High expression of Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) predicted poor prognosis in Glioblastoma multiforme

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Research Article

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Abstract

Purpose: Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) played an important role in inflammatory reactions. Recently the roles of TREM-1 in cancer had been explored, but not in Glioblastoma multiforme (GBM). The study was performed to explore the significance of TREM-1 in GBM.

Methods: Immunohistochemical staining of TREM-1 was performed in 91 operative patients diagnosed with GBM. Clinicopathological characteristics and survival time were recorded. Besides, TREM-1 expression and its effect on prognosis were analyzed using online GEPIA, TCGA and CGGA databases. Then the expression

profile of TCGA-GBM cohort was used to perform functional enrichment analysis. CIBERSORT method and TIMER database were used to estimate the tumor-infiltrating immune cells (TIICs). ESTIMATE algorithm was used to estimate the immune-stromal score. Lastly, the relationships between TREM-1 and TIICs, immune-stromal score and immune checkpoint genes (ICGs) were analyzed.

Results: The expression of TREM-1 was up-regulated in GBM and high TREM-1 expression predicted poor prognosis. TREM-1, IDH1, surgical resection, postoperative radiotherapy and temozolomide chemotherapy were associated with the survival time of GBM patients, but only surgical resection and TREM-1 expression were independent prognostic factors. GBM with high TREM-1 expression exhibited more infiltrate of neutrophils and macrophage. TREM-1 was positively associated with immune-stromal score and multiple ICGs, most of whom were involved in immunosuppressive responses.

Conclusions: The study revealed high expression of TREM-1in GBM was an independent poor prognosis factor and TREM-1 was associated with the immunosuppressive microenvironment. Blocking TREM-1, or combing block TREM-1 and ICGs may be a strategy for enhancing GBM immune response.

Introduction

Glioblastoma multiforme (GBM) was the most malignant glioma and belonged to grade IV according to World Health Organization classification (WHO) classification. Since most GBM was invasive and had no obvious boundary with normal tissue, it cannot be treated by surgery alone(Cuddapah et al., 2014). Postoperative radiotherapy and chemotherapy were generally supplemented, but the overall clinical efficacy and prognosis were still not satisfactory. The median overall survival time(mOS) of GBM after standardized treatment was only 14.2 months (Stupp et al., 2005) and it was urgent to seek new treatment method to improve the prognosis(Weller et al., 2015)

The occurrence and development of tumors were not only determined by the tumor cells themselves, but also the inflammatory and immune components in the tumor microenvironment (Hanahan & Weinberg, 2011). The major features of cancer-related inflammation included the infiltration of white blood cells, prominently tumor-associated macrophages (TAMs) and the presence of polypeptide messengers of inflammation [cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, chemokines such as

CCL2 and CXCL8] (Colotta et al., 2009). On the one hand, inflammation favored carcinogenesis, malignant transformation, tumor growth, invasion, and metastatic spread; on the other hand, inflammation could stimulate immune effector mechanisms that might limit tumor growth(Multhoff et al., 2011).

Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) was recognized play an important role in the occurrence and amplification of inflammatory reactions and innate immune responses, which was first discovered in 2000 and mainly expressed on neutrophils and monocytes/macrophages(Bouchon et al., 2000; Colonna, 2003). Many previous studies had explored the significance of TREM-1in infection and aseptic inflammation, including both acute (ischemia-reperfusion, hemorrhagic shock, pancreatitis, psoriasis and cystic fibrosis) and chronic (inflammatory bowel diseases, rheumatic diseases, and atherosclerosis) forms(Bouchon et al., 2001; Liu et al., 2019; Tammaro et al., 2017).

In recent years, the role of TREMI in tumors had received increasing attentions. The prognostic significance of TREM-1 has been explored in a variety of tumors.

In hepatocellular carcinoma (HCC), TREM-1 was associated with the aggressive migratory ability of HCC cells and the high density of peritumoral TREM-1 was associated with poor OS and elevated risks of recurrence(Liao et al., 2012).

The high expression of TREM-1 in renal cell carcinoma (RCC) was associated with poor outcome relative to patients with low or negligible TREM-1 expression(Ford et al., 2021).

Recent study on breast cancer indicated that increased TREM-1 expression is prognostic of inferior breast cancer outcomes and may contribute to myeloid-mediated breast cancer progression and immune suppression(Pullikuth et al., 2021). TREM-1 expression in papillary thyroid cancer (PTC) was also significantly higher than that in normal tissues and associated with $BRAF^{V600E}$ profiles and advanced tumor stages.

Overexpression of TREM-1 in PTC cells promotes an immunosuppressive microenvironment by enhancing Treg infiltration(Zhao et al., 2022). In non-small-cell lung cancer (NSCLC), TREM-1 expression in tumor-associated macrophages (TAMs) associated with cancer recurrence and poor survival(Ho et al., 2008). Qinchuan Wu, et al. demonstrated hypoxia inducible factor 1α induced increased expression of TREM-1 in TAMs resulting in immunosuppression in HCC (Wu et al., 2019). Besides, TREM-1 could mediate macrophage polarization by regulating the PI3K/AKT signaling pathway in HCC (Chen et al., 2021). TAMs (mainly M2 Macrophages) were predominantly inflammatory cells and closely related to the immune response in tumor microenvironment, which was recognized as antitumor suppressors(Aras & Zaidi, 2017). These studies suggest that TREM-1 may promote tumor development and metastasis by promoting tumor immune microenvironment.

With the great success of immunotherapy in a variety of tumors, especially the immune checkpoint inhibitors (ICIs)(Larkin et al., 2015; Motzer et al., 2015; Ready et al., 2019; Reck et al., 2019), there were

also many studies explored the application prospect of immunotherapy in GBM(Cloughesy et al., 2019; Kong et al., 2017; O'Rourke et al., 2017; Schalper et al., 2019). However, due to the high secretion of transforming growth factor-β(TGF-β) and indoleamine 2,3-dioxygenase 1 (IDO), GBM was thought to be with a significant immunosuppressive microenvironment(Gong et al., 2021; Zhai et al., 2021), which may be one of the main factors affecting the efficacy of immunotherapy(Bagley et al., 2018).

Given the immunosuppressant microenvironment of GBM and the carcinogenic role of TREM-1 in mediating inflammatory-immune responses, we investigated the prognostic impact and immunological correlates of TREM-1 expression in GBM.

Simultaneous analysis of The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA), databases confirmed that high TREM-1 expression was indicative of poor prognosis. TREM-1 was significantly associated with multiple tumor-infiltrating immune cells and immune checkpoint genes (ICGs) using the TCGA database and multiple online networks.

Materials And Methods

Database

The mRNA-seq data in Count format of TCGA-GBM cohort was obtained from the TCGA dataset (https://portal.gdc.cance r.gov/).

The mRNA microarray data of 301 glioma patients and mRNA sequencing data of 693 glioma and 325 glioma patients were obtained from CGGA data portal (http://www.cgga.org.cn/). Patients histologically classified as GBM were screened for subsequent analysis.

Online GEPIA (http://gepia.cancer-pku.cn/) was used to compare the mRNA expression of TREM-1 in GBM and normal tissues, which using a standard processing pipeline combined the RNA sequencing expression data of tumors and normal samples from TCGA and the Genotype Tissue Expression (GTEx) projects.

Patients and samples

Clinical and pathological data were retrospectively collected from 91 operative patients diagnosed with GBM at the Henan Provincial People's Hospital between January 2016 to December 2019. Patients included 53 males and 38 females aged 10–81 years. All patients were classified according to the 2016 WHO classification system. Overall survival time (OS) was calculated from the date of diagnosis until death or the end of the study. Progression free survival time (PFS) was calculated from the time of operation to the time of first progression. Patients were followed up until death or the end of the study (December 2021). At the end of data analysis, the follow-up time was from 1 to 68.9 months, with a median follow-up of 12 months.

Immunohistochemical staining and scoring

All specimen tissues were formalin fixed, paraffin embedded and sectioned into 4-μm serial sections. After dewaxing and hydration, antigen repair, and blocking endogenous peroxidase, the sections were incubated with primary TREM-1 rabbit monoclonal antibody (ab225861; Abcam, Cambridge, UK) at 4°C overnight. Then the sections were washed with sterile phosphate-buffered saline (PBS) and incubated with secondary antibodies for 1 h at room temperature. After adding substrate and hematoxylin staining, the slides were observed by microscope and interpreted by qualified pathologists, staining scoring criteria were as previously described(Li et al., 2021). Tissue sections with a final staining score ≥ 3 was considered to be positive.

Identification of differentially expressed genes and functional enrichment analysis

The transcription data of 153 samples from TCGA were divided into low- and hig-TREM-1 groups according to the TREM-1 level. The analyses of differentially expressed genes (DEGs), Gene Ontology (GO) and Encyclopedia of Genes and Genomes (KEGG) were performed according to the previous article (Li et al., 2021; Newman et al., 2015). The volcano plot of DEGs was drawn using “ggplot2” package. Besides, the gene set enrichment analysis (GSEA) was performed based on JAVA platform using TREM-1 level as the phenotype and “hallmark gene sets” as the reference gene set, which was obtained from the MSigDB database (http://software.broadinstitute.org/gsea/msigdb/). The P values were adjusted by FDR method and FDR < 0.25 was considered the significant enrichment pathways.

CIBERSORT algorithm

To understand the differential profiles of tumor-infiltrating immune cells (TIICs) between low- and high-TREM-1groups in GBM patients, we downloaded the “CIBERSORT” scripts (https://cibersort.stanford.edu/) and running “CIBERSORT” package with 1000 permutations (Newman et al., 2015). After the samples were filtrated through P > 0.05, a total of 57 samples were selected to perform the immune cells analysis (Table S1), including 29 low-TREM-1samples and 28 high-TREM-1 samples. Specific fractions of 22 immune cells in each GBM sample were shown by box plot. The differential infiltrating density between low- and high-TREM-1 groups was analyzed by Wilcoxon rank-sum test, which was generated as a violin plot using the ggplot2 R package.

TIMER Database

TIMER is a comprehensive resource for systematic analysis of immune infiltrates across multiple malignancies (https://cistrome.shinyapps.io/timer/) (Li et al., 2016). Based on the public resources, we could analyze the correlations between TREM-1 and the infiltrating level of different subsets of immune cells and the Pearson’s correlation coefficient and the estimated P value were calculated.

ESTIMATE Score

Immune cells and stromal cells are two main types in tumor microenvironment, which play important roles in the occurrence and development of tumors. ESTIMATE algorithm had been used to calculate the immune scores and stromal scores(Yoshihara et al., 2013). So, we used R software package to estimate

the immune scores and stromal scores of each tumor sample, and then to observe the relationship between TREM-1 expression and immune score in GBM.

Correlation analysis of immune checkpoint genes (ICGs)

We further analyzed the relationship between TREM-1 and 46 common ICGs based on the TCGA database by using “Psych” package. The Pearson’s correlation coefficient and the estimated P value were calculated.

Protein-protein interaction (PPI) network analysis

The STRING database is a database that searches for known and predicted interactions between proteins. Twenty-six ICGs associated with TREMI based on the TCGA database was used to map the PPI network using the online tool STRING (https://www.string-db.org/). PPI network was constructed by setting medium confidence at 0.400. All the mentioned active interaction sources were included.

Statistical analysis

Student’s t test was used for continuous variables, while categorical variables were compared by χ2 test. Cox analysis and the log-rank test were performed using the survival package. The Wilcoxon rank-sum test was used to compare two groups. All statistical analyses were performed in RStudio (Version 3.6.3), and p value < 0.05 was considered to be statistically significant.

Results

TREM-1 was up-regulated in GBM

Online GEPIA analysis revealed that the mRNA expression of TREM-1 in GBM was much higher than that in the normal tissues (Fig. 1A). Then we performed immunohistochemistry staining for TREM-1 in GBM and normal brain tissues. Immunostaining of TREM-1 was observed in the cytoplasmic or membranous (Fig. 1B-D). The protein expression of TREM-1 was much higher in GBM samples than that in the normal brain tissue ($\chi^2 = 4.193$, $P = 0.041$). The positive staining rate in GBM was 61.5% (56/91), while the positive rate in normal brain tissues was 33.3% (5/15). Correlation analysis of TREM-1 expression with clinicopathological features of GBM patients showed that TREM-1 expression was significantly associated with the status of IDH1 and P53(Table I).

High expression of TREM-1 predicted poor prognosis

Data from both TCGA and CGGA all showed that GBM patients with high TREM-1 expression exhibited much worse survival time than who with low expression of TREM-1 (Fig. 2A-D). In the present study, the median OS in TREM-1 high expression group was 10.5 months, while that in the low expression group was 18.5 months. The median PFS in TREM-1 high expression group was 6.0 months, while that in the

low expression group was 13.5 months. GBM patients with high expression of TREM-1 had shorter OS (Fig. 2E) and PFS (Fig. 2F) than who with low TREM-1 expression.

Univariate and Multivariate Survival Analyses

Univariate analyses showed that complete surgical resection, postoperative radiation therapy, postoperative temozolomide (TMZ) chemotherapy, isocitrate dehydrogenase-1 (IDH1) mutation and low TREM-1 expression were beneficial prognostic factors (Fig. 2E, Fig. 3A-3D). And complete surgical resection, postoperative radiotherapy, low TREM-1 expression, negative glial fibrillary acidic protein (GFAP) expression and wild P53 were associate with the longer PFS (Fig. 2F, Fig. 3E-3H). Multivariate analysis revealed both TREM-1 expression and complete surgical resection were independent predictors of OS and PFS (Table II, Table III). The results proved that thoroughness of surgery was a very important factor in deterring the survival of GBM patients.

Functional enrichment analysis

By dividing GBM patients from TCGA database into low- and high- TREM-1expression groups according to the medium value, there were 614 DEGs were identified (Table S2; Fig. 4A). GO enrichment analysis revealed the DEGs mainly as components of extracellular matrix, play roles in receptor ligand activity and receptor regulator activity, participate in the neutrophil activation and neutrophil mediated immunity (Fig. 4B). KEGG enrichment analyses showed that the DEGs mainly involved in the procession of Cytokine – cytokine receptor interaction (Fig. 4C). Besides, we performed GSEA by using TREM-1 level as the phenotype, the major signaling pathways were exhibited in Fig. 4D and 4E.

Associations of TREM-1 with tumor-infiltrating immune cells (TIICs)

TREM-1 was recognized as an immune related signature (8), so we further analyzed its role in tumor immune microenvironment. Firstly, we utilized the CIBERSORT algorithm to determine the estimated fractions of 22 immune cells in each sample (Fig. 5A). M2 Macrophages was the highest immune cell in GBM, followed by M0 Macrophages and Monocytes. Additionally, Wilcoxon rank-sum test was applied to accurately compare the difference between high-low TREM-1groups. The results showed that the infiltrating levels of Neutrophils ($P < 0.001$) and Mast cells activated($p = 0.029$) were much higher in the high TREM-1 group, while the infiltrating levels of Macrophages M1 ($p = 0.031$) and Mast cells resting($p = 0.017$) were significantly lower in the high TREM-1 group (Fig. 5B).

Then, based on the TIMER database, we found that TREM-1 expression was significantly associated with the infiltrating immune cells of Neutrophils ($p = 4.44e-14$), Macrophage ($p = 3.33e-02$), B cell plasma (3.88e-02) and Myeloid dendritic cell (6.91e-05) (Fig. 6A-F).

Lastly, we used the ESTIMATE algorithm estimating the score of tumor immune microenvironment. The results showed that both stromal score (Fig. 6G) and immune score (Fig. 6H) were positively associate with the expression of TREM-1. Naturally, there was also a positive correlation with the sum of the immune score and stromal score (Fig. 6I).

Associations of TREM-1 with immune checkpoint genes (ICGs)

Immune checkpoints are co-stimulators or co-suppressors required to maintain self-tolerance and produce an immune response(Pardoll, 2012). Tumors may activate immune checkpoint pathways to prevent being recognized by the immune system and inhibit immune responses(Beatty & Gladney, 2015). ICIs by blocking the immune checkpoints had breakthrough the treatment of a variety of malignant tumors(Larkin et al., 2015; Motzer et al., 2015; Ready et al., 2019; Reck et al., 2019). Therefore, we analyzed the relationship of TREM-1 to multiple ICGs based on the gene expression from TCGA database. Here shown in Fig. 7 were all statistically different genes. We found that TREM-1 was positively correlated with the expression of majority ICGs.

PPI network

Select 26 ICGs that correlated with the expression of TREM-1, we constructed the PPI network. In the PPI network (Fig. 8), there were 27 nodes, 153 edges and the p-value were < 1.0e-16. Among the ICGs, only CD86 was directly interact with TREM-1.

Discussion

TREM-1, as a novel inflammatory receptor, played an important role in inflammatory and immune responses in the tumor microenvironment(Bouchon et al., 2000; Colonna, 2003). In the present study, we proved the expression of TREM-1 in GBM was much higher than that in normal brain tissue and high expression of TREM-1 in GBM predicted poor prognosis.

The results are consistent with previous studies in HCC(Liao et al., 2012), RCC(Ford et al., 2021) and breast cancer(Pullikuth et al., 2021). In NSCLC, high TREM-1 expression in TAMs and high soluble TREM-1 level were also the poor prognostic factor (Ho et al., 2008; Kuemmel et al., 2018). Both in human lung cancer xenografts and pancreatic cancer xenograft mouse models, TREM-1 inhibitors all could exhibit strong antitumor effect(Shen & Sigalov, 2017; Sigalov, 2014). In pancreatic cancer, the anti-tumor effect correlated significantly with increased survival and suppressed TAM infiltration. Blockade of TREM-1 significantly reduced the serum levels of IL-1α, IL-6 and macrophage colony-stimulating factor (M-CSF) (Shen & Sigalov, 2017).

Functional enrichment analysis showed TREM-1 was predominantly involved in inflammatory response, TNF signaling via NF-κB and JAK-STAT signaling pathways, which in consistent with the previous reports on the function and mechanisms of TREM-1. TREM-1 can stimulate neutrophil and monocyte-mediated inflammatory responses through the transmembrane adapter protein DAP12 and induces secretion of inflammatory chemokines and cytokines. After phosphorylation of DAP12, the production of chemokines and cytokines was induced, such as TNF-α and IL-1β(Carrasco et al., 2019; Duan et al., 2015; Tessarz & Cerwenka, 2008). NF-κB was a key orchestrator of innate immunity/inflammation and aberrant NF-κB regulation had been observed in many cancers(Karin, 2006). In both tumor and inflammatory cells, NF-κB was activated downstream of the toll-like receptor (TLR)-MyD88 pathway and of the inflammatory

cytokines TNF-α and IL-1β. TREM-1 possesses the ability to amplify signaling by the TLR4 or TLR2(Arts et al., 2011). STAT3 was a point of convergence for numerous oncogenic signaling pathways(Yu et al., 2007). TREM-1 blockade could prolong survival of rats with polymicrobial sepsis and attenuate systematic inflammatory responses through the JAK2/STAT3 signaling pathway(Ford et al., 2021).

TNF played a major role in the proinflammatory cytokines. Tumor promotion by this cytokine could involve different pathways: TNF enhances tumor growth and invasion, leukocyte recruitment, angiogenesis and facilitate epithelial to mesenchymal transition(Balkwill, 2009; Kulbe et al., 2007). Caer et al. showed TNF correlated with TREM-1-expressing monocytes and TREM-1 pathway may act in concert with other factors in the intestine of Crohn's disease patients, such as TNF, to influence the inflammatory environment (Caer et al., 2021).

Inflammasome-dependent release of cytokines and antigen can activate, shape or even inhibited adaptive immune responses(Deets & Vance, 2021). So, the relationship between TREM-1 and tumor-infiltrating immune cells, ICGs were further explored in GBM.

Macrophages, as important antigen presenting cells, can determine the preference of T cell response, and the cytokine pattern secreted by activated T cells is determined by the cytokine pattern secreted by M1/M2(Aras & Zaidi, 2017). TAMs are closely related to immune response in tumor microenvironment and recognized as antitumor suppressors. TIICs analysis showed M2 Macrophages was the highest immune cell in GBM. High macrophage infiltration can mediate the tumor immunosuppressive microenvironment in a variety of ways to promote tumor genesis, development and metastasis(Aras & Zaidi, 2017). Up-regulation of TREM-1 was observed frequently in dendritic cells and occasionally in TAMs(Ho et al., 2008; Klesney-Tait et al., 2006). Additionally, TREM-1 can mediate macrophage polarization by regulating the PI3K/AKT signaling pathway in HCC(Chen et al., 2021). But, in the present study, TREM-1 expression was positively associated with the infiltrating level of Macrophage (TIMER) and negatively associated with the M1 Macrophages (CIBERSORT algorithm), but not the M2 Macrophages. So, the relationship between TREM-1 and M1/M2 Macrophages in GBM needs to be further verified in tissue specimens.

In addition to immune cells, the immune microenvironment also includes non-immune stromal components, which also closely associated with oncogenesis and malignant behaviors of tumors(Bremnes et al., 2011). TREM-l was positively associate with the stromal score and immune score. In the TCGA-GBM cohort, the survival time of both high stromal score and immune score groups were shorter than that in the low groups. But there was no statistical difference(Jia et al., 2018). Zeng's study showed that immune score was linked to favorable OS, while stromal scores were linked to unfavorable OS, and patients with low immune score and high stromal score exhibited the worse survival time(Zeng et al., 2021). Ren et al reported that interaction between stromal cells and epithelial cancer cells affected the cancer progress in pancreatic cancer(Ren et al., 2018).

TREM-1 was highly expressed in GBM, in which immune therapy were less effective due to the immune suppression microenvironment (Bagley et al., 2018). Study found that in HCC, blocking the TREM-1

pathway can not only inhibit tumor development, but also improve the efficacy of PD-L1 by reducing the recruitment of CCR6 + Foxp3 + Tregs (Wu et al., 2019). TREM-1 inhibitors may be an effective adjuvant that enhances anti-PD-1-mediated immunogenic cell death in microsatellite stable (MSS) colorectal cancer (CRC)(Roh et al., 2021). These studies suggest that TREM-1 may be involved in the resistance of ICIs. And if there were relationships between TREM-1 expression and immune checkpoint genes, as well as PD-L1? In our analysis of the TCGA GBM cohort, we observed TREM-1 significantly correlate with not only PD-L1(CD274), but also multiple ICGs.

Among the ICGs, CD80(CD86)/CD28/ ICOS (the receptor of B7h), PD-L1/PD-L2(PDCD1LG2) and TMIGD2(the receptor of HHLA2) were the members of B7-CD28 family and belonged to groups 1, 2 and 3 respectively(Janakiram et al., 2017). It was well established that engagement of CD28 by its ligands CD80 or CD86 provides key costimulatory signals to activate the T cell activation(Janakiram et al., 2017). B7-1/B7-2/CD28/CTLA-4 pathway was important in modulating central immune tolerance while PD-L1/PD-L2/PD-1, B7-H3, B7x, and HHLA2 were important in peripheral immune regulation(Janakiram et al., 2017). ICIs targeting CTLA4 and PD-1/PD-L1 been explored in GBM(Cloughesy et al., 2019; Schalper et al., 2019; Youssef & Dietrich, 2020).

In the correlation analysis, TREM-1 was significantly associated with the expression of CD86, as well as in the PPI network. Previous study showed that TREM-1 inhibitory peptide can attenuate proinflammatory subtype transition of microglia, which was evidenced by the decreased levels of markers including CD68, CD16, CD86(Wu et al., 2021).

TNFR/TNF family had a diverse and complex set of interactions with the immune system and could influence T cell responses in a number of ways, and many inhibitors targeting TNF family had been explored(Croft et al., 2013). Among the significant associations of TREM-1 with ICGs, TNFSF9, TNFRSF9, TNFSF14, TNFRSF14, TNFRSF18, TNFSF15, CD40 and CD70 were the members of TNFR/TNF family. TNFRSF14 expression in GBM is associated with worse overall survival and disease-free survival and a lower Th1 response(Lombardo et al., 2020). TNFRSF9 is strongly overexpressed in human diffuse gliomas as compared with the normal central nervous system(Blank et al., 2015) and the antitumoral effects and prolonged survival were observed(Kim et al., 2001). In lung cancer, low TNFRSF9 expression level in Tregs was associated with enhanced overall survival rate and response to anti-PD-1 immunotherapy, proposing that TNFRSF9 promotes immune suppressive activity of Tregs in tumor (Cho et al., 2021).

CD70 had also been identified have (Wischhusen et al., 2002) the ability to induce immunosuppression

through tumor-induced apoptosis of T-lymphocytes in glioblastoma. Different from the above members of TNFR/TNF family, high CD40 expression was recognized as a poor clinical outcome, but a good performance in predicting ICI response(Yan & Richmond, 2021). High expression level of TREM-1 indicated a poor response to anti-TNF drugs, which may be associated with the decreased differentiation to M2 type regulatory macrophages inflammatory bowel disease (IBD)(Prins et al., 2021). These studies

suggest that TREM-1 may have staggered interactions with TNF signaling pathway, which was consistent with our previous functional enrichment analysis.

In addition to those mentioned above, several related ICGs were involved in immunosuppressive responses, such as HAVCR2(TIM-3), LGALS9, CD48(SLAMF2) and ligands CD244(SLAMF4), VSIR and LAIR1. The ICGs could exert immunosuppressive effect through different functions and were new research targets of cancer immunotherapy(Katabathula et al., 2022; McArdel et al., 2016; Peng et al., 2020; Solinas et al., 2019; Sun et al., 2021; Wang et al., 2020). The correlation between TREM-1 and multiple ICGs in tumor microenvironment suggested the potential immunosuppressive effect of TREM-1 in GBM. Combined blocking of TREM-1 and other ICGs may be a strategy for enhancing GBM immune response.

Declarations

Ethical approval

The study was approved by the Ethics Committees of the Henan Provincial People's Hospital. All the patients selected for our study were fully informed about our experiment protocols and signed an informed consent to participate in this study. The study was conducted in accordance with the Declaration of Helsinki.

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Conflict of interest

The authors declare that there are no competing interests to disclose.

Author contributions

Ke Ma, Yanxin Li and Qianqian Guo designed the study. Ke Ma was responsible for data analysis and wrote the manuscript. Yanxin Li collected the clinical and pathological data. Xianwei Zhang provided pathological section and staining analysis. Qianqian Guo provided guidance in the smooth implementation of the study and the revision of the paper.

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Tables

Table1: Associations between TREM-1 expression and GBM patient clinicopathological characteristics.

		Negative		Positive			
Variables	No.	No.	%	No.	%	χ2	*P*-value
Gender						0.072	0.788
Male	53	21	39.6	32	60.4		
Female	38	14	36.8	24	63.2		
Ages						0.361	0.548
≤60 years	51	21	41.2	30	58.8		
>60 years	40	14	35.0	26	65.0		
TMZ						1.189	0.276
Yes	56	24	42.9	32	57.1		
No	35	11	31.4	24	68.6		
Complete resection						0.427	0.514
Yes	64	26	40.6	38	59.3		
No	27	9	33.3	18	66.7		
Radiation therapy						2.032	0.154
Yes	46	21	45.7	25	54.3		
No	45	14	31.1	31	68.9		
IDH1 status						35.348	0.000
Mutation	31	25	80.6	6	18.4		
Wild	60	10	16.7	50	83.3		
GFAP status						1.056	0.304
Positive	76	31	40.8	45	59.2		
Negative	15	4	26.7	11	73.3		
P53 status						18.356	0.000
Mutation	54	11	20.4	43	79.6		
Wild	37	24	64.9	13	35.1		
Ki-67						0.291	0.589
<50%	54	22	40.7	32	59.3		
>50%	37	13	35.1	24	64.9		

Note: Radiation therapy, postoperative radiation therapy; TMZ, postoperative temozolomide chemotherapy.

Abbreviations: GBM, glioblastoma multiforme; TMZ, temozolomide; IDH1, isocitrate dehydrogenase-1, GFAP, glial fibrillary acidic protein.

Table III. Multivariate analysis of OS for GBM (N=91).

Variable	B	SE	Wald	P value	Hazard Rate (95%CI)
TMZ chemotherapy	0.358	0.246	2.110	0.146	1.430 (0.882, 2.318)
Complete Resection	0.730	0.272	7.213	0.007	2.075 (1.218, 3.535)
Radiation therapy	0.416	0.259	2.573	0.109	1.515(0.912, 2.517)
TREM-1	-0.739	0.265	7.781	0.005	0.477 (0.284, 0.803)
IDH1 status	0.525	0.346	2.304	0.129	1.691 (0.858, 3.331)

Note: TMZ chemotherapy, postoperative temozolomide chemotherapy; Radiation therapy, postoperative radiation therapy.

Abbreviations: GBM, glioblastoma multiforme; TMZ, temozolomide; TREM-1, triggering receptor expressed on myeloid cells 1; IDH1, isocitrate dehydrogenase-1; OS, overall survival time.

Table III. Multivariate analysis of PFS for GBM (N=91).

Variable	B	SE	Wald	P value	Hazard Rate (95%CI)
Complete resection	0.593	0.275	4.653	0.031	1.810 (1.056, 3.104)
Radiation therapy	0.438	0.260	2.835	0.092	1.549(0.931, 2.579)
TREM-1	-0.731	0.257	8.080	0.004	0.481 (0.291, 0.797)
GFAP	-0.687	0.361	3.620	0.057	0.503 (0.248, 1.021)
P53	-0.503	0.278	3.273	0.070	0.605 (0.351,1.043)

Note: Radiation therapy, postoperative radiation therapy.

Abbreviations: GBM, glioblastoma multiforme; TREM-1, triggering receptor expressed on myeloid cells 1; GFAP, glial fibrillary acidic protein; PFS, progression free survival time.

Figures

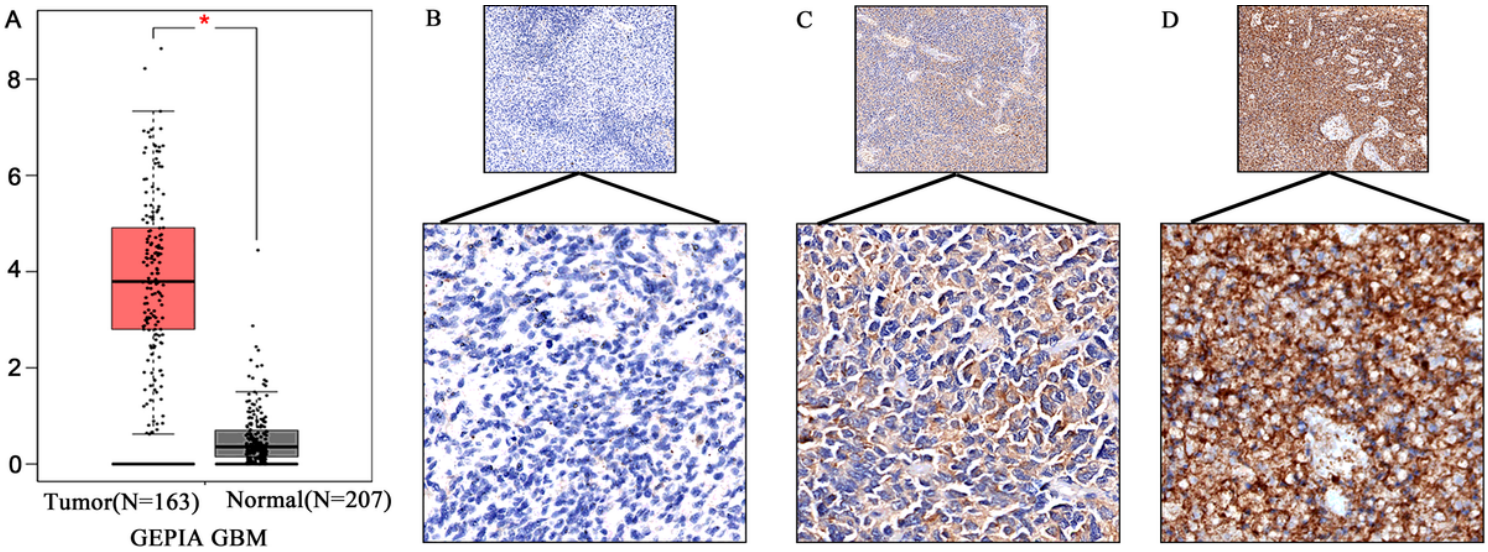

Figure 1

The expression of TREM-1 in GBM. A: The mRNA expression of TREM-1 was much higher in GBM than that in normal brain tissues. *P < 0.05.

B-D: Representative immunohistochemical staining of TREM-1 in GBM tissues (upper, X100: below X400). (B) weak, (C) moderate and (D) intense immunostaining of TREM-1 in GBM tissues.

Abbreviations: TREM-1, triggering receptor expressed on myeloid cells 1; GBM, glioblastoma multiforme.

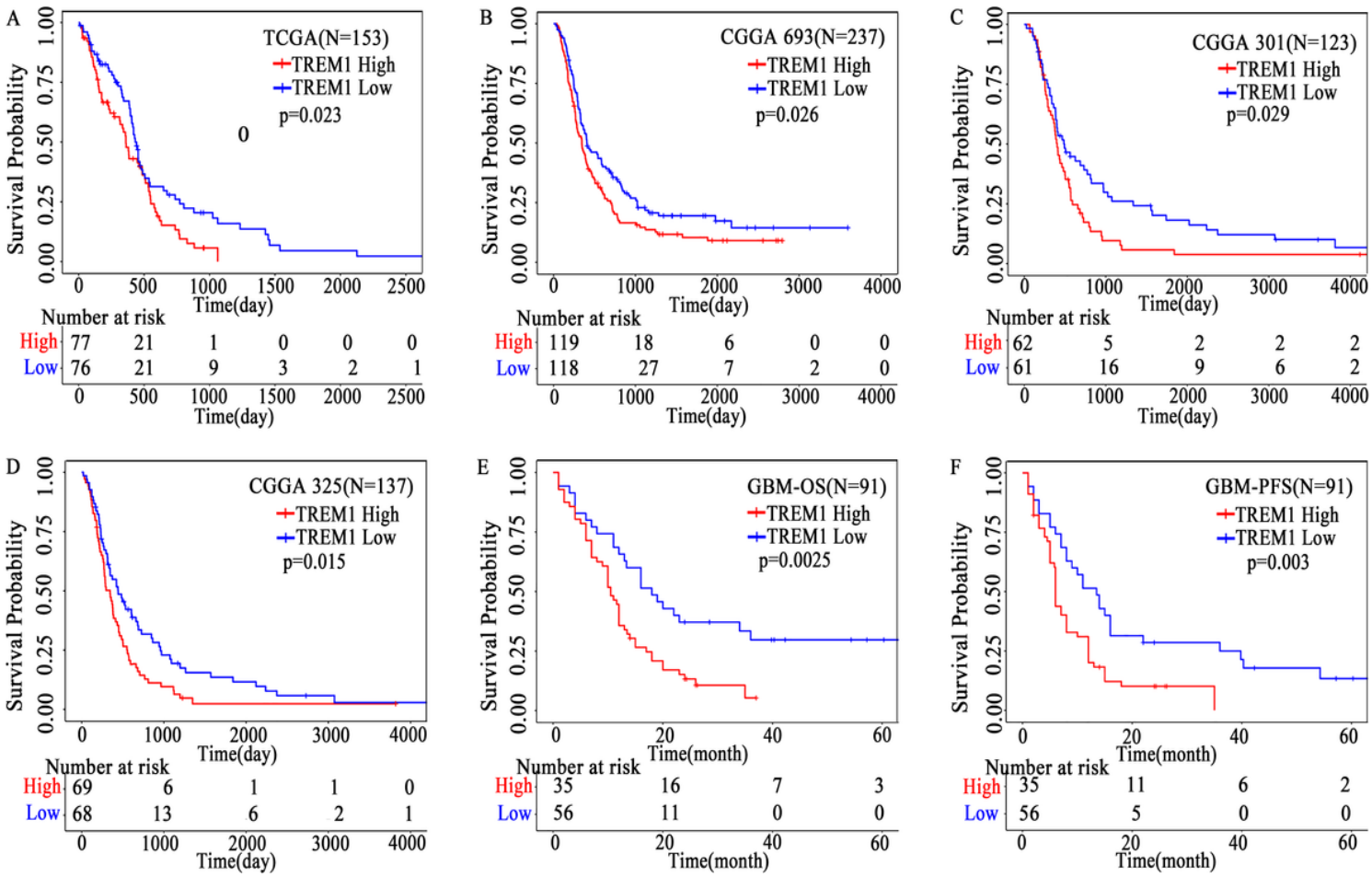

Figure 2

High expression of TREM-1 predicted poor prognosis. A: Kaplan–Meier curve of TREM-1 based on TCGA database. B-D: Kaplan–Meier curve of TREM-1based on CGGA database. E-F: Kaplan–Meier curve of TREM-1 for 91 GBM patients.

Abbreviations: TREM-1, triggering receptor expressed on myeloid cells 1; GBM, glioblastoma multiforme; TCGA, The Cancer Genome Atlas; CGGA, Chinese Glioma Genome Atlas (CGGA); OS, overall survival time; PFS, progression free survival time.

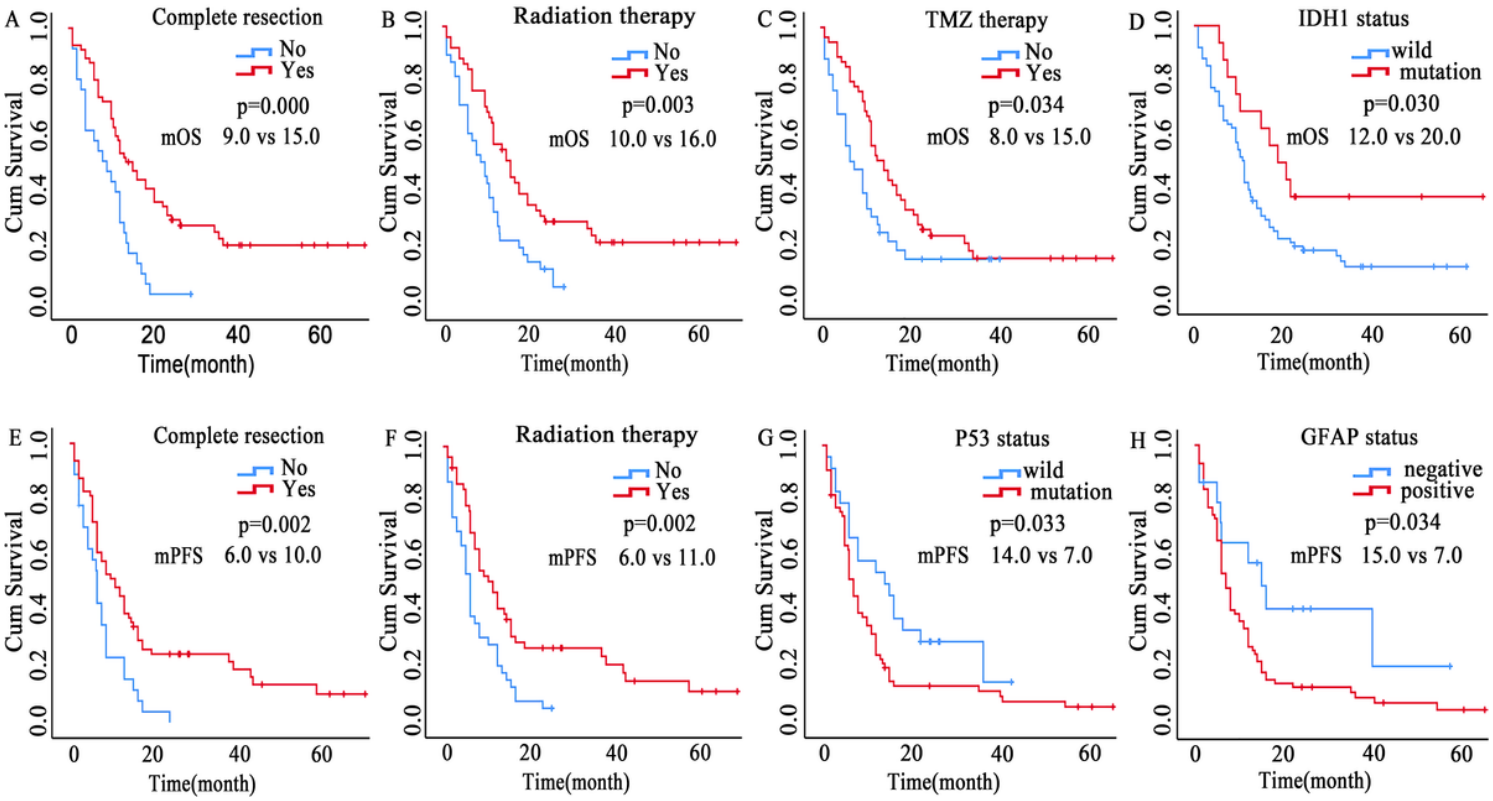

Figure 3

Univariate survival analyses of GBM. Univariate analyses showed complete surgical resection(A), postoperative radiation therapy(B), postoperative TMZ chemotherapy(C) and *IDH1* mutation (D) exhibited longer OS. Besides, complete surgical resection(E), postoperative radiation therapy (F), wild P53(G) and negative GFAP expression (H) were less likely to develop disease progression.

Abbreviations: GBM, glioblastoma multiforme; TMZ, temozolomide; IDH1, isocitrate dehydrogenase-1, GFAP, glial fibrillary acidic protein; mOS, median overall survival time; mPFS, median progression free survival time.

Figure 4

Differentially expressed genes and pathway enrichment analysis. A: Volcano plot showing the differentially expressed genes. B-C: Bubble chart of the results of GO and KEGG enrichment analysis. D-E: GSEA showed the representative signaling pathways in the high- and low-TREM-1 expression groups.

Abbreviations: FDR, false discovery rate; BP, biological process; CC, cellular component; MF, molecular function; GO, Gene Ontology; KEGG, Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis.

Figure 5

Evaluation of tumor-infiltrating immune cells using CIBERSORT algorithm. A: Summary of estimated fractions of 22 immune cell subtypes in GBM. Each Bar chart exhibited the cell proportions of each patient. B: Wilcoxon rank-sum test revealed the infiltration levels of 22 immune cell subtypes in low- and high- TREM-1 groups.

Abbreviations: TREM-1, triggering receptor expressed on myeloid cells 1; GBM, glioblastoma multiforme.

Figure 6

Associations of TREM-1 with tumor-infiltrating immune cells and immune scores. A-F: Using TIMER analyze the associations of TREM-1 with neutrophil(A), macrophage(B), T cell CD4+(C), B cell plasma(D), myeloid dendritic cell (F) and T cell CD8+(F). G-I: TREM-1 was positively related with the stromal score(G), immune score(H) and estimate score(I).

Abbreviations: TREM-1, triggering receptor expressed on myeloid cells 1; GBM, glioblastoma multiforme.

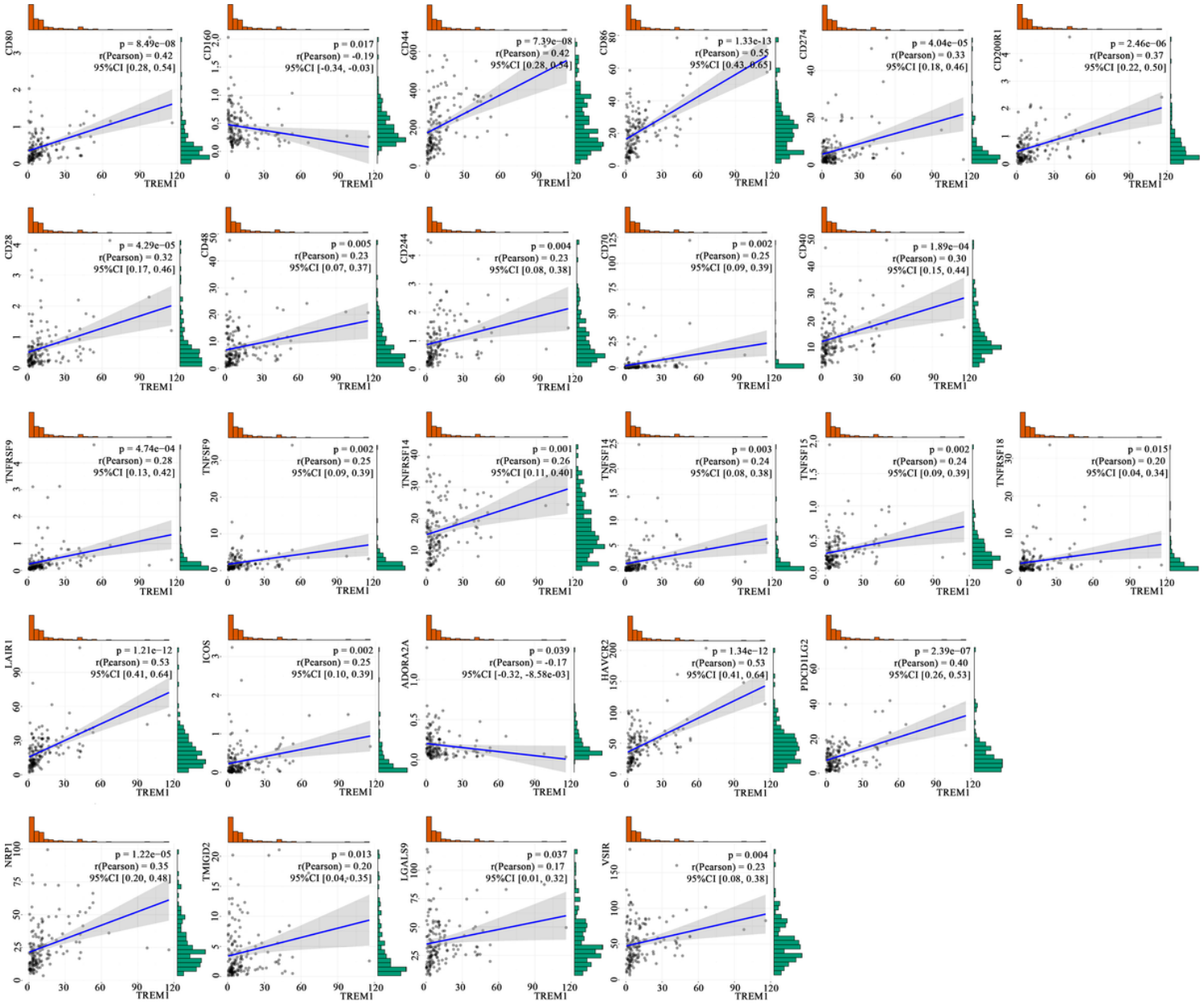

Figure 7

TREM-1 expression was statistically correlated with the expression of 26 common immune checkpoint genes based on TCGA database.

Abbreviations: TREM-1, triggering receptor expressed on myeloid cells 1; CI, confidence interval; TCGA, The Cancer Genome Atlas.

Figure 8

Protein–protein interaction (PPI) network analysis was constructed from the

selected 26 immune checkpoint genes and TREM-1 using online STRING database.

Supplementary Files

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

- TableS1.txt
- TableS2.txt
- TableS3.txt
- TableS4.txt
- TableS5.txt