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Integrative analysis identified the key role of LAG3 in T cell exhaustion in glioma

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Abstract

Lymphocyte-activation gene 3 (LAG3) is an immune checkpoint expressed on the surface of T cells. We conducted a comprehensive molecular characterization and clinical analysis of LAG3 expression in gliomas. Across the CGGA and TCGA glioma datasets, we observed that LAG3 expression increased with higher glioma grade. Further investigation into the molecular subtypes revealed that LAG3 exhibited the highest expression in the Mesenchymal subtype, similar to other immune checkpoints. CIBERSORT analysis showed a significant positive correlation between LAG3 expression and macrophages, suggesting LAG3's involvement in shaping the immune microenvironment. Single-cell data indicated that LAG3 expression appeared to be complementary pattern to other immune checkpoints, implying multiple mechanisms of T cell exhaustion. Importantly, clinical data analysis demonstrated that elevated LAG3 expression is an independent poor prognostic factor for patient survival. These findings suggest that LAG3 may play a crucial role in regulating the immune microenvironment in gliomas and contribute to T cell exhaustion, alongside other immune checkpoints such as the PD-L1/PD-1 pathway. As such, LAG3 represents a potential therapeutic target for glioma immunotherapy.

Keywords Glioma, Immune check point, LAG3, Immunotherapy, Immune evasion

1 Introduction

Lymphocyte-activation gene 3 (LAG3) is an immune checkpoint predominantly expressed on the surface of various immune cells, including T cells, natural killer cells, and certain myeloid cells [1]. LAG3 has been reported to play a role in the regulation of T cell activity during different immune responses [2, 3]. The extracellular region of LAG3 contains four immunoglobulin superfamily domains, which share structural homology with CD4, enabling LAG3 to bind to class II major histocompatibility complex (MHC) and execute its immunoregulatory functions. LAG3 Expression Correlates with immunotherapy response in melanoma patients and serves as a prognostic and predictive biomarker [4]. Combined blockade of LAG3 and PD1 has demonstrated significant efficacy in advanced melanoma. The median progression-free survival (mPFS) was significantly longer in the relatlimab (anti-LAG3) plus nivolumab (anti-PD1) group

compared to the nivolumab monotherapy group [5, 6]. LAG3 is highly expressed in the tumor microenvironment of non-small cell lung cancer (NSCLC). Its expression is associated with immune cell infiltration (e.g., CD8+ T cells) and MHC-I expression, suggesting its potential as a predictive biomarker for immunotherapy response. Furthermore, non-invasive monitoring of LAG3+ tumor-infiltrating lymphocytes (TILs) via radionuclide imaging can be utilized to assess early response to immunotherapy [7, 8]. As a key member of the immune checkpoint family, the primary biological function of LAG3 is to suppress the effector functions of T cells through interactions with co-stimulatory factors [1]. While other immune checkpoints, such as PD-L1 [9, 10], PD-L2 [11], and B7-H3 [12, 13], have been extensively studied in the context of tumor biology, LAG3 has been relatively less explored in the case of glioma.

The cell type-specific expression pattern of LAG3 across different glioma subtypes remains poorly understood. Furthermore, the mechanisms underlying the interactions between LAG3 and its ligands within the glioma tumor microenvironment have not been elucidated.In order to characterize the expression pattern of LAG3 in glioma, we integrated clinical and molecular data including single-cell data, and performed a comprehensive analysis. It was found that LAG3 was universally expressed in T cells within the tumor microenvironment. Interestingly, LAG3 expression appeared to be mutually exclusive to other classical immune checkpoints, such as PD-1 and CTLA-4, in glioma samples. This suggests that LAG3 may play a distinct role in regulating T cell exhaustion within the glioma immune landscape, potentially through alternative mechanisms compared to other well-studied immune checkpoint pathways. These findings warrant further investigation into the specific function of LAG3 in modulating the glioma immune microenvironment and its potential as a therapeutic target.

2 Materials and methods

2.1 Data sources

RNA expression profile data (in FPKM format) from 325 glioma patients covering all WHO grades (2–4) were obtained from the Chinese Glioma Genome Atlas (CGGA, https://www.cgga.org.cn) database [14, 15]. Core clinical information, including age, sex, pathological grade, IDH mutation status, and survival follow-up data, was also collected. Inclusion criteria: Pathological diagnosis confirmed as glioma; Complete clinical data with no missing key information.

RNA sequencing data (in RSEM format) and corresponding clinical information for 670 glioma patients were downloaded from The Cancer Genome Atlas (TCGA, https://www.cbioportal.org/) database [16, 17]. Inclusion criteria were consistent with those for the CGGA dataset. Cases with suspected sample contamination or abnormal sequencing data were excluded.

Three publicly available single-cell datasets were integrated: The CGGA single-cell dataset previously published by our team [18]; The dataset published by Suva et al. [19]; The dataset published by Richards et al. [20]. Detailed information, such as age, gender, clinical stage and key mutation status, are also provided in supplementary tables. FPKM data from CGGA and RSEM data from TCGA were normalized. RSEM data were first converted to FPKM format for consistency. Subsequently, all data were log2(FPKM + 1) transformed to reduce distribution skewness. For single-cell data, we re-grouped all the cells according to key cell type markers combined with Seurat clustering results, and

divided the cells into four major categories: tumor cells, myeloid cells, T cells, and oligodendrocytes. All the subsequent single-cell analysis was based on this refined cell type classification. These comprehensive datasets allowed us to perform a combined clinical-molecular analysis to characterize the expression pattern and clinical relevance of LAG3 in glioma.

2.2 Differentially expressed genes and functional enrichment analysis

The limma package was employed to analyze the differential expression of LAG3 across different pathological grades, molecular subtypes, and IDH mutation statuses. The screening criteria were set as |log2 fold change (FC)| > 1 and adjusted P-value (FDR) < 0.05. Genome-wide Pearson correlation analysis was performed on LAG3 expression levels within the CGGA and TCGA datasets separately to identify gene sets correlated with LAG3 expression. Gene Set Enrichment Analysis (GSEA) was conducted using the fgsea package, with the Hallmark gene sets and KEGG pathway gene sets from the MSigDB database serving as the reference. An FDR < 0.05 was considered indicative of significant enrichment.

2.3 Immune cell infiltration analysis

The composition of immune cells within the tumor microenvironment was analyzed using CIBERSORT, xCell, and MCP-counter. The input data consisted of a log2-transformed FPKM matrix. The Pearson correlation coefficient was calculated between LAG3 expression levels and the proportions of various immune cell subsets (e.g., M1 macrophages, M2 macrophages, CD8+ T cells, etc.). The significance of these correlations was tested ($P < 0.05$).

2.4 Survival analysis

Based on the median LAG3 expression level, patients in the CGGA and TCGA datasets were stratified into high-expression and low-expression groups. Kaplan-Meier survival curves were plotted using the survival package, and the log-rank test was applied to compare overall survival (OS) differences between the two groups. A multivariate Cox proportional hazards regression model was constructed, incorporating age, gender, pathological grade, IDH mutation status, and LAG3 expression level as covariates, to determine whether LAG3 was an independent prognostic factor ($P < 0.05$).

2.5 Statistical analysis

All statistical analyses were performed using R language (v4.4.2). Key packages utilized included ggplot2, pheatmap, fgsea, enrichplot, CIBERSORT, xCell, MCP-counter, Seurat, survival, and circlize. Continuous variables are presented as mean ± standard deviation. Comparisons between two groups were performed using the t-test, while comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). Correlation analyses employed Pearson or Spearman correlation coefficients. Survival analyses utilized the Kaplan-Meier method and Cox regression models. All statistical tests were two-sided, and a p-value < 0.05 was considered statistically significant.

3 Results

3.1 The expression level of LAG3 increases with glioma pathological grade

We reclassified the glioma samples (WHO grades 2, 3, 4) according to the 2021 fifth edition of the WHO Classification of Tumors of the Central Nervous System (CNS) [21]. IDH mutation, the most important molecular marker for glioma classification, was also included in the analysis. We found that in both the CGGA and TCGA bulk tumor RNA sequencing datasets, the expression of LAG3 showed a significant upregulation with increasing tumor grade (Fig. 1A, B and t-test), suggesting that the intensity of the immune reaction within the tumor as the tumor grade progresses. However, we did not observe a stable correlation between LAG3 expression and IDH mutation status in the two datasets, although it appeared that LAG3 expression was generally higher in IDH-mutant gliomas (Fig. 1C, D and t-test). This is in stark contrast to our previous research on the PD-L1/PD-1 axis [9, 10]. These findings suggest that the upregulation of LAG3 expression is associated with higher-grade gliomasand may reflect changes in the tumor immune microenvironment during glioma progression. Due to the limited number of IDH-mutant grade 4 gliomas and the consequent limited statistical power, the relationship between LAG3 expression and IDH status in grade 4 gliomas requires further investigation.

3.2 LAG3 is most significantly expressed in the mesenchymal subtype

Molecular subtypes based on expression profile usually indicates the preferred biological process of glioma. We further analyzed the expression of LAG3 according to the

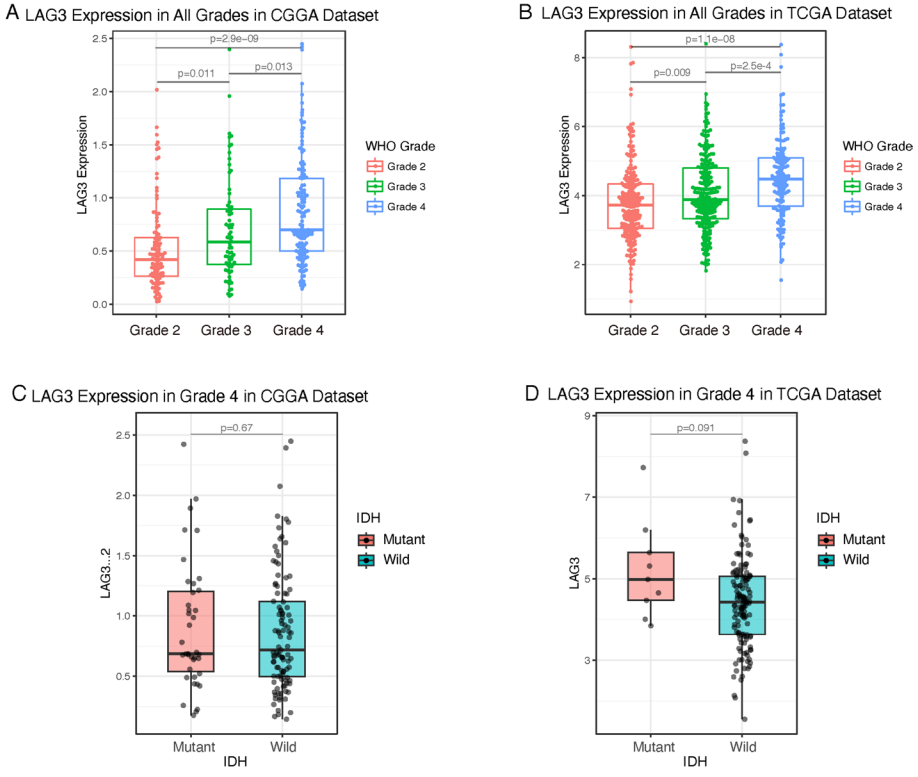

Fig. 1 Comparison of LAG3 expression in different WHO grade gliomas in CGGA (**A**) and TCGA (**B**) bulk tumor RNA-seq datasets. No significant effect of IDH on LAG3 expression was observed in both datasets (**C**, **D**). P value are generated with t-test comparing two groups of continuous values

molecular subtypes [22]. In both CGGA and TCGA bulk tumor datasets, we observed that the expression of LAG3 was most significantly expressed in the mesenchymal subtype (Fig. 2A and B), which was particularly similar to the expression characteristics of PD-L1 as described in our previous study [9], suggesting that LAG3 may play a more significant role in mesenchymal subtype glioma than in that of others. This result was in consistence with the immune infiltrative features of mesenchymal subtype. As an immune checkpoint, LAG3 probably was involved in the formation of mesenchymal transition of glioma.

3.3 LAG3 plays different biological role in terms of IDH mutation status

To further elucidate the biological role of LAG3 in glioma, we conducted functional enrichment analysis using two bulk tumor RNA-sequencing datasets. Whole genome Pearson correlation was performed for LAG3 expression in two datasets. Then R value was used as the input for fgsea package for functional analysis.

Notably, LAG3 exhibited distinct biological roles based on IDH mutation status. In IDH mutant gliomas, LAG3 expression was primarily linked to cell proliferation, with key biological processes involving DNA replication, the cell cycle, and nuclear division (Fig. 3A–C, CGGA dataset; Figs. 3D–F, TCGA dataset). In IDH wild-type gliomas, LAG3 was associated with adaptive immunity, T cell proliferation, and γ-interferon-related immune responses (Fig. 3G–I, CGGA dataset; Fig. 3J–L, TCGA dataset).

To further investigate the intrinsic function of LAG3, we refocused on the immunological role of LAG3 in glioma. Using the online tool CIBERSORT, we performed immune component analysis on the expression data from the two bulk tumor datasets, CGGA and TCGA. The CIBERSORT results showed that the proportion of macrophages, especially M1 type, wassignificantly but weakly correlated correlated with LAG3 expression in both datasets (Pearson's R values were 0.1901, p-value = 0.0128 in CGGA IDHmt, Fig. 4A; Pearson's R values were 0.1469, p-value = 0.0023 in TCGA IDHmt, respectively, Fig. 4C). In IDH wild-type gliomas, LAG3 was also highly associated with macrophage M1 type (Pearson's R values were 0.1842, p-value = 0.0222 in CGGA IDHwt, Fig. 4B; Pearson's R values were 0.2143, p-value = 0.0010 in TCGA IDHwt, Fig. 4D). Furthermore, MCP-counter and xCell were employed to enhance the analysis of immune cell populations associated with LAG3(Supplementary Figs. 1, 2), and the relationships between LAG3 expression and immune score, stromal score, ESTIMATE score, and tumor purity were evaluated in IDH-mutant and wild-type gliomas, respectively(Supplementary

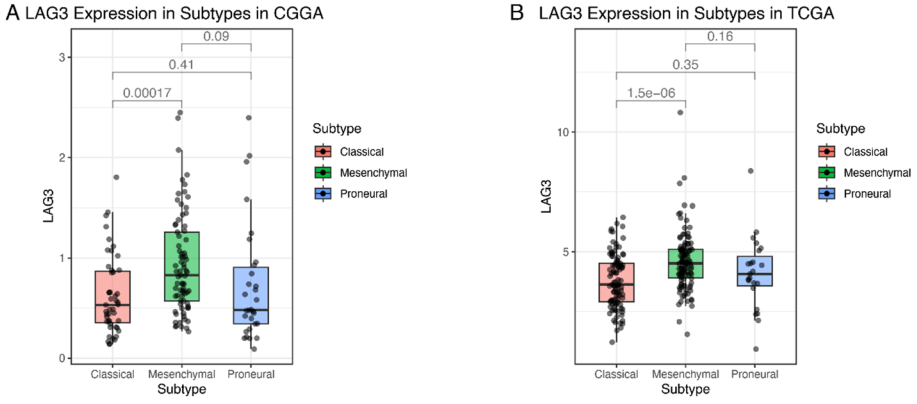

Fig. 2 Comparison of LAG3 expression levels in different molecular subtypes (**A**, **B**)

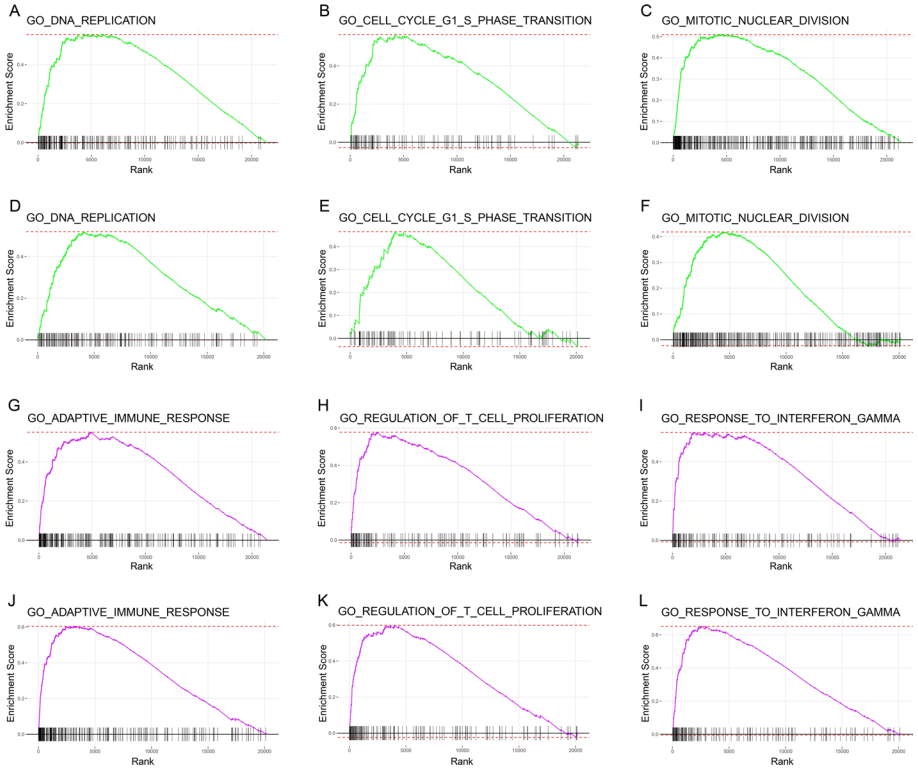

Fig. 3 LAG3 is mainly associated with cell proliferation in IDH-mutant gliomas (**A**–**C** CGGA dataset; **D**–**F** TCGA dataset), while is mainly associated with immune responses in IDH wild-type gliomas (**G**–**I** CGGA dataset; **J**–**L** TCGA dataset)

Figs. 3–6). LAG3 expression was positively correlated with immune score, stromal score, and ESTIMATE score, and negatively correlated with tumor purity. These findings further indicate that high expression of this gene is accompanied by increased infiltration of immune cells in the tumor microenvironment, and that its expression primarily originates from immune cells within the tumor microenvironment rather than from the tumor cells themselves.

3.4 Single-cell data reveals that LAG3 is mainly expressed on T cells and myelodis

To further verify the expression and distribution characteristics of LAG3, we utilized the CGGA single-cell dataset [18], as well as two published single-cell datasets - the Suva 2019 dataset [19] and the Richards dataset [20]. We re-grouped the single cells according to key markers combined with Seurat clustering into four major cell categories: tumor cells, myeloid-derived cells (myeloids), T cells, and oligodendrocytes (Fig. 5A–C). Across these three single-cell glioma datasets, we found that LAG3 was predominantly expressed on T cells and myeloids, with especially high expression on T cells. This was in good agreement with the patterns we observed in the bulk tumor RNA profiling analysis (Fig. 4B, D). The single-cell data were obtained from EGAS00001004656 (https://ega-archive.org/studies/EGAS00001004656), GSE89567 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE89567), and GSE131928 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131928).

The expression characteristics of LAG3 were similar to that of PD-1 (PDCD1) [10], while ITGAM (CD11b) was mainly expressed in myeloids. CD274 (PD-L1) was widely

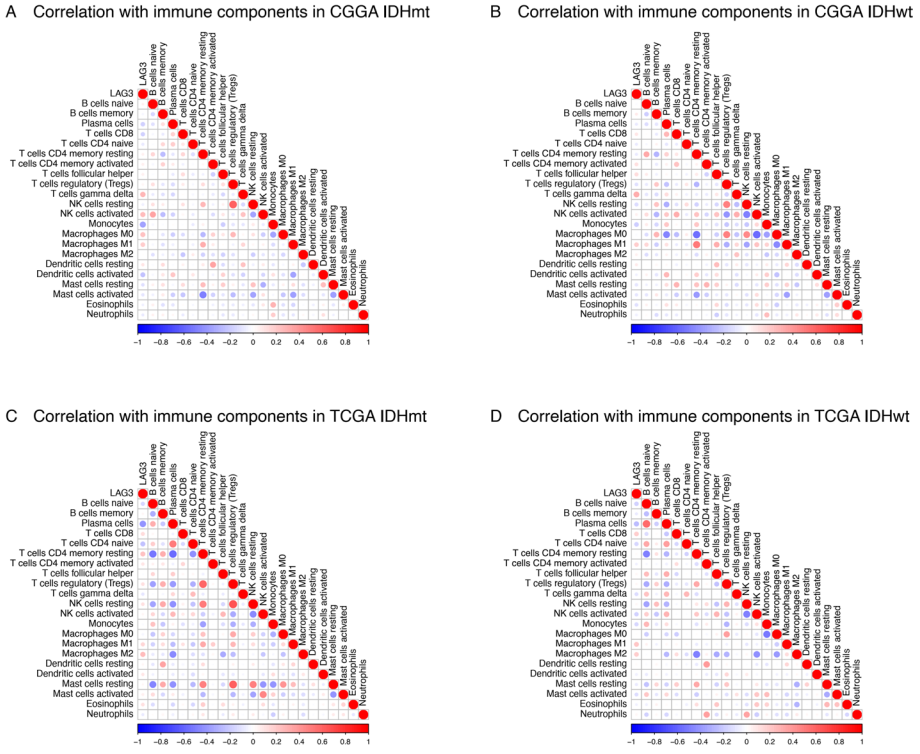

Fig. 4 Analysis of immune cell components by CIBERSORT revealed that LAG3 expression was predominantly associated with macrophages (M1) in IDH-mutant gliomas (**A** CGGA dataset; **C** TCGA dataset). In IDH wild-type gliomas, LAG3 expression was also mainly associated with macrophages (**B** CGGA dataset; **D** TCGA dataset)

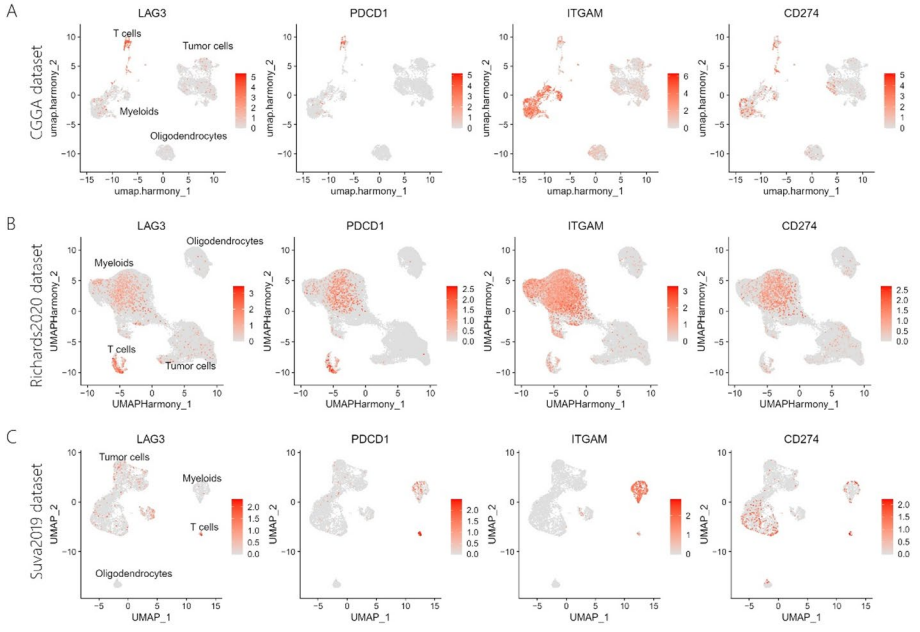

Fig. 5 The expression of classical immune markers LAG3, PDCD1 (PD-1), ITGAM (CD11b), and CD274 (PD-L1) in the glioma single cells in three datasets. We found that LAG3 and PDCD1 were mainly expressed in T cells, ITGAM (CD11b) was mainly expressed in myeloids, while CD274 (PD-L1) was relatively widely expressed among tumor cells, myeloids and T cells

expressed, but predominantly in tumor cells (Fig. 5). These findings suggest that both myeloids and T cells simultaneously express immune checkpoint receptors such as LAG3 and PD-1, which may result in the exhaustion of their cytotoxic functions within the glioma microenvironment.

3.5 The expression of different immune checkpoints on T cells often exhibits a complementary pattern

To explore the relationship between LAG3 and PD-1 (PDCD1) expression in T cells, we extracted the T cell populations from the single-cell datasets and performed a co-expression analysis of these two classical immune checkpoint receptors across the three datasets (Fig. 6A–C). As shown in Fig. 6, except for a small number of cells that co-expressed the two markers (yellow-colored cells), LAG3 and PD-1 were largely expressed in a mutually exclusive manner, with most T cells expressing only one of the two immune checkpoint receptors. This suggested that LAG3 and PD-1 were less likely to be simultaneously expressed on the same individual T cells.

To further validate this finding, we performed a more comprehensive analysis by including additional canonical immune checkpoint receptors (LAG3, PDCD1, HAVCR2, CTLA4, KLRG1, CD244, and BTLA) (Fig. 7).The expression heatmap of these molecules across T cells (Fig. 7A–C) clearly revealed a complementary expression pattern among the major checkpoint receptors in most cases.Based on their single-cell expression profiles, we conducted pairwise Spearman correlation analysis. The results, visualized using circus plots, further supported a predominantly negative correlation between the expression levels of different checkpoint receptors (Fig. 7D–F).

These results indicate that, within the glioma microenvironment, individual T cells tend to express only a subset of the available immune checkpoint receptors, rather than co-expressing multiple checkpoints simultaneously. This may have important implications for understanding T cell exhaustion and the potential for combination immunotherapy strategies.

3.6 The expression of LAG3 is significantly correlated with prognosis of glioma patients

Subsequently, we obtained the clinical survival data of the patients. It can be seen from the Kaplan-Meier curves that in the CGGA and TCGA datasets, patients with higher LAG3 expression in their tumors tended to suffer shorter overall survival, and there is no significant correlation with IDH mutation status (Fig. 8A–D). Multivariate Cox

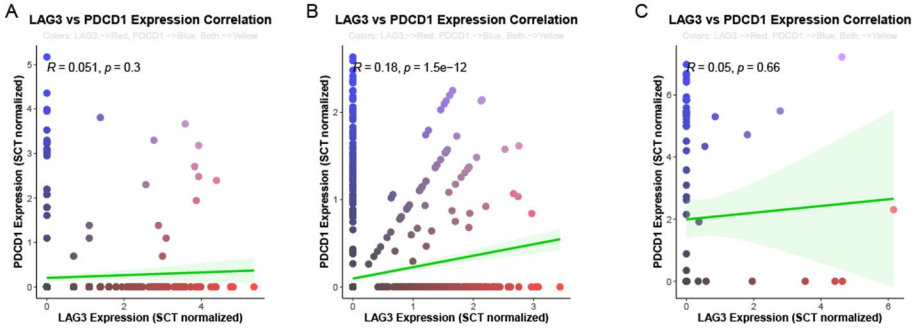

Fig. 6 LAG3 and PDCD1 expression pattern on T cells in three single cell datasets (**A**–**C**). Only a small amount of cell co-expressed two immune checkpoints

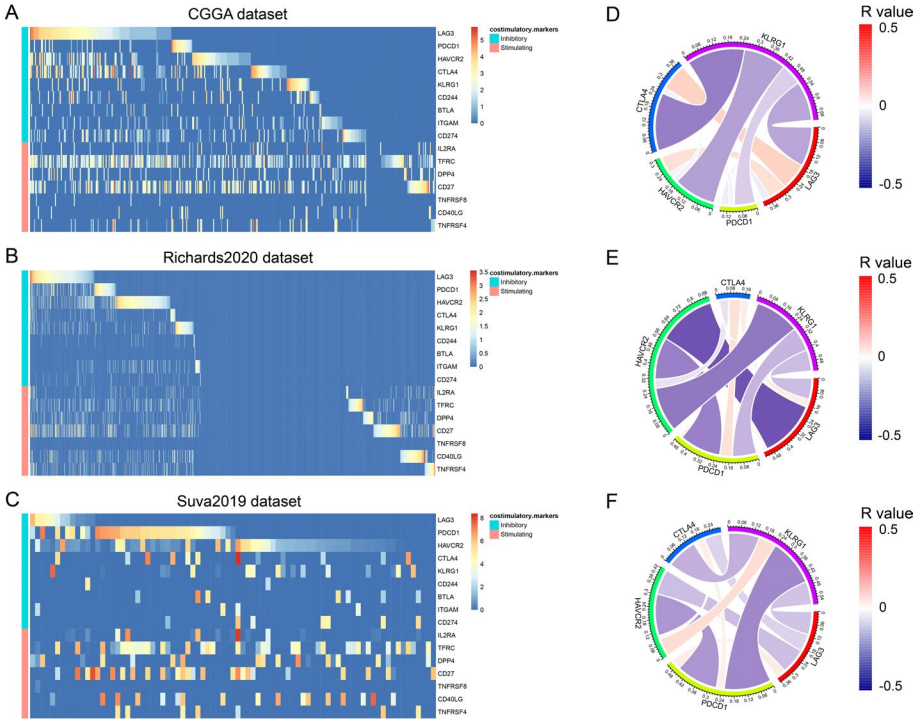

Fig. 7 Heatmaps (**A**–**C**) and correlation analysis (**D**–**F**) of T cell surface immune checkpoint expression patterns. **D**–**F** Green strips indicate negative correlations and red strips indicate positive correlations. Both strip color depth and strip width represent the absolute value of the R value. The outer ring of the circus plot represents the sum of the absolute r values of the corresponding marker correlating with all the markers

proportional hazards model also further confirmed the significance of LAG3 as independently prognosis makers (Tables 1 and 2).

4 Discussion

Although the brain has been recognized as an immune privileged organ, increasing studies have shown that gliomas often exhibit strong immunogenicity due to their intrinsic genetic, transcriptomic, and proteomic heterogeneity [23, 24]. This leads the immune system to recognize glioma cells as antigenic targets and mount an immune response against them [25]. However, the continuous progression of gliomas typically indicates a complete failure of the body's immune defense, ultimately allowing the tumors to escape immune surveillance [26].

The mechanisms underlying glioma immune evasion are complex, involving not only the tumor cells themselves, but critically, their dynamic interactions and crosstalk with various immune cell populations [27, 28]. For example, glioma cells can downregulate MHC expression, upregulate immune checkpoint receptors, and secrete immunosuppressive cytokines. Additionally, the tumor-associated macrophages are often polarized towards an M2 phenotype, which further promotes an immunosuppressive tumor microenvironment by secreting anti-inflammatory mediators and factors that support tumor growth [29, 30]. Furthermore, the function of infiltrating T cells is often severely impaired, leading to a state of T cell exhaustion [31–33].

Under the combined effects of these multifaceted immune evasion mechanisms, gliomas are able to ultimately escape the cytotoxic effects of the body's immune system, enabling their uncontrolled malignant progress. Understanding these complex immune

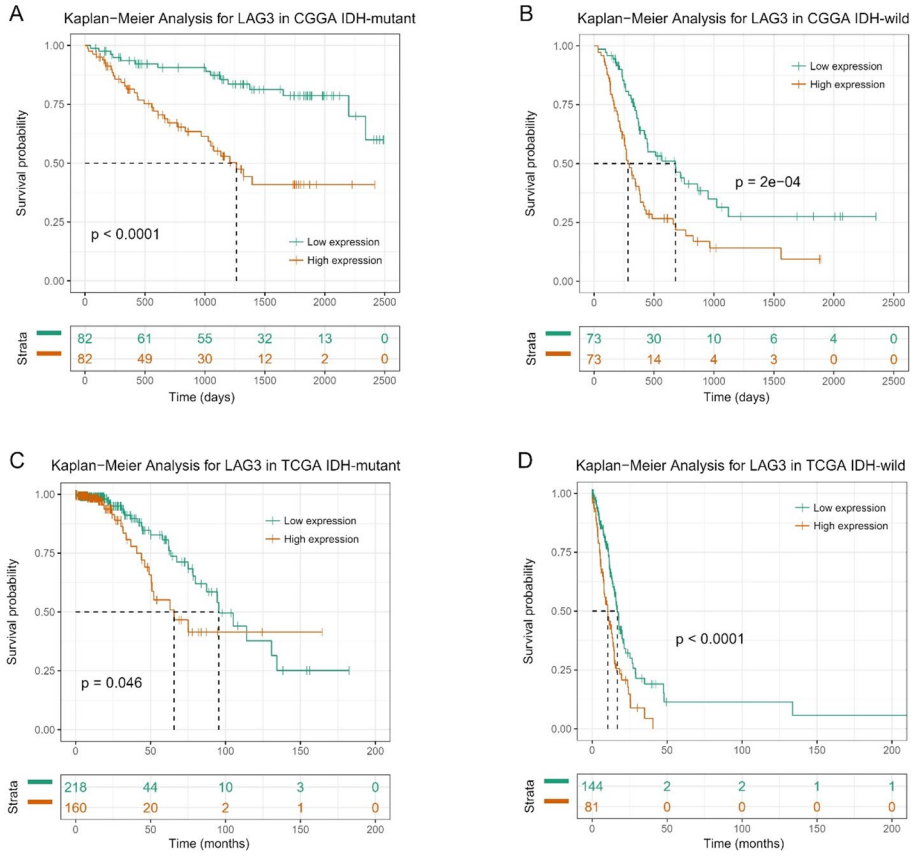

Fig. 8 Survival prognostic analysis. Higher LAG3 expression indicates significantly worse survival outcome (**A**, **C** IDH-mutant glioma; **B**, **D** IDH-wild glioma)

Table 1 Multi-variates cox proportional hazards model in CGGA dataset

	Coef	Exp (coef)	Lower0.95	Upper0.95	*p* value
Age	0.005902	1.005919	0.9905	1.0216	0.454585
Gender (male)	0.108712	1.114841	0.783	1.5873	0.546479
Grade	0.9865	2.681831	2.0459	3.5155	9.11×10^{-13}
IDH	-0.615078	0.540599	0.3547	0.8239	0.004219
LAG3	0.581851	1.789347	1.2999	2.463	0.000358

Table 2 Multi-variates cox proportional hazards model in TCGA dataset

	Coef	Exp (coef)	Lower 0.95	Upper 0.95	*P* value
Age	0.04941	1.05065	1.037	1.0645	1.52×10^{-13}
Gender (male)	0.04346	0.95747	0.7	1.3097	0.7857
IDH	1.88445	0.15191	0.102	0.2263	$<2\times10^{-16}$
LAG3	0.15737	1.17043	1.023	1.3395	0.0223

dynamics within the glioma microenvironment is crucial for developing more effective immunotherapeutic strategies.

While progress has been made in targeting immune checkpoint pathways, especially in tumors such as melanoma [34, 35], kidney cancer [36, 37], and lung cancer [38, 39], little advancement has been achieved in the glioma setting. Treatment regimens focused on classical immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have not yielded satisfactory results in glioma [40, 41]. This warrants additional efforts to investigate

other immune checkpoint receptors and explore novel targeting treatment options for this devastating disease.

In this context, we performed an integrated clinical and molecular analysis of the immune checkpoint receptor LAG3 in glioma. We performed multi-dataset integration and validation to enhance the reliability of the conclusions. For the first time, we systematically analyzed the impact of IDH mutation status on LAG3 function, untangling its dual biological roles. By integrating bulk and single-cell data, we comprehensively elucidated the mechanisms of LAG3 action at both the tissue and cellular levels. Supplemented with multi-platform immune cell infiltration analysis, this study provides more robust evidence supporting LAG3 as a potential immunotherapeutic target for glioma. Through these comprehensive datasets, we found that LAG3 expression, similar to PD-1/PD-L1, increased with the degree of glioma malignancy. However, unlike PD-1/PD-L1, LAG3 expression levels were not stably associated with IDH mutation status, suggesting that LAG3 expression was independent of IDH mutation. This indicates that the tumor microenvironment, with or without IDH mutation, did not significantly affect LAG3 expression. Intriguingly, the biological role of LAG3 appears to differ between IDH-mutant and IDH wild-type gliomas. In IDH wild-type tumors, LAG3 expression was often associated with tumor cell proliferation, while in IDH-mutant gliomas, the primary role of LAG3 was related to immune suppression. These findings suggest that LAG3 may play distinct biological roles in different glioma molecular subgroups and their corresponding microenvironments. The molecular characteristics of IDH-mutant and IDH-wild-type gliomas differ, leading to distinct regulatory mechanisms for LAG3 expression. The oncometabolite 2-hydroxyglutarate produced by IDH mutation interferes with epigenetic modifications, linking LAG3 expression to cell proliferation pathways [42]. Due to the limited immune infiltration in IDH-mutant gliomas, LAG3 expression is primarily dependent on tumor cell proliferation signals. In contrast, IDH-wild-type gliomas exhibit a high tumor mutational burden and strong antigen presentation, resulting in extensive T-cell infiltration and subsequent exhaustion. Consequently, LAG3 expression in these tumors is regulated by T-cell activation signals and is closely associated with immune processes [43].

It should be noted that discrepancies in LAG3 expression results may arise from differences in sample sources and processing standards across databases. The inclusion criteria for samples in the CGGA and TCGA may vary. For instance, CGGA likely includes a higher proportion of samples from the Chinese population, whereas TCGA encompasses a broader range of global population samples. Differences in genetic backgrounds among populations may influence LAG3 expression levels. Furthermore, variations in RNA extraction methods, sequencing platforms, and data normalization procedures during sample processing could introduce systematic biases in the detected LAG3 expression values. Consequently, these factors may lead to inconsistent conclusions regarding expression differences between IDH-mutant and wild-type gliomas, as well as between the MES (mesenchymal) and PN (proneural) subtypes.Single-cell data analysis revealed that LAG3 was almost exclusively expressed on the surface of T cells, in contrast to other immune checkpoint receptors such as PD-1, TIM-3, and CTLA4. Moreover, the expression of these classical immune checkpoint receptors on T cells tends to be mutually exclusive. This phenomenon suggests that T cells within the glioma microenvironment typically express a limited subset of immune checkpoint receptors,

rather than co-expressing multiple checkpoints simultaneously. Similar patterns are also observed in gastric caners. The authors found that immune checkpoint molecules function via different but complementary mechanisms [44].

This unique expression pattern of immune checkpoints on glioma-infiltrating T cells has important implications. It cautions us that when treating glioma patients with immune checkpoint inhibitors, we need to be particularly aware of the potential for multiple checkpoint receptor expression on T cells. This property also provides a strong theoretical basis for the concomitant use of various immune checkpoint inhibitors as a combination immunotherapy approach for glioma patients.

Consistent with the role of most immune checkpoint receptors, higher expression levels of LAG3 were associated with worse patient survival, and this prognostic value was independent of IDH mutation status and other clinical factors. Collectively, these findings suggest that LAG3 may hold significant promise as a target for the development of novel immune checkpoint inhibitor therapies for glioma [45–47].

5 Conclusion

Through the integrated analysis of the clinical characteristics and molecular data of LAG3, it is revealed that LAG3 is an important immune checkpoint in glioma, participates in the immune evasion of glioma, and is an independent unfavorable prognostic factor for glioma patients. It can be used as a promising candidate target for immune checkpoint inhibitors other than PD-1/PD-L1.

5.1 Limitations

This study has several limitations. First, the dataset size is relatively limited, which may restrict the robustness of the statistical analyses, particularly for rare outcome events. Second, we only used data from public database and did not perform external validation with independent datasets, which may affect the generalizability of our results to other populations.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s12672-026-04416-3.

Supplementary Material 1.

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Author contributions

Conceptualization: Zheng Wang, Haitao Fu, Zhong Zhang, Liyun Zhong, Wei Yan; Data Curation: Zheng Wang, Haitao Fu, Yuwei Li, Zewei Liu, Wenhua Fan, Yuqing Liu, Chuanbao Zhang, Jingshan Liang; Methodology: all the authors; Supervision: all the authors; Writing—original draft: all the authors; Writing – review & editing: all the authors.

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Data availability

TCGA bulk tumor RNAseq data was obtained from cBioportal (https://www.cbioportal.org/), CGGA bulk tumor RNAseq data was obtained from CGGA website [Chinese Glioma Genome Atlas (https://www.cgga.org.cn)]. The single-cell data were obtained from EGAS00001004656 (https://ega-archive.org/studies/EGAS00001004656), GSE89567 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89567), and GSE131928 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131928).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Publish declaration
We, the corresponding author and all co-authors of the manuscript titled "Integrative analysis identified the key role of LAG3 in T cell exhaustion in glioma"(submitted to Discover Oncology), hereby declare the following regarding the publication of this manuscript: The content is original, has not been published previously, and is not under consideration by any other journal. There is no duplicate publication issue. If accepted, we will sign the copyright agreement as required by the journal, acknowledging that authors retain copyright, Springer Nature holds the right to publish, and the manuscript will be subject to the Creative Commons open access license. We understand the journal's open access requirements, will pay the Article Processing Charge (APC) as required, and agree that the manuscript will be freely accessible upon publication.

Competing interests
The authors declare no competing interests.

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