TIGIT is Positively Associated with Advanced Human Glioma and Displays an Immunosuppressive Microenvironment

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Research Article

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Abstract

Background: Diffuse glioma is a malignant human brain cancer that is hard to overcome. This represents a high risk of mortality. The current challenge is limited to the control of tumor progression and survival improvement.

Immunotherapy consists of stimulating the immune system in order to eliminate the non-self-elements that damage the human body, including cancer cells. However, in human glioma, the current immunotherapeutic targets did not show significant benefit.

In this study, we aimed at evaluating the expression and potential role of a new immunosuppressive molecule, TIGIT in glioma patients.

Patients and Methods: A cohort of 667 patients from the TCGA database along with a cohort of 53 Moroccan patients, were analyzed in order to assess the role of TIGIT in human glioma progression and to estimate whether blocking this immune checkpoint molecule would be of a potential therapeutic benefit.

Real time RT-PCR from fresh human biopsies and bioinformatics approaches were both used in this study.

Results: Our results showed that high expression of TIGIT had prognostic value with some known clinical glioma risk factors such as sex, age and mutation status. High expression of TIGIT was positively associated with an advanced grade of glioma. Moreover, elevated levels of TIGIT were correlated to higher rates of FoxP3, a regulatory T-cell marker that reflects a strong immunosuppressive microenvironment. Interestingly, TIGIT showed strong association with Treg cell-secreted cytokines (TGF-beta and IL-10), supporting the likely involvement of TIGIT in the exhaustion of the intra-tumoral immune cells. Finally, elevated rates of TIGIT were significantly associated to elevated rates of other inhibitory immune checkpoint molecules (PD-1, VISTA and Tim-3) in human glioma patients.

Conclusions: TIGIT blockade might be of valuable therapeutic benefit in patients with advanced gliomas.

Introduction

Gliomas regroup all tumors that originate from glial cells, astrocytic tumors, oligodendroglioma and ependymoma. Gliomas are the common primary malignant brain tumors (with around 80%), where Glioblastoma (GBM) is the most frequent one and the one, which represents the worst prognosis for patients [1].

Immunotherapy is taking place as a strong approach for the treatment of cancer. Several studies suggest the potential of targeting immune checkpoints pathways to overcome tumor progression. However, the current two main targeted immunosuppressive molecules in treating cancer, CTLA-4 & PD-1, did not result

in favorable outcomes in glioma patients [2, 3]. It is therefore, important to explore new potential immunotherapeutic targets.

Human gliomas are characterized by an immunosuppressive microenvironment [4]. Several ongoing clinical studies encourage the use of immune checkpoint inhibitors to overcome gliomas. However, Nivolumab and Ipilimumab, the two most tested immunotherapeutic antibodies, resulted in a very limited outcome [5]. It was therefore important to identify novel molecules, which function as immune checkpoints and which could be used as new targets either solely or in combination with others, in order to improve the anti-tumor immune response in glioma patients.

T cell immunoglobulin and ITIM domain (TIGIT), also known as Washington University Cell Adhesion Molecule (WUCAM), V-set and transmembrane domain-containing protein 3 (Vstm3) and V-set and immunoglobulin domain-containing protein 9 (VSIG9) [6–8], is an immune checkpoint identified on the surface of T cells [9, 10] and NK cells [11]. This molecule is composed of three domains, an extracellular, which is made up of an immunoglobulin-like domain, a transmembrane type 1 domain and a cytoplasmic domain that contains both ITIM *(Immunoreceptor Tyrosine-based Inhibitory Motif*) and ITT (*Ig Tail-Tyrosine like motif*) motifs, which are responsible for the negative signaling [7]. TIGIT delivers co-inhibitory signals following the stimulation with the ligand CD155, and after a competition with CD96 and CD226 that also bind to the same ligand CD155 [8]. Several studies reported that a higher expression of TIGIT is correlated with a bad prognosis in different cancer types [12–15], and the blockade of this molecule would allow the immune system to regain its defensive capacities [16–18].

Regulatory T cells are known to be an immunosuppressive subtype of CD4 + T-cells. In the tumor microenvironment, these cells produce immunosuppressive cytokines that cooper in the inhibition of the immune system. In the context of glioma, we showed that FoxP3, which is a regulatory T cell specific transcription factor [19], is positively associated with higher grades of glioma [20].

In this study, we report evidence for the implication of TIGIT in human glioma progression. We started by evaluating TIGITs' levels of expression and its association with few clinical features including grades and IDH mutation status. We then explored the potential link of TIGIT with markers of immunosuppression, especially those related with regulatory T-cells. Our results showed that high expression of TIGIT is related to poor prognosis in human glioma patients. These findings support a therapeutic targeting of TIGIT in glioma patients.

Patients And Methods

Patients:

A total of 53 glioma patients approved their involvement in our study with written informed consent for the use and experimentation of their clinical data and biopsies. Fresh specimens were isolated at the time of the removal of the tumor at the Neurosurgery Service of the University Hospital Center Ibn Rochd, Casablanca Morocco. Metastatic tumors were excluded.

TCGA Data Analysis:

A total of 667 including 154 Glioblastoma Multiform (GBM) and 513 Low-Grade Glioma (LGG) patients were explored through the cBioportal for Cancer Genomics https://www.cbioportal.org/, by evaluating clinical data and converted Log2 mRNA-seq database of The Cancer Genome Atlas (TCGA).

Real-Time RT-PCR assays:

Total RNA was extracted from conserved biopsies using a home-developed protocol using TRIzol solution (Bioline, France). After being dosed on a NanoVueTM plus Spectrophotometer (GE Healthcare, UK), 0.5µg of total RNA was then converted to cDNA in two steps. Step one was for inhibiting RNA's secondary structures by incubation of RNA for 10min at 70°C with Random Hexamer Primers (1µl; Bioline, France) and RNase Free Water (4µl). The step two was to synthesis the cDNA by incubating the product from step one for 10min at 25°C, 30min at 45°C and 5min at 85°C with 4 µl Tetro Reverse Transcriptase buffer, 4 µl of dNTP (10 mM), 0.5 µl of RNase Inhibitor (40U/µl; Invitrogen, France), 0.5 µl Tetro Reverse Transcriptase Enzyme (10000U; Bioline, France) and 1 µl of RNase-Free Water. To evaluate mRNA gene expression, qPCR experiment was performed using Fast 7500 machine using 10µl of fluorescent dye SYBR ™ Green PCR Master Mix (Thermo Fischer), 7µl of H2O, and 0.5µl of each primer (forward and reverse).

The primers used for experimentations were:

beta-actin Forward: 5'-TGGAATCCTGTGGCATCCATGAAAC-3'

beta-actin Reverse: 5'-TAAAACGCAGCTCAGTAACAGTCCG-3'

TIGIT Forward: 5'-CGTGAACGATACAGGGGAGT-3',

TIGIT Reverse: 5'-ACGATGACTGCTGTGCAGAT-3',

FoxP3 Forward: 5'TCTTCCTTGAACCCCATGCC-3'

FoxP3 Reverse: 5'GCATGAAATGTGGCCTGTCC-3'

Statistical analysis:

IBM SPSS Statistics (version 26) predictive analytics software was used to check the normality of distribution of each cohort. Statistical analyses and graph designs were performed on GraphPad Prism 6.0 software (GraphPad Software, USA). Mann-Whitney t-test was used to compare differences between medians for the expression of distinct sets of genes. Correlations were performed by Spearman test and ANOVA was used to analyze the differences among means. All analysis were conducted by two independent researchers before validating results.

Results

TIGIT was significantly up-regulated in advanced human gliomas.

When analyzing the expression of *TIGIT* depending on clinical and histological parameters available in TCGA database (Table 1), the results of non-parametric t-test showed that *TIGIT* was linked to sex, age, high grade and IDH wild type status ($p = 0.0145$; $p = 0.0006$; $p < 0,0001$; $p = 0,0041$ respectively) but not with the overall survival status ($p = 0.1896$).

According to the TCGA database, and depending on the 2016' WHO classification of histological status (Fig. 1.A), *TIGIT* was highly expressed in GBM compared to astrocytoma (GII & GIII) ($p < 0.0001$), oligoastrocytoma (GII & GIII) ($p < 0.0001$) and oligodendroglioma (GII & GIII) ($p = 0.0016$). In contrast, no significant differences were detected when comparing the other subtypes (astrocytoma vs oligoastrocytoma; $p = 0.6269$), (astrocytoma vs oligodendroglioma; $p = 0.1618$) and (oligoastrocytoma vs oligodendroglioma $p = 0.5209$). These data indicated that high expression of *TIGIT* was positively associated with the most malignant subtypes of human gliomas.

ANOVA test revealed a significant difference between the means of the three available grades of Astrocytoma in TCGA database (GII, GIII and GIV/GBM) ($p < 0.0001$) (Table 1). Also, t-test was performed to evaluate the expression of *TIGIT* depending on the grades from TCGA database. Results showed higher expression in glioma Grade IV (GBM) compared to grade II (Astrocytoma, Oligoastrocytoma and Oligodendroglioma) ($p < 0.0001$) and grade III (Astrocytoma, Oligoastrocytoma and Oligodendroglioma) ($p < 0.0001$). In contrast, no statistical difference was observed between grade II and grade III ($p = 0.1639$) (Fig. 1B). These results corroborated those reported in (Fig. 1A) and showed that elevated levels of *TIGIT* were associated to the most advanced grade of human gliomas.

Table 1
Expression of *TIGIT* depending on distinct patients' pathophysiological parameters in the TCGA cohort.

Clinical parameters	**TCGA Cohorts (n)**	**Median of *TIGIT* mRNA**	***p value***
Sex	Women (n = 285)	2.284	*0.0145*
	Man (n = 382)	2.557	
Age	< 40 (n = 262)	2.179	*0.0006*
	> 40 (n = 393)	2.585	
IDH mutation	Wild Type (n = 169)	2.972	*0.0041*
	Mutant (n = 99)	2.362	
Overall Survival Status	Living (n = 441)	2.337	*0.1896*
	Deceased (n = 225)	2.585	
Grade	Low grade (GII-GII) (n = 514)	2.221	*< 0.0001*
	High grade (GIV) (n = 152)	3.000	
Expression Subtype (GBM)	Mesenchymal (n = 49)	3.700	*0,0101*
	Classical (n = 39)	2.585	
	Neural (n = 26)	2.989	
	Proneural (n = 29)	2.322	
	G-CIMP (n = 8)	2.953	
Histological Diagnosis	Astrocytoma GII (n = 63)	1.958	*< 0,0001*
	Astrocytoma GIII (n = 131)	2.312	
	Astrocytoma GIV (*GBM*) (n = 152)	3.170	
	Oligoastrocytoma GII (n = 74)	2.350	*0.1223*
	Oligoastrocytoma GIII (n = 55)	2.219	
	Oligodendroglioma GII (n = 112)	2.274	*0.9708*
	Oligodendroglioma GIII (n = 79)	2.323	

*GBM: Glioblastoma multiform

**p* value < 0.05 indicates a significant statistical test

The second cohort used in this study was related to the Moroccan patients, and which was composed of 53 human glioma patients (Table 2). In this cohort, *TIGIT* showed similar trends as those observed in the TCGA cohort. However, *TIGIT* expression did not reach significant difference when comparing groups of patients depending on age and sex ($p = 0.8734$; $p = 0,1268$ respectively).

Table 2
Expression of *TIGIT* depending on distinct patients' pathophysiological parameters in the Moroccan cohort.

Clinical data	Cohorts (n)	Median of *TIGIT* mRNA	*p value*
Sex	Female (n = 23)	1.480	*0.1268*
	Male (n = 30)	1.955	
Age	< 40 (n = 28)	1.705	*0,8734*
	> 40 (n = 24)	1.820	
Grade	Low grade* (n = 24)	1.310	*0.0423*
	High grade* (n = 29)	2.060	

*Low grade (Grade I-Grade II)

*High grade (Grade III-Grade IV)

*p value < 0.05 indicates a significant statistical test

Interestingly, when exploring the 53 Moroccan glioma biopsies (Fig. 1C), *TIGIT* expression was significantly higher in advanced grades (GIII & GIV, n = 29) compared to lower grades of gliomas (GI & GII, n = 24) ($p = 0.0423$), confirming data from the TCGA cohort.

TIGIT expression was higher in IDH-wildtype glioma and was associated to mesenchymal-molecular subtype.

TIGIT expression was assessed according to the glioma molecular status. The expression of *TIGIT* was first evaluated depending on *IDH* gene status, *IDH wildtype* versus *mutant* in patients from TCGA database (Table 1). The results showed that *IDH-wildtype* patients displayed higher expressions of *TIGIT* compared to patients with *IDH-mutant* ($p = 0.0041$). This analysis could not be achieved in the second cohort (the Moroccan cohort) because of the small sample size (*Wildtype*, n = 3; *Mutant*, n = 4).

Subsequently, Kruskal-Wallis test was used to assess the difference in the expression of GBM molecular subtypes (Mesenchymal, Classical, Neural, Proneural and G-CIMP) and reported a significant difference between these groups ($p = 0,0101$) (Supplementary Fig. 1). Furthermore, when performing several t-tests between the five subtypes above, we found that *TIGIT* was significantly higher in the mesenchymal molecular subtype compared to Proneural and Classical (p = 0,0073; p = 0,0043 respectively) (Supplementary table 1).

Higher TIGIT **expression was linked to higher expression of immune T cell markers and was positively correlated with** FoxP3.

As previously mentioned in the introduction, several studies confirmed a higher expression of *TIGIT* on T-cells in advanced grades of different tumor sites. While exploring TCGA database, we wanted to assess whether a high expression of *TIGIT* could be associated with a higher infiltration of *CD4 + T-cells*, *CD8 + T*-cells and *regulatory T-cells* (via *FoxP3*) in glioma (Fig. 2). Our results showed that indeed, markers of *CD4*, *CD8* and *regulatory T-cells* were upregulated in patients with high expression of *TIGIT* versus cases with lower expression of *TIGIT* in human glioma ($p < 0.0001$). Furthermore, we evaluated the correlation of *TIGIT* with the expression levels of the three cell markers (Table 3), and found that high expression of TIGIT was positively correlated with *FoxP3*, *CD8* and *CD4* expression (r = 0.4269, $p < 0.0001$; r = 0.2565, $p < 0.0001$; r = 0.1926, $p = 0.0004$). This suggested that high expression of *TIGIT*, whose expression was found to be associated with advanced subtypes of glioma from previous results, also was positively correlated with high infiltration of T cells.

Table 3: Correlation between the expression of T-cells markers with high *TIGIT* expression profile depending on TCGA database.

	High TIGIT	
	r	*p*
CD4 T-cells	0.1926	*0.0004*
CD8 T-cells	0.2565	*< 0,0001*
FoxP3 Tregs	0.4269	*< 0,0001*

*p value < 0.05 indicates a significant statistical test

Subsequently, we wanted to check if the data from the Moroccan cohort were similar to those observed with the TCGA database. 18 patients having available mRNA expression data for both *TIGIT* and *Foxp3* were analyzed to evaluate their correlation in the context of human glioma (Fig. 3). The result joined those of Table 3 and corroborated that *TIGIT* was positively correlated to *FoxP3* (r = 0.534; $p = 0.0225$).

TIGIT **was associated with an immunosuppressive microenvironment.**

Since the regulatory T cell marker (*FoxP3*) exhibited the highest rate of correlation with *TIGIT*, we checked whether higher *TIGIT* expression would correlate with an immunosuppressive microenvironment in human glioma, globally. We therefore analyzed the level of expression of other known and potent regulatory T cell secreting immunosuppressive cytokines (Fig. 4), and found that the expression of both *IL-10* and *TGF-beta* was upregulated in the case of *high TIGIT* compared to *low TIGIT* conditions ($p < 0.0001$ and $p < 0.0001$, respectively). This suggested that *TIGIT* highly contributes to the setting up of a potent immunosuppressive microenvironment in human glioma.

TIGIT **was significantly upregulated along with other immune checkpoints in human glioma.**

To assess the immunosuppressive status of human gliomas according to *TIGIT* expression level, three immune checkpoints (*PD-1*, *VISTA*, *Tim-3*) were assessed. Interestingly, and according to TCGA database (Fig. 5), we found that in the context of low *TIGIT*, the immune checkpoints *PD-1*, *VISTA* and *Tim-3* exhibited lower expression compared to the context of high *TIGIT* expression where all the three immune checkpoints were upregulated ($p < 0.0001$). This showed that in human glioma's microenvironment, the expression level of the three inhibitory molecules *PD-1*, *VISTA* and *Tim-3* goes along with the level of expression of *TIGIT*, supporting the important role of *TIGIT* as a potential potent immunosuppressive immune checkpoint in human glioma.

Discussion

Glioma is a general term that describes different tumors that initiate from glial cells, including astrocytes, oligodendrocytes, and ependymal cells. This tumor is known to be the most common primary brain cancer and represents a very bad prognosis with high rates of recurrence [21]. Lower median survival is registered, especially in advanced grade glioma, even with several choices of treatments, including surgery, chemotherapy and radiotherapy [22].

In this work, we describe the involvement of *TIGIT* in human glioma development in two independent cohorts (TCGA database and Moroccan patients). We found that this newly identified co-inhibitory molecule is strongly associated with a malignant type of glioma.

TIGIT expression was upregulated in higher grades especially in the glioblastoma. Our study reports an elevated rate of *TIGIT* in higher compared to lower age. Also, Tian et al. showed that female patients with GBM have higher cancer specific survival compared to male. The study suggests an implication of female hormones to prevent the malignity of glioma [23]. Consistently, our results revealed an elevated rate of expression of this inhibitory molecule in male compared to female. A link between *TIGIT* upregulation and hormones is likely. Further studies need to be conducted to evaluate whether there is a direct link.

IDH mutation represents a critical genomic alteration, which could indicate tumor prognosis [24]. The *IDH-wildtype* patients tends to display an aggressive phenotype compared to the *IDH mutant* patients [25]. Interestingly, our results reported that *TIGIT* was significantly more prevalent in patients with the *IDH-wildtype* gene compared to patients with the *IDH mutant* gene. Moreover, Phillips et al. and Kafess et al. shed light on the several molecular subtypes of glioblastoma, reporting that the mesenchymal subtype represents the most aggressive phenotype [26, 27]. Our results were consistent with these two studies. First, ANOVA test showed a significant difference of the expression pattern of *TIGIT* between different molecular subtypes with an elevated expression in the mesenchymal group. This observation was subsequently confirmed using t-test. This suggests that *TIGIT* could be used as a potential biomarker for the mesenchymal molecular subtype. Therefore, the two findings join previous results to confirm the association of *TIGIT* with poor prognosis in human glioma.

It is believed that tumors are positively associated with immune cell infiltration, including T lymphocytes (Tumor Infiltrating Lymphocytes) [28]. The upregulation of immune checkpoints on these TILs drive them to a state of exhaustion due to chronic activation [29]. For this reason, we evaluated the expression of *TIGIT* in different T cell subtypes, *CD4+*, *CD8 +* and *FoxP3/* regulatory T-cells, via their specific markers (*CD4*, *CD8A* and *FoxP3*, respectively). Our results showed a significant upregulation of the three markers of T-cells in the case of high *TIGIT* compared to low *TIGIT*, indicating a likely role of *TIGIT* in suppressing the immune system and limiting its anti-tumor capacity.

It has been reported that glioma displays an immunosuppressive microenvironment [30]. Therefore, we wanted to check whether *TIGIT* represents a bad prognosis to glioma patients. We evaluated the potential association between *TIGIT* and *FoxP3*, as a regulatory T cell marker [31]. We found that *TIGIT* was positively correlated to *FoxP3+*-regulatory T cells in both the Moroccan patients' cohort and the TCGA database. Moreover, explored immunosuppressive cytokines (*IL-10* and *TGF-beta*) have been confirmed in previous studies to be implicated in advanced grades of glioma [32–34]. Our results reported that both *IL-10* and *TGF-beta* were also significantly upregulated in the context of high *TGIT* compared to low *TIGIT*, suggesting a key role for *TIGIT* in setting up an immunosuppressive environment.

Several studies explored the role of *TIGIT* in different cancer sites such as bladder, colorectal, melanoma and blood [10, 12, 13, 35]. *TIGIT* was also explored to show difference in expression between GBM and Multiple Sclerosis [36], and as a potential therapeutic target upon a dual blockade of anti-TIGIT with anti-PD-1 in a murine GBM model [18, 37]. At the best of our knowledge, our study is the first one to assess the role of *TIGIT* in the progression of human glioma.

PD-1, *VISTA* and *Tim-3* are a set of immune checkpoints that are upregulated on TILs in different sites of tumor including glioma [38–40]. The three studies suggest that these molecules have an impact on tumor progression via the modulation of the immune response in the tumor microenvironment. Our results show a significant association between the upregulation of *PD-1, VISTA and Tim-3* along with the upregulation of *TIGIT*, suggesting that *TIGIT* should also be considered as an appropriate immunotherapeutic target within the human glioma microenvironment.

As we mentioned previously, current immunotherapeutic targets show only limited outcome. *TIGIT* exhibited a significant association to tumor proliferation in several tumor locations. Furthermore, the blockade of *TIGIT* was associated with an inhibition of tumor progression and an improvement of the overall survival in a murine model [16, 41].

Nevertheless, our study has also limitations. A major one is related to the small size of the Moroccan cohort, which did not help to better assess the possible association of *TIGIT* with several other clinical features. In addition, our findings need to be confirmed by assessing *TIGIT* protein expression. Finally, further studies need to evaluate the impact of blocking *TIGIT* in human glioma, especially in improving patients' overall survival.

Conclusion

In summary, our data suggest that *TIGIT* expression was associated to human glioma progression and that the blockade of *TIGIT* could help reducing the immunosuppression state in the glioma microenvironment and therefore, allow the reactivation of the anti-tumor immune response.

Abbreviations

ANOVA
Analysis Of Variances
CD
Cluster of Differentiation
cDNA
Complementary Deoxyribonucleic Acid
CTLA-4
cytotoxic T-lymphocyte-associated protein 4
FoxP3
Forkhead box P3
GBM
Glioblastoma Multiform
G-CIMP
Glioma CpG Island Methylator Phenotype
IDH
Isocitrate Dehydrogenase
IL-10
Interleukin-10
LGG
Low Grade Glioma
mRNA
messenger Ribonucleic Acid
NK
Natural Killer
PD-1
Programmed cell death 1
qPCR
quantitative Polymerase Chain Reaction
RT-PCR
Reverse Transcription Polymerase Chain Reaction
SPSS
Statistical Package for the Social Science

TCGA
The Cancer Genome Atlas
TIGIT
T-cell immunoglobulin and ITIM domain
TILs
Tumor Infiltrating Lymphocyte
Tim-3
T-cell Immunoglobulin and mucin domain
TGF-b
Transforming growth factor-beta
VISTA
V-domain Ig suppressor of T-cell Activation
WHO
World Health Organization.

Declarations

Availability of data and materials

Datasets analyzed during the current study are freely available via https://www.cbioportal.org/. Other data are available from the corresponding author on reasonable request.

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Contributions:

All the authors cooperated sufficiently to take part of the content. Ahmed Qandouci collected, analyzed and interpreted data, also wrote the manuscript. Amina Ghouzlani collected and analyzed data, Abdou-Samad Kone analyzed data Abdelhakim Lakhdar collected and analyzed data, Abdallah Badou designed and supervised the study, analyzed and interpreted data and revised the final draft of the manuscript. All authors approved the final version of the manuscript.

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Ethics declarations

Ethics approval and consent to participate

The consent was verified and approved by the ethical committee of the Ibn Rochd University Hospital Center, Casablanca. Written informed consent was provided and signed by all patients

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest in this work.

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Figures

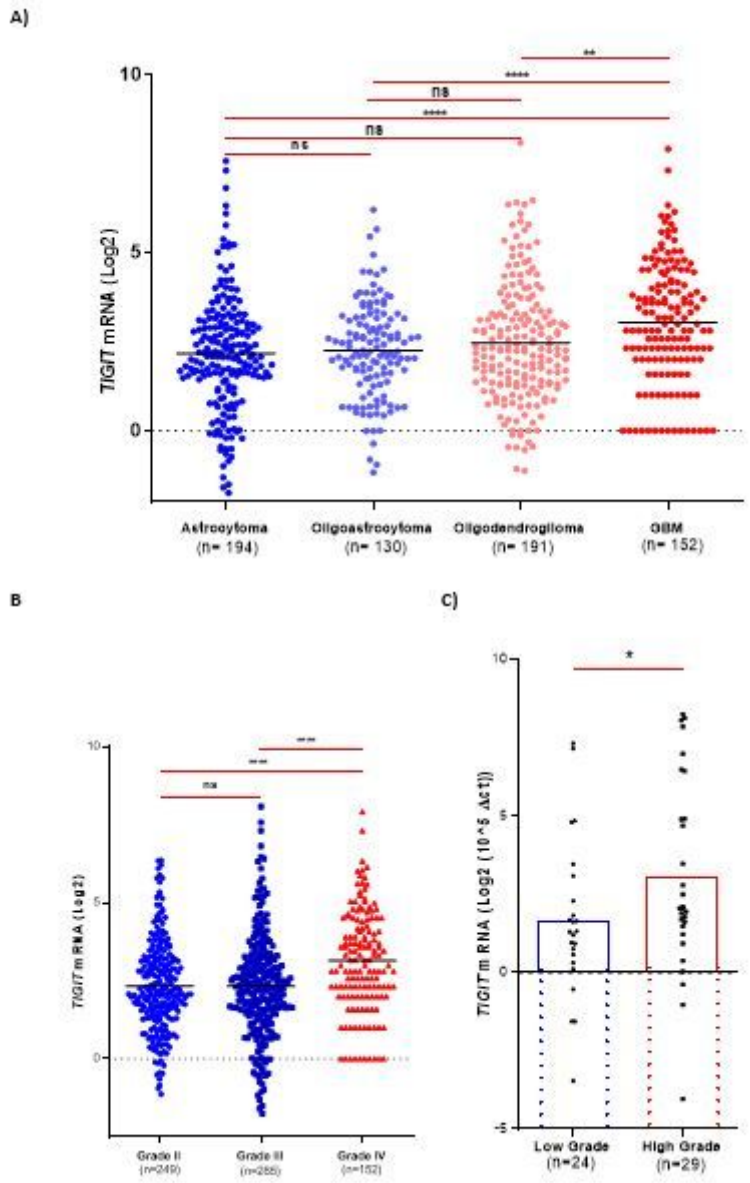

Figure 1

TIGIT* is positively associated with severe malignant glioma.** mRNA expression of *TIGIT* was analyzed in two different cohorts to explore its levels depending on histological subtypes and grades **A)** Tumors from glioblastoma multiform patients of the TCGA dataset exhibited higher expression of TIGIT. **B)** *TIGIT* expression in TCGA glioma patients (GII: n= 249; GIII: n= 265; GIV: n= 152) exhibited its highest levels in GIV. **C)**. *TIGIT* expression in our home cohort is highly expressed in high grade (n=29) compared to low grade (n=24) glioma. Significant differences are indicated (* for $p < 0.0001$, *** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$)

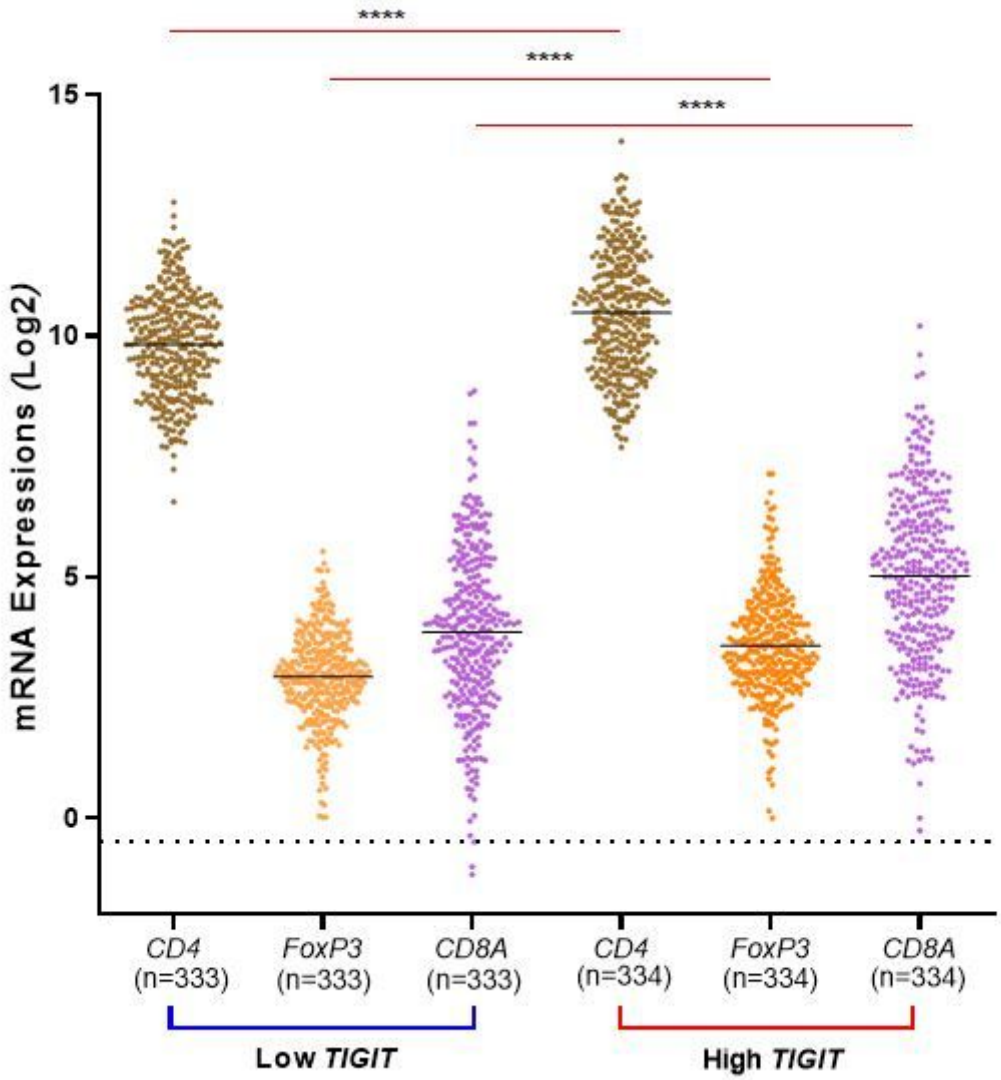

Figure 2

T cell subtypes marker expression (*CD4*, *CD8A* and *Tregs/FoxP3*) depending *TIGIT* expression status. Patients from TCGA database were distributed into two cohorts for each marker of T-cells and depending on the level of expression of *TIGIT*. For each marker, non-parametric t-test was used to compare the level of expression depending on high versus low profile of *TIGIT*. The three markers of T-cells exhibited their higher expression levels in the condition of high *TIGIT*, compared to the condition of low expression of *TIGIT* where their expressions were at lower levels. Significant statistical differences are indicated (**** for $p < 0.0001$).

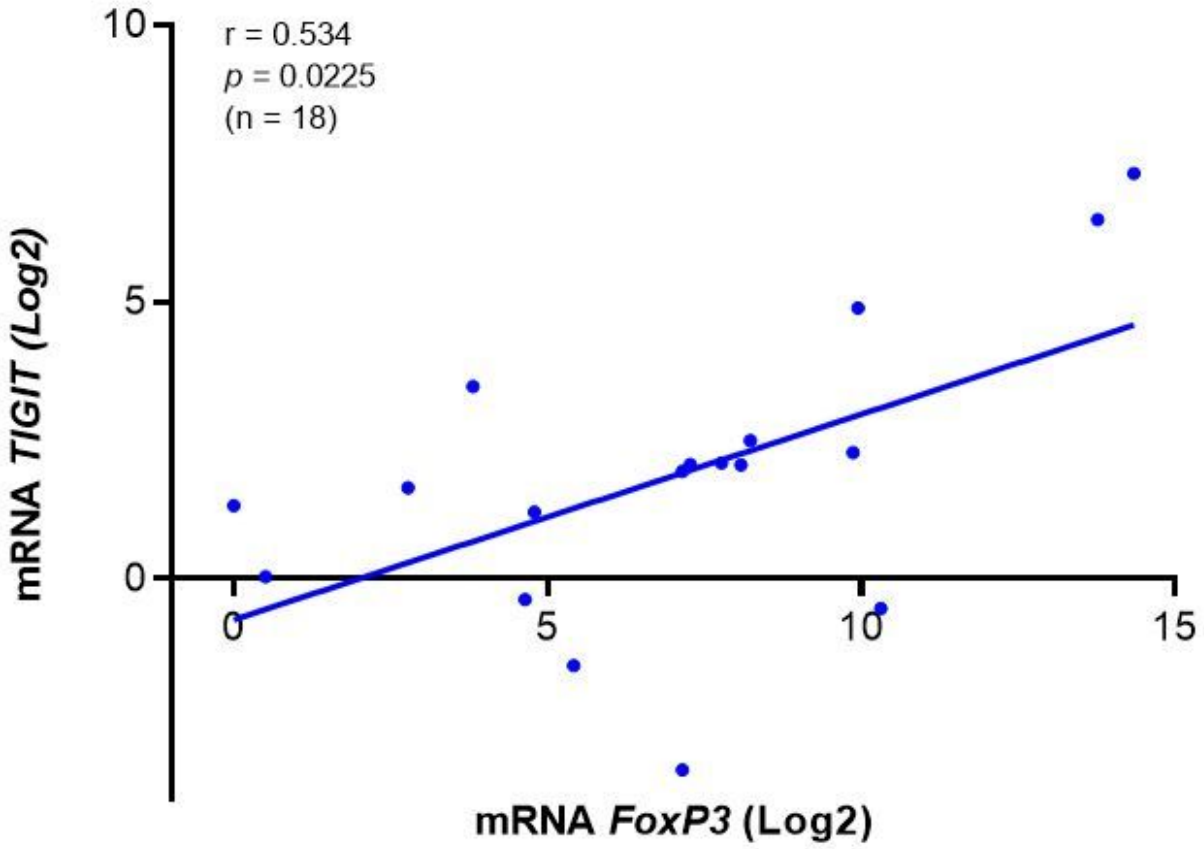

Figure 3

Correlation between expression of *TIGIT* and the expression of *FoxP3* in human glioma patients of Morocco. 18 human glioma patients from the Moroccan cohort were included in the Spearman r test to evaluate the correlation between *TIGIT* and *FoxP3*. *TIGIT* expression was positively correlated to *FoxP3* in the Moroccan cohort. Positive correlation was mentioned on the graph (r=0.534). The statistical significance of the test was indicated too ($p < 0.05$).

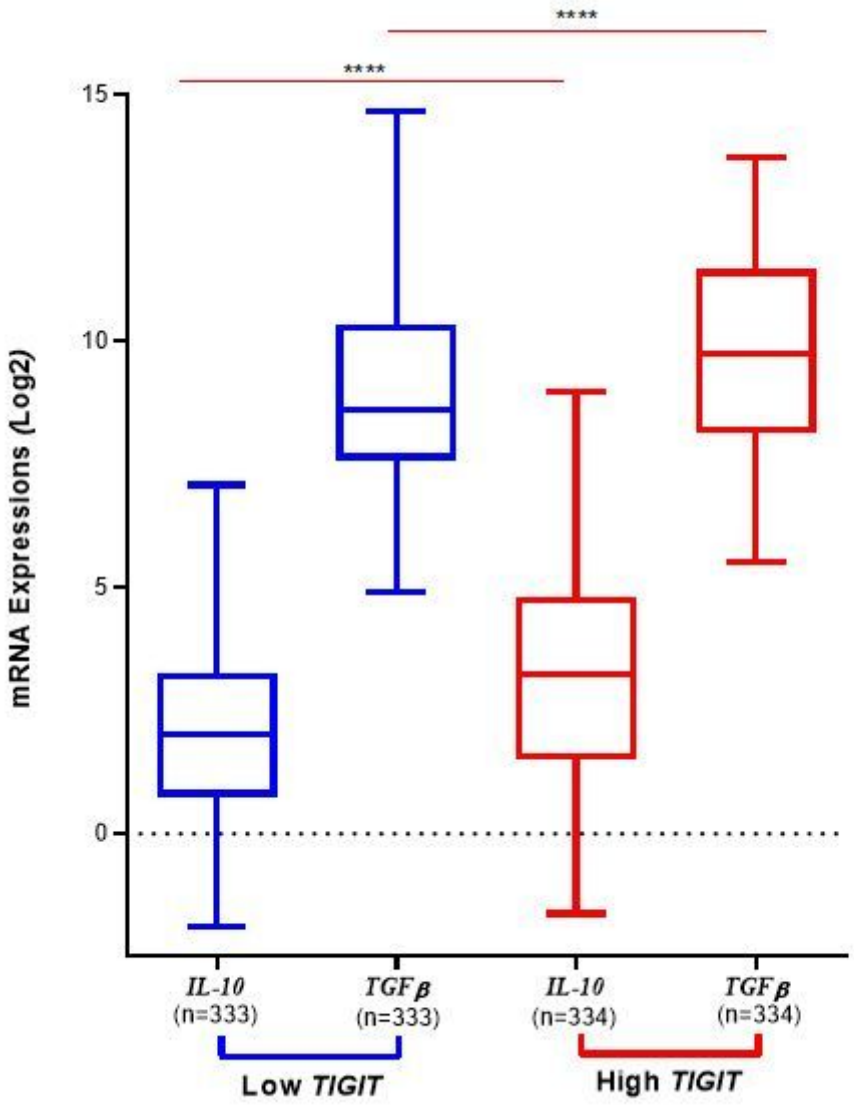

Figure 4

High expression of *TIGIT* transcripts is associated to high expression of anti-inflammatory cytokines. According to TCGA database, *IL-10* and *TGF-beta* showed strong levels of expression in glioma patients in the context of high versus low *TIGIT* expression profile, indicating the strong link between these molecules in inhibiting the anti-tumor response. The statistical significance of the two tests was indicated (**** for $p < 0.0001$).

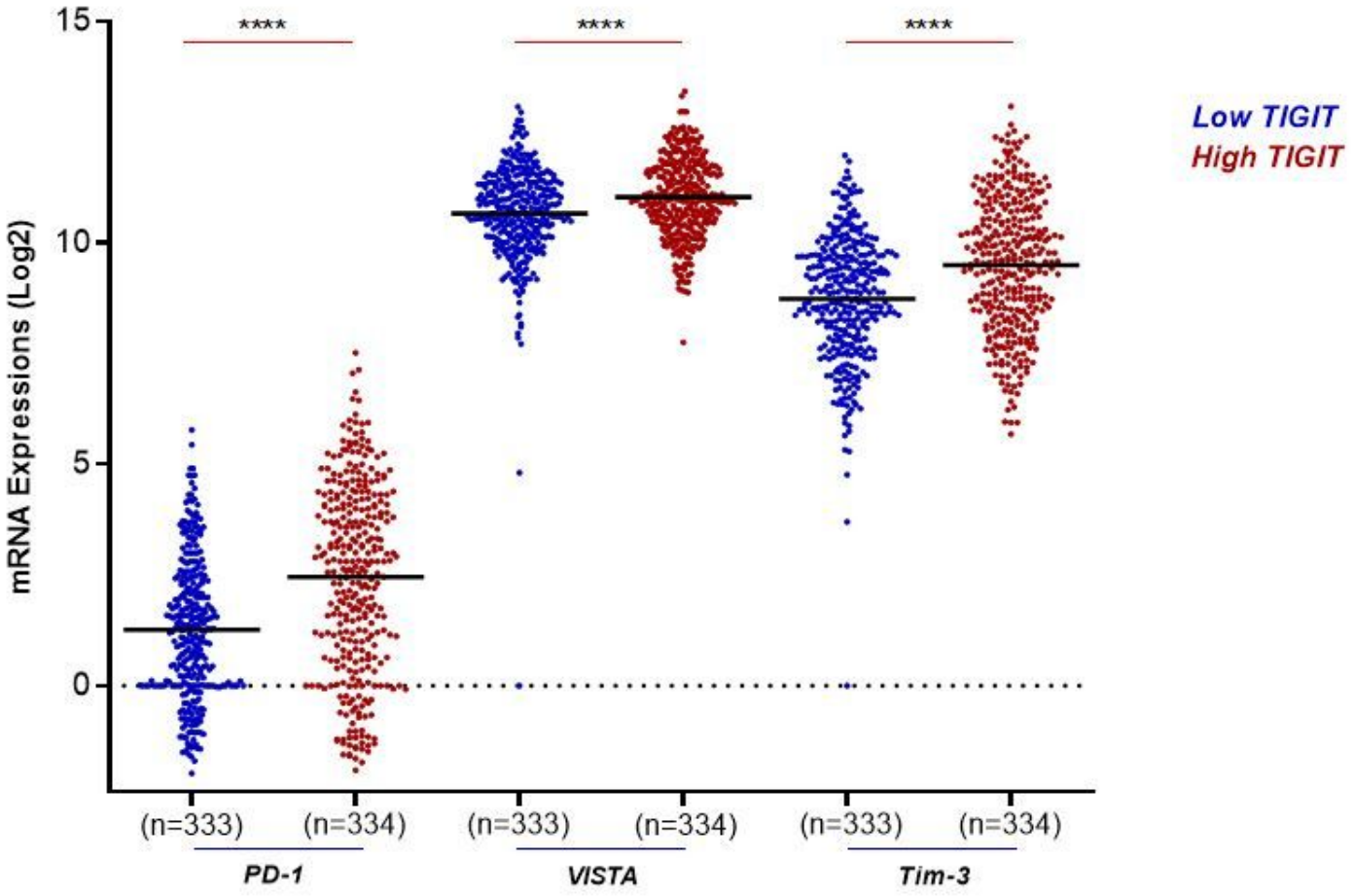

Figure 5

TIGIT* expression is positively associated with critical immune checkpoint regulators.** TCGA dataset was explored to evaluate the levels of expression of three inhibitory molecules. Results showed an upregulation of *PD-1*, *VISTA* and *Tim-3* along with the upregulation of *TIGIT* in the TCGA database. The blue color indicates patients expressing low levels of *TIGIT*. The red color indicates patients expressing high levels of *TIGIT*. Significant differences are indicated for each molecule (* for $p < 0.0001$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupFigure1.docx
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