug resistance (41). This property stems from its expression in neoplastic cells and its role in the tumor microenvironment. Tim-3/Gal-9 induces NLRC4 inflammasome formation and activation; the expression levels of these molecules increase with glioma grade, and this relationship correlates with poor survival, according to Sim et al. (29). Another study found that Tim-3 not only regulates the malignant behavior of glioma cells but also plays a role in inducing macrophage migration and the transition to anti-inflammatory/protumorigenic phenotypes via Tim-3/IL6 signaling by activating NF-κB, one of the primary regulators of IL6 (11). Zhang et al. found that methylation of the MGMT promoter in tumor tissue and lower Tim-3 expression in mesenchymal immune cells were associated with improved survival (41). However, while Tim-3 expression in the mesenchymal immune cells of HGG samples was comparable with our findings, they did not investigate the effect of Tim-3 expression alone on survival. IDH status is not considered in studies investigating the effect of Tim-3 expression on prognosis in HGGs, and its prognostic impact in IDH wild-type tumors has not been extensively examined (15,40). As a result, we also performed survival analyses on the IDH-1-negative cohort. While there was no relationship between Tim-3 expression and OS and PFS in the whole cohort, in the IDH-1-negative cohort, an expression percentage of >12% and a high IDS were associated with better OS (p=0.028 and p=0.029, respectively) and PFS (p=0.013 and p=0.017, respectively). Furthermore, the extent of Tim-3 expression (>12%) was found to be an independent predictor of improved OS (HR: 2.059, p=0.008) and PFS (HR: 1.885, p=0.022).

Tim-3's association with a favorable prognosis could be due to strong immune recognition, which complicates tumor escape (5,45). Tim-3 serves similar functions in tissues, and its targeting or silencing has identical effects. As a result, it is emphasized in many studies where high Tim-3 expression is associated with good (5,34,37) and poor (22,23) prognoses that Tim-3 can be a potential treatment candidate. In this context, the high expression of immune checkpoint molecules in IDH wild-type gliomas due to DNA hypomethylation suggests that immune checkpoint inhibitors may be beneficial in IDH wild-type gliomas that are unresponsive to standard therapy (19). When an anti-Tim-3 antibody is combined with an anti-PD-1 antibody in an animal model, 100% survival (100th day after implantation) is achieved with stereotactic radiosurgery (13). To investigate this synergistic effect, a phase I clinical trial in patients with recurrent HGGs was launched (NCT03961971).

It has been proposed that Rel-B expression in neural precursor cells during mammalian development is consistent with the possibility that increased Rel-B activity plays a role in the pathogenesis of central nervous system cancers (14). Emerging evidence suggests that Rel-B is more expressed in IDH wild-type gliomas, which supports the expression of mesenchymal genes in glial tumors, and is associated with pathological grade progression and that Rel-B suppression inhibits glioma cell migration and invasion (14,28,39). Rel-B is expressed more in neoplastic tissues than in nonneoplastic tissues, and high expression levels in many solid tumors (10,24,25,30,44), including gliomas (14,39), have been associated with poor prognosis. Only one study has found that increased expression is associated with a better prognosis (9).

In this study, expression was only found in neoplastic tissue, not nonneoplastic glial parenchyma. This finding is consistent with molecular studies, which found higher Rel-B expression in glioblastoma samples than in non-malignant glial tissue (35). We found a higher expression of Rel-B in IDH-1-negative samples, similar to Tim-3 and previous studies (39), but the result was not statistically significant (p=0.288). Despite its relationship with neoplastic processes and its proclivity overexpressed in IDH wild-type gliomas, there was no significant relationship with survival time in the whole or IDH-1 negative cohorts. Even if it is not found to be significantly associated with a good prognosis, the fact that high expression of Tim-3 is observed in patients with longer survival and the significant relationship between them and Tim-3 expression supports the expression-enhancing relationship between NF-κB (Rel-B) and immune checkpoint (Tim-3). Zeng et al. found that PD-1, PD-L1, Tim-3, and B7-H3 expression levels were positively correlated with Rel-B expression, thus supporting this hypothesis (39).

A recent study found that NF-κB regulates the expression of transcriptional and posttranslational immune checkpoint mediators, allowing tumors to evade the immune system (3). On the other hand, overexpression of Tim-3 in breast cancer activates the NF-κB/STAT3 pathway and its downstream genes and increases paclitaxel chemoresistance (7). Guo et al. also reported that using an IL6R inhibitor (Tocilizumab) to block the Tim-3/IL6 signaling pathway, which occurs through

NF-κB induction by Tim-3, preventing the effects of this pathway and suppressing glioma tumorigenicity *in vivo* may provide a new therapeutic strategy (11).

CONCLUSION

This study revealed novel information about the clinicopathological significance of NF-κB and immune checkpoint mechanisms, particularly Rel-B and Tim-3, in grade 4 diffuse gliomas. This is the first study to emphasize the role of the Rel-B domain in Tim-3 molecule interaction with the NF-κB transcription factor. Furthermore, it is the first scientific publication to report that Tim-3 expression correlates with improved survival and that Rel-B expression is found in patients who live longer. Confirming these findings with larger and more comprehensive patient series will provide more accurate prognostic prediction and potential treatment options, particularly for patients with high-grade gliomas.

ACKNOWLEDGMENTS

This study was produced from the Medical Specialization thesis of Dr. Fatih Yilmaz, which was completed under the supervision of Dr. Evrim Yilmaz at the Faculty of Medicine, Pathology Department, Eskisehir Osmangazi University.

Declarations

Funding: This study was supported by Eskisehir Osmangazi University Scientific Research Projects Coordination Unit within project number 202011A223.

Availability of data and materials: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure: The authors declare that there is no conflict of interest.

AUTHORSHIP CONTRIBUTION

Study conception and design: FY, EY

Data collection: FY, MA, EO, BY

Analysis and interpretation of results: FY, EY, CB

Draft manuscript preparation: FY, EY, DA

Critical revision of the article: EY, CB, FC, DA

Other (study supervision, fundings, materials, etc...): DA, FC, MA, BY

All authors (FY, EY, DA, FC, BY, MA, EO, CB) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Aggarwal P, Luo W, Pehlivan KC, Hoang H, Rajappa P, Cripe TP, Cassady KA, Lee DA, Cairo MS: Pediatric versus adult high grade glioma: Immunotherapeutic and genomic considerations. Front Immunol 22:13:1038096, 2022. https://doi.org/10.3389/fimmu.2022.1038096
2. Arrieta VA, Dmello C, McGrail DJ, Brat DJ, Lee-Chang C, Heimberger AB, Chand D, Stupp R, Sonabend AM: Immune checkpoint blockade in glioblastoma: From tumor heterogeneity to personalized treatment. J Clin Invest 133:1332023, 2023. https://doi.org/10.1172/JCI163447
3. Betzler AC, Theodoraki MN, Schuler PJ, Döscher J, Laban S, Hoffmann TK, Brunner C: NF-κB and its role in checkpoint control. Int J Mol Sci 21:3949, 2020. https://doi.org/10.3390/ijms21113949
4. Bikfalvi A, da Costa CA, Avril T, Barnier JV, Bauchet L, Brisson L, Cartron PF, Castel H, Chevet E, Chneiweiss H, Clavreul A, Constantin B, Coronas V, Daubon T, Dontenwill M, Ducray F, Enz-Werle N, Figarella-Branger D, Fournier I, Frenel JS, Gabut M, Galli T, Gavard J, Huberfeld G, Hugnot JP, Idbaih A, Junier MP, Mathivet T, Menei P, Meyronet D, Mirjolet C, Morin F, Mosser J, Moyal EC, Rousseau V, Salzet M, Sanson M, Seano G, Tabouret E, Tchoghandjian A, Turchi L, Vallette FM, Vats S, Verreault M, Virolle T: Challenges in glioblastoma research: Focus on the tumor microenvironment. Trends in Cancer 9:9-27, 2023. https://doi.org/10.1016/j.trecan.2022.09.005
5. Burugu S, Gao D, Leung S, Chia SK, Nielsen TO: TIM-3 expression in breast cancer. OncoImmunology 7:e1502128, 2018. https://doi.org/10.1080/2162402X.2018.1502128
6. Cao Y, Zhou X, Huang X, Li Q, Gao L, Jiang L, Huang M, Zhou J: Tim-3 expression in cervical cancer promotes tumor metastasis. PLoS One 8:e53834, 2013. https://doi.org/10.1371/journal.pone.0053834
7. Cong Y, Liu J, Chen G, Qiao G: The emerging role of T-cell immunoglobulin Mucin-3 in breast cancer: A promising target for immunotherapy. Front Oncol 11:723238, 2021. https://doi.org/10.3389/fonc.2021.723238
8. Curley J, Conaway MR, Chinn Z, Duska L, Stoler M, Mills AM: Looking past PD-L1: Expression of immune checkpoint TIM-3 and its ligand galectin-9 in cervical and vulvar squamous neoplasia. Mod Pathol 33:1182-1192, 2020. https://doi.org/10.1038/s41379-019-0433-3
9. Espinoza-Sánchez NA, Győrffy B, Fuentes-Pananá EM, Götte M: Differential impact of classical and non-canonical NF-κB pathway-related gene expression on the survival of breast cancer patients. J Cancer 10:5191-5211, 2019. https://doi.org/10.7150/jca.34302
10. Giopanou I, Lilis I, Papadaki H, Papadas T, Stathopoulos GT: A link between Rel B expression and tumor progression in laryngeal cancer. Oncotarget 8:114019-114030, 2017. https://doi.org/10.18632/oncotarget.23109
11. Guo Q, Shen S, Guan G, Zhu C, Zou C, Cao J, Cheng W, Xu X, Yu J, Lin Z, Wang G, Chen L, Cheng P, Wu A: Cancer cell intrinsic TIM-3 induces glioblastoma progression. iScience 25:105329, 2022. https://doi.org/10.1016/j.isci.2022.105329
12. Kim HS, Chang CY, Yoon HJ, Kim KS, Koh HS, Kim SS, Lee SJ, Kane LP, Park EJ: Glial TIM-3 modulates immune responses in the brain tumor microenvironment. Cancer Res 80:1833-1845, 2020. https://doi.org/10.1158/0008-5472.CAN-19-2834
13. Kim JE, Patel MA, Mangraviti A, Kim ES, Theodros D, Velarde E, Liu A, Sankey EW, Tam A, Xu H, Mathios D, Jackson CM, Harris-Bookman S, Garzon-Muvdi T, Sheu M, Martin AM, Tyler BM, Tran PT, Ye X, Olivi A, Taube JM, Burger PC, Drake CG, Brem H, Pardoll DM, Lim M: Combination therapy with anti-PD-1, anti-TIM-3, and focal radiation results in regression of murine gliomas. Clin Cancer Res 23:124-136, 2017. https://doi.org/10.1158/1078-0432.CCR-15-1535

14. Lee DW, Ramakrishnan D, Valenta J, Parney IF, Bayless KJ, Sitcheran R: The NF-κB RelB protein is an oncogenic driver of mesenchymal glioma. PLoS One 8:e57489, 2013. https://doi.org/10.1371/journal.pone.0057489
15. Li G, Wang Z, Zhang C, Liu X, Cai J, Wang Z, Hu H, Wu F, Bao Z, Liu Y, Zhao L, Liang T, Yang F, Huang R, Zhang W, Jiang T: Molecular and clinical characterization of TIM-3 in glioma through 1,024 samples. Oncoimmunology 6:e1328339, 2017. https://doi.org/10.1080/2162402X.2017.1328339
16. Liu H, Zeng Z, Sun P: Prognosis and immunoinfiltration analysis of angiogene-related genes in grade 4 diffuse gliomas. Aging 15:9842-9857, 2023. https://doi.org/10.18632/aging.205054
17. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, Chernova T, Manning S, Greenfield EA, Coyle AJ, Sobel RA, Freeman GJ, Kuchroo VK: Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature 415:536-541, 2002. https://doi.org/10.1038/415536a
18. Moore M, Ring KL, Mills AM: TIM-3 in endometrial carcinomas: An immunotherapeutic target expressed by mismatch repair-deficient and intact cancers. Modern Pathol 32:1168-1179, 2019. https://doi.org/10.1038/s41379-019-0251-7
19. Mu L, Long Y, Yang C, Jin L, Tao H, Ge H, Chang YE, Karachi A, Kubilis PS, De Leon G, Qi J, Sayour EJ, Mitchell DA, Lin Z, Huang J: The IDH1 mutation-induced oncometabolite, 2-hydroxyglutarate, may affect DNA methylation and expression of PD-L1 in gliomas. Front Mol Neurosci 11:82, 2018. https://doi.org/10.3389/fnmol.2018.00082
20. Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, Barnholtz-Sloan JS: CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2015-2019. Neuro Oncol 24 Suppl 5:v1-v95, 2022. https://doi.org/10.1093/neuonc/noac202
21. Ostrom QT, Shoaf ML, Cioffi G, Waite K, Kruchko C, Wen PY, Brat DJ, Barnholtz-Sloan JS, Iorgulescu JB: National-level overall survival patterns for molecularly defined diffuse glioma types in the United States. Neuro Onco 25:799-807, 2023. https://doi.org/10.1093/neuonc/noac198
22. Peng PJ, Li Y, Sun S: On the significance of Tim-3 expression in pancreatic cancer. Saudi J Biol Sci 24:1754-1757, 2017. https://doi.org/10.1016/j.sjbs.2017.11.006
23. Pu F, Chen F, Zhang Z, Qing X, Lin H, Zhao L, Xia P, Shao Z: TIM-3 expression and its association with overall survival in primary osteosarcoma. Oncol Lett 18:5294-5300, 2019. https://doi.org/10.3892/ol.2019.10855
24. Qin H, Zhou J, Xu J, Cheng L, Tang Z, Ma H, Guo F: The nuclear transcription factor RelB functions as an oncogene in human lung adenocarcinoma SPC-A1 cells. Cancer Cell Int 18:88, 2018. https://doi.org/10.1186/s12935-018-0580-5
25. Qin H, Zhou J, Zhou P, Xu J, Tang Z, Ma H, Guo F: Prognostic significance of RelB overexpression in non-small cell lung cancer patients. Thoracic Cancer 7:415-421, 2016. https://doi.org/10.1111/1759-7714.12345
26. Romagnoli G, D'Alessandris QG, Capone I, Tavilla A, Canini I, Lapenta C, Buccarelli M, Giordano M, Tirelli V, Sanchez M, Fragale A, Giannetti S, Di Bonaventura R, Lauretti L, Biffoni M, Ricci-Vitiani L, Pallini R, Gabriele L: CD8+CD103+PD1+TIM3+ T cells in glioblastoma microenvironment correlate with prognosis. Immunology 171:198-211, 2024. https://doi.org/10.1111/imm.13710
27. Sauer N, Janicka N, Szlasa W, Skinderowicz B, Kołodzińska K, Dwernicka W, Oślizło M, Kulbacka J, Novickij V, Karłowicz-Bodalska K: TIM-3 as a promising target for cancer immunotherapy in a wide range of tumors. Cancer Immunol Immunother 72:3405-3425, 2023. https://doi.org/10.1007/s00262-023-03516-1
28. Shen F, Guo Q, Hu Q, Zeng A, Wu W, Yan W, You Y: RelB, a good prognosis predictor, links cell-cycle and migration to glioma tumorigenesis. Oncol Lett 15:4404-4410, 2018. https://doi.org/10.3892/ol.2018.7894
29. Sim J, Park J, Kim S, Hwang S, Sung K, Lee JE, Yang S, Cho K, Lee S, Moon JS, Ahn J, Lim J: Association of Tim-3/Gal-9 axis with NLRC4 inflammasome in glioma malignancy: Tim-3/Gal-9 induce the NLRC4 inflammasome. Int J Mol Sci 23:232022, 2022. https://doi.org/10.3390/ijms23042028
30. Singh MK, Singh L, Chosdol K, Pushker N, Saini N, Meel R, Bakhshi S, Sen S, Kashyap S: Differential expression of p52 and RelB proteins in the metastatic and non-metastatic groups of uveal melanoma with patient outcome. J Cancer Res Clin Oncol 145:2969-2982, 2019. https://doi.org/10.1007/s00432-019-03052-5
31. Sørensen MD, Nielsen O, Reifenberger G, Kristensen BW: The presence of TIM-3 positive cells in WHO grade III and IV astrocytic gliomas correlates with isocitrate dehydrogenase mutation status. Brain Pathology 31:e12921, 2021. https://doi.org/10.1111/bpa.12921
32. Soubannier V, Stifani S: NF-κB signalling in glioblastoma. Biomedicines 5:29, 2017. https://doi.org/10.3390/biomedicines5020029
33. Su H, Xie H, Dai C, Ren Y, She Y, Xu L, Chen D, Xie D, Zhang L, Jiang G, Chen C: Characterization of TIM-3 expression and its prognostic value in patients with surgically resected lung adenocarcinoma. Lung Cancer 121:18-24, 2018. https://doi.org/10.1016/j.lungcan.2018.04.009
34. Wang Y, Zhao S, Zhang X, Zhu H, Ji X, Jiang Y, Meng J, Shi H, Gao X, Zhang X, Li H: Higher T cell immunoglobulin mucin-3 (Tim-3) expression in cervical cancer is associated with a satisfactory prognosis. Translat Cancer Res 9:2801-2813, 2020. https://doi.org/10.21037/tcr.2020.02.41
35. Waters MR, Gupta AS, Mockenhaupt K, Brown LN, Biswas DD, Kordula T: RelB acts as a molecular switch driving chronic inflammation in glioblastoma multiforme. Oncogenesis 8:37, 2019. https://doi.org/10.1038/s41389-019-0146-y
36. Wolf Y, Anderson AC, Kuchroo VK: TIM3 comes of age as an inhibitory receptor. Nature reviews. Immunology 20:173-185, 2020. https://doi.org/10.1038/s41577-019-0224-6
37. Wuerdemann N, Pütz K, Eckel H, Jain R, Wittekindt C, Huebbers CU, Sharma SJ, Langer C, Gattenlöhner S, Büttner R: LAG-3, TIM-3 and VISTA expression on tumor-infiltrating lymphocytes in oropharyngeal squamous cell carcinoma-Potential biomarkers for targeted therapy concepts. Int J Mol Sci 22:379, 2021. https://doi.org/10.3390/ijms22010379

38. Xia Y, Shen S, Verma IM: NF-kappaB, an active player in human cancers. Cancer Immunol Res 2:823-830, 2014. https://doi.org/10.1158/2326-6066.CIR-14-0112
39. Zeng F, Wang K, Huang R, Liu Y, Zhang Y, Hu H: RELB: A novel prognostic marker for glioblastoma as identified by population-based analysis. Oncol Lett 18:386-394, 2019. https://doi.org/10.3892/ol.2019.10296
40. Zhang J, Sai K: Wang Xl, Ye SQ, Liang LJ, Zhou Y, Chen Zj, Hu WM, Liu JM: Tim-3 expression and MGMT methylation status association with survival in glioblastoma. Front Pharmacol 11: 584652, 2020. https://doi.org/10.3389/fphar.2020.584652
41. Zhang J, Wang Z, Ye Y, Jiang Y, Zhu Z, Zhou J, Lv Y, Hu W, Yang Y: Glioma cells with Tim-3 expression induce resistance to chemotherapy drug. Int J Clin Exp Med 11:13442-13447, 2018
42. Zhao Y, Chen D, Wang W, Zhao T, Wen J, Zhang F, Duan S, Chen C, Sang Y, Zhang Y, Chen Y: Significance of TIM-3 expression in resected esophageal squamous cell carcinoma. Ann Thoracic Surg 109:1551-1557, 2020. https://doi.org/10.1016/j.athoracsur.2019.12.017
43. Zhou E, Huang Q, Wang J, Fang C, Yang L, Zhu M, Chen J, Chen L, Dong M: Up-regulation of Tim-3 is associated with poor prognosis of patients with colon cancer. Int J Clin Exp Pathol 8:8018-8027, 2015
44. Zhou X, Shan Z, Yang H, Xu J, Li W, Guo F: RelB plays an oncogenic role and conveys chemo-resistance to DLD-1 colon cancer cells. Cancer Cell Int 18:181, 2018. https://doi.org/10.1186/s12935-018-0677-x
45. Zhuang C, Ni B, Zhang ZZ, Zhao WY, Tu L, Ma XL, Yang LX, Cao H, Wang M: Low distribution of TIM-3+ cytotoxic tumor-infiltrating lymphocytes predicts poor outcomes in gastrointestinal stromal tumors. J Immunol Res 2021:6647292, 2021. https://doi.org/10.1155/2021/6647292