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Expression and prognostic value of PIM-1 kinase in gliomas

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Summary. Objective. This study aimed to explore the correlation of PIM-1 with the clinicopathological features and prognosis of patients.

Methods. The MTERF3 mRNA and protein expression levels in tissues were detected by western blot and immunohistochemistry. The expression and survival of PIM-1 in patients with glioma were analysed using the Gene Expression Profiling Interactive Analysis database, the Gene Expression Database of Normal and Tumor Tissues 2, and the Chinese Glioma Genome Atlas database. The relationship between PIM-1 expression and immune cells and chemokines was analysed using the Tumor Immune Estimation Resource Version 2.0 tool and the Tumor and Immune System Interactions Database. A Kaplan–Meier plot was used to estimate the correlation between PIM-1 expression and the survival of patients with glioma.

Results. The expression of PIM-1 was upregulated in glioma and was positively correlated with tumour grade. The expression of PIM-1 was significantly inhibited on the second day after transfection ($p<0.05$), and the inhibition was most obvious on the sixth day ($p<0.01$). The results of the co-expression pattern of PIM-1 showed that the expression of 5,012 genes was positively correlated with PIM-1, while the expression of 3,651 genes was negatively correlated with PIM-1. Macrophages ($p<0.001$), myeloid dendritic cells ($p<0.001$), NK cells ($p<0.001$), CD4 T cells ($p<0.001$), cancer-associated fibroblasts ($p<0.001$), and neutrophils ($p<0.001$) were positively correlated with the expression of PIM-1 in low-grade glioma.

Conclusion. PIM-1 is overexpressed in glioma and is related to the prognosis of glioblastoma multiforme, and PIM-1 may be a prognostic biomarker and therapeutic target for glioma.

Key words: PIM-1, Glioma, Expression, Isocitrate dehydrogenase 1, Prognosis

Introduction

Gliomas are the most common primary brain tumours, and the highly malignant and most aggressive subtype is glioblastoma multiforme (GBM) (Tan et al., 2020; Ostrom et al., 2021). However, due to the heterogeneity and tremendous invasive capacity of GBM, the average life expectancy of most patients with GBM is 12-15 months (Tan et al., 2020; Ostrom et al., 2021). Precision oncology and immunotherapy, based on an enhanced understanding of molecular biology and the immune microenvironment of glioblastoma, have contributed to prolonging survival. Therefore, it is imperative to find new potential targets for gliomas that will improve our ability for early diagnosis and personalised treatment for gliomas.

PIM-1, a serine/threonine kinase, was originally identified in mouse T-cell lymphomas and has a regulatory effect on cell cycle and apoptosis and a role in promoting tumour development (Nawijn et al., 2011). Studies have shown that PIM-1 is expressed at abnormal levels in a variety of cancers, including acute myeloid leukaemia (Fathi et al., 2012), osteosarcoma (Liao et al., 2016), non-triple-negative breast cancer cell lines (Brasó-Maristany et al., 2016), prostate cancer (Wang et

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al., 2010; Eerola et al., 2021), and multiple myeloma (Wu et al., 2021). Although less investigated in GBM, there is evidence that PIM-1 promotes GBM cell development. PIM-1 is associated with apoptosis in GBM cells, and inhibition of PIM-1 combined with ABT-737 induced apoptosis in GBM cells (Remy et al., 2019). Furthermore, inhibition of PIM-1 enhanced the sensitivity of GBM cells to tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) and induced TRAIL-mediated activation of the apoptotic pathway in GBM cells (Serrano-Saenz et al., 2019). In addition, blockade of the kinase activity of PIM-1 was found to inhibit the viability of GBM stem cells and the expression of stem cell markers (Seifert et al., 2021). A study by Iqbal et al. (2016) further revealed the interaction of PIM-1 with the PI3k/mTOR signalling pathway, and combined inhibition of PI3k and PIM-1 could more effectively inhibit the proliferation of GBM cells.

Given the potential role of PIM-1 in glioma, this study aimed to examine PIM-1 expression levels in eight GBM cell lines, 53 human GBM samples, and GBM tissue microarrays (TMAs). Considering the importance of the isocitrate dehydrogenase (IDH) mutation in the prognostic evaluation of glioma, this study also investigated the association between PIM-1 expression and IDH mutation. At the same time, the study preliminarily investigated the effect of PIM-1 on GBM cell proliferation (van den Bent et al., 2017) to provide more experimental and theoretical support for understanding the role of PIM-1 in GBM development. Through these studies, we hope to further elucidate the role of the PIM-1 gene in the development and progression of human GBM to provide an experimental and theoretical basis.

Materials and methods

Collection of glioma tissues and human glioma cell lines

Between January 2018 and August 2021, 53 tumour tissues including World Health Organization (WHO) grade I (9 cases), II (14 cases), III (15 cases), IV (15 cases), and 7 non-tumour tissues were collected from the Tissue Bank, Institute of Neurosurgery, Tianjin Huanhu Hospital. All samples were pathologically diagnosed, and the histopathological grading of the tumours was evaluated according to the 2016 WHO classification of adult central nervous system tumours. The IDH1 mutational status was evaluated in 51 samples; 22 samples were IDH1 mutated, while 29 samples were IDH1 wild type.

The A172, SNB19, LN229, U87MG, U251, TJ899, TJ905, and LN18 human glioblastoma cell lines and human astrocytes were used. Human glioblastoma A172, SNB19, LN229, U87MG, U251MG, and LN18 human astrocyte cell lines were purchased from China Academia Sinica Cell Repository, (Shanghai, China), while TJ899 and TJ905 were established and characterised by the Laboratory of Neuro-oncology, Tianjin Medical University General Hospital. All cells were maintained in Dulbecco's Modified Eagle Medium with 10% foetal bovine serum at 37°C and 5% CO_2.

Glioma tissue microarray

The glioma tissue microarray was prepared by WellBio Technology Co. Ltd. (ZL-BraG180sur01, WellBio Technology Co., Shanghai, China). The TMA slide contained 180 cases of glioma, including 98 cases of WHO grade IV, 27 grade III, and 55 grades I/II; no non-tumorous brain tissue was included. The IDH1 mutational status had been evaluated in 92 samples; 16 samples were mutated, while 76 samples were wild-type. There were 115 male and 65 female cases.

Immunohistochemical staining

For immunohistochemical analysis of formalin-fixed paraffin-embedded tissue sections, sections were first placed in xylene to remove paraffin and progressively hydrated through graded ethanol. Antigens were then retrieved by heating in citrate buffer at 95°C for 15 min. Sections were subsequently incubated overnight at 4°C with PIM-1 monoclonal antibody (Thermo, MA, USA) diluted to 1:100. Following incubation, sections were allowed to recover for 1h at room temperature and then incubated with biotin-labelled secondary antibodies (ZSGB-BIO, Beijing, China). Finally, colour development was performed using a DAB staining kit (ZSGB-BIO, Beijing, China) and contrast staining was performed with haematoxylin. Stained sections were imaged by light microscopy.

Protein extraction and western blot analysis

For protein extraction from glioma cells and tissue samples, radioimmunoprecipitation assay buffer containing protease inhibitors (Solarbio, Beijing, China) was used. The concentration of extracted protein was determined using a bicinchoninic acid assay kit. Next, equal amounts of protein samples were separated by sodium dodecyl-sulphate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, USA). The membranes were blocked using 5% skim milk in phosphate-buffered saline with Tween 20 for 1h at room temperature to reduce non-specific binding. Following this, the membranes were incubated overnight at 4°C with PIM-1 antibody (Thermo, MA, USA) diluted to 1:1,000 and glycolytic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Unibody, Tianjin, China) diluted to 1:2,000. Following PIM-1 incubation, incubation was continued for 1h at room temperature using horseradish peroxidase-labelled anti-mouse and anti-rabbit secondary antibodies (Unibody, Tianjin, China). Eventually, the detection of protein bands was performed using the ChemiDoc XRS system (Bio-Rad, Hercules, CA), in which GAPDH was normalised as an internal reference protein.

Small interfering ribonucleic acid transfection and cell counting kit-8 assay

To inhibit the expression of PIM-1 protein in glioma cells, small interfering ribonucleic acid (siRNA) technology was used for manipulation. Specifically, U87MG cells were seeded in 96-well plates at a density of 3×10^3 cells per well. Subsequently, a 50 nM concentration of PIM-1-specific siRNA (5'-GAUAUGGUGUGGAGAUAdTdT-3', provided by Shanghai, Hanbang Biotechnology Co., Ltd.) was transfected into cells using Lipofectamine 3000 transfection reagent. Following the transfection process, after three days of culture, cellular protein samples were collected to assess the effect of siRNA on PIM-1 expression. In addition, to assess the effect of siRNA transfection on cell proliferation, a cell counting kit-8 (CCK-8) assay was performed. In the experiment, cell viability and proliferation were evaluated by measuring the absorbance at 450 nm using a spectrophotometer. With these experimental steps, the effect of PIM-1 silencing by siRNA in glioma cells could be effectively assessed.

Analysis of PIM-1 expression and survival of patients with glioma via the GEPIA, GENT2, and CGGA databases

The Gene Expression Profiling Interactive Analysis (GEPIA) (Tang et al., 2017) and Gene Expression Database of Normal and Tumor Tissues 2 (GENT2) (Park et al., 2019) databases were used to compare the expression of the *PIM-1* gene in different tumour tissues and healthy controls. In addition, Kaplan-Meier survival curve analysis was performed using the Chinese Glioma Genome Atlas (CGGA) database (Zhao et al., 2021) to assess the association between *PIM-1* gene expression and survival in patients with glioma.

Analysis of Correlations between PIM-1 expression and clinicopathology in the TCGA-LGG and TCGA-GBM cohorts

The dataset was combined from The Cancer Genome Atlas (TCGA) brain low-grade glioma (LGG) and GBM datasets. The TCGA LGG and GBM gene-level copy number variation (CNV) were estimated using the genomic identification of significant targets in the cancer version 2 (GISTIC2) method. The copy number profile was measured experimentally using a whole genome microarray at a TCGA genome characterisation centre. Subsequently, the TCGA Firehose pipeline was used to apply the GISTIC2 method to produce segmented CNV data, which was then mapped to genes to produce gene-level estimates. Genes were mapped onto the human genome coordinates using the University of California, Santa Cruz Xena HUGO probeMap tool. Curated survival data was obtained from the Pan-Cancer Atlas paper titled 'An Integrated TCGA Pan-Cancer Clinical Data Resource (TCGA-CDR) to drive high-quality survival outcome analytics'. The paper highlights four types of carefully curated survival endpoints and recommends using overall survival (OS), progression-free interval, disease-free interval, and disease-specific survival for each TCGA cancer type.

Searching PIM-1-relevant genes in the LinkedOmics and STRING databases

To identify genes associated with the differential expression of the *PIM-1* gene in GBM and LGG, the LinkFinder tool was employed (Vasaikar et al., 2018). By calculating Pearson correlation coefficients, the association of these genes with PIM-1 was analysed and the results were visualised using volcano plots and heatmaps. In addition, to gain insight into the functional and biological significance of these genes, the LinkInterpreter tool was used to perform gene ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and gene set enrichment analysis (GSEA) to annotate co-expressed genes of *PIM-1*.

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database is a widely recognised platform for predicting interactions between proteins, including direct physical links and indirect functional associations (Szklarczyk et al., 2021). The STRING database was utilised to screen proteins that interact with PIM-1 to further explore their potential mechanisms of action in tumours.

The immune response of PIM-1 expression analysed using TIMER2.0 and TISIDB

In this study, gene expression analysis of glioma samples from the TCGA database was performed to estimate the relative abundance of tumour-infiltrating immune cells using the deconvolution algorithm in the Tumor Immune Estimation Resource Version 2 (TIMER2.0) tool (Li et al., 2020). In addition, the Tumor and Immune System Interactions Database (TISIDB) was used to predict tumour interactions with the immune system and possible responses to immunotherapy (Ru et al., 2019). By combining TIMER2.0 and TISIDB analyses, the correlation between *PIM-1* gene expression and immune cell infiltration and chemokine expression was investigated to reveal its role in the immune microenvironment of gliomas.

Statistical analysis

Data analysis was performed using SPSS software (version 22.0, SPSS Inc, Chicago, IL, USA) and GraphPad Prism software (version 8.0, GraphPad Software, USA). Experimental results were presented as mean ± SD, and each experiment was independently repeated three times. To assess differences between the two sets of data, a Student's t-test was used. In addition, to investigate the association between PIM-1 expression

and clinicopathological features, the Pearson chi-squared test was performed. OS and disease-free survival (DFS) were analysed by Kaplan–Meier survival curves and differences between survival curves were assessed using the log-rank test. In statistical analysis, p values of <0.05 were considered statistically significant.

Results

The expression of PIM-1 in glioma cell lines

The expression of PIM-1 in eight glioma cell lines was analysed by western blot analysis (Fig. 1). PIM-1 was upregulated in glioma cell lines compared with astrocytes. PIM-1 expression in the A172 cell line was the highest among cell lines, followed by SNB19 and U87MG cells, while in TJ899 and LN18 cells, PIM-1 expression levels were similar to those in astrocytes.

Expression of PIM-1 in glioma tissues

PIM-1 expression in 53 human glioma specimens and 7 non-neoplastic brain tissues was examined by western blotting. PIM-1 expression was upregulated in gliomas compared with non-tumorous brain tissues and

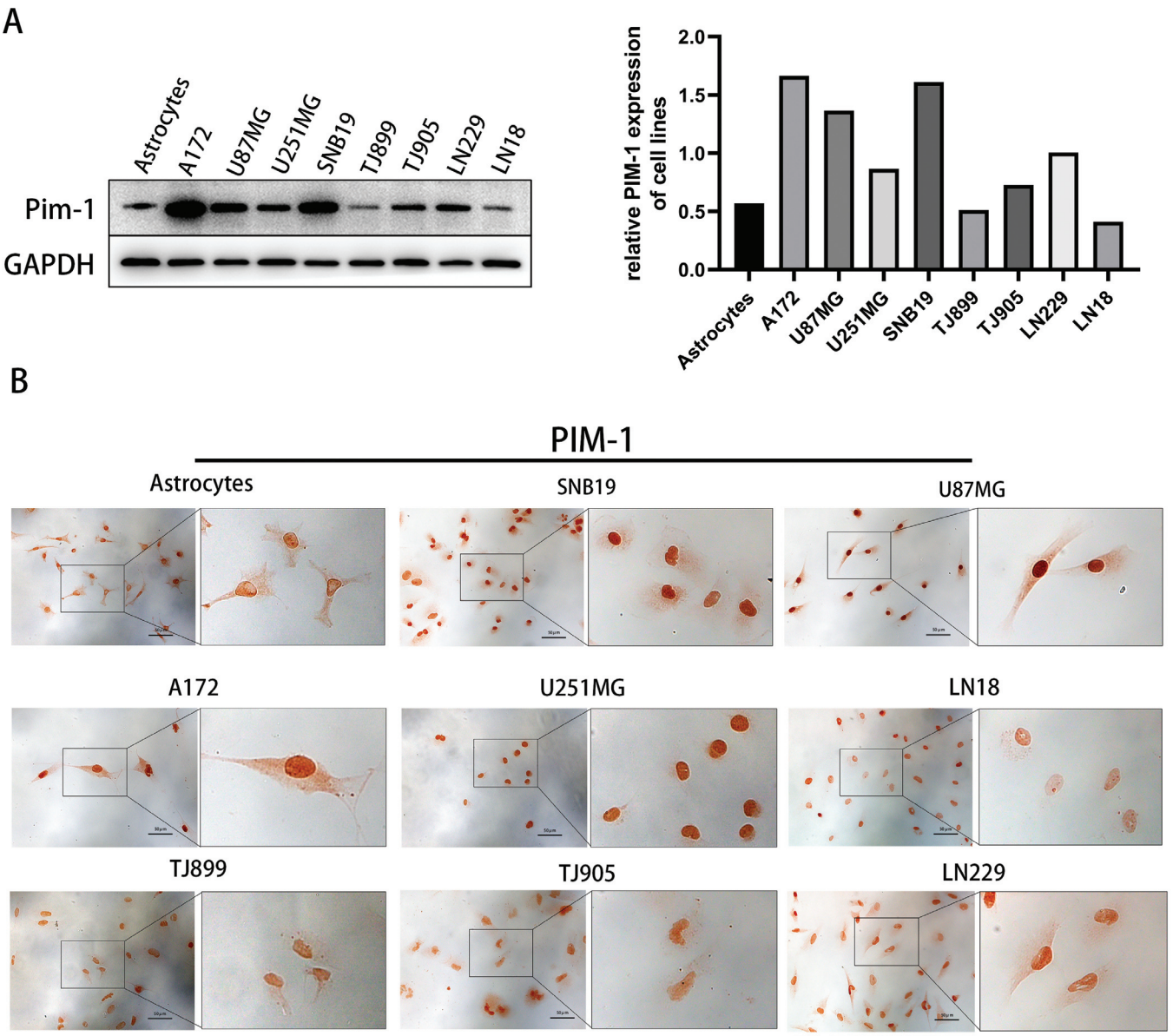

Fig. 1. Expression of PIM-1 in human GBM cell lines. **A.** Expression of PIM-1 in human GBM cell lines analysed by western blot analysis. GAPDH was used as a loading control. **B.** Expression of PIM-1 in human GBM cells.

was positively correlated with tumour grade (Fig. 2A). Next, the correlation of PIM-1 expression with IDH mutational status was investigated; the result indicated that PIM-1 expression was higher in IDH1 wild-type specimens than mutant samples (Fig. 2B).

The expression of PIM-1 in tissue microarrays and its relation to glioma prognosis

The expression level of PIM-1 in glioma TMAs was further evaluated, which included 180 glioma specimens and examination by immunocytochemical staining. Since no non-tumorous brain tissue had been supplied by the glioma TMA, seven non-tumorous brain tissues were collected from the Tissue Bank, Institute of Neurosurgery, Tianjin Huanhu Hospital, and served as controls. We found that 114 out of 180 glioma tissues (63.3%) showed higher PIM-1 expression than those in non-tumorous brain tissues. The representative images of immunohistochemistry staining indicated that PIM-1 expression in grades III/IV was stronger than in grade II glioma (Fig. 3A). The ratio of PIM-1 upregulation was also significantly higher in grades III/IV than in grade I/II gliomas (Table 1). These findings revealed that PIM-1 expression levels were positively correlated with tumour grades (p=0.047). The immunochemical staining results of TMA also demonstrated that the signal intensity of PIM-1 staining in IDH1-wild-type gliomas was stronger than in IDH1-mutant gliomas (Fig. 3B), the proportion of high PIM-1-expression cells in IDH1-wild-type glioma was much higher than in IDH1-mutant gliomas; high PIM-1 expression was significantly associated with IDH1 wild-type (p=0.020) (Table 1). IHC staining results for PIM-1 in glioma specimens appeared to have stronger nuclear and lower cytoplasmic staining.

Further analysis was performed to determine whether PIM-1 expression levels in gliomas correlated with patients' clinical characteristics and prognosis. We found that PIM-1 expression did not correlate with age or gender (p>0.05, Table 1). To identify the association between PIM-1 expression and glioma patient survival, Kaplan-Meier curves for survival analysis of the TMA cohort were plotted. Interestingly, in the first year of the follow-up study, patients with GBM with higher PIM-1 expression tended to have a poorer prognosis, however, the correlation did not reach statistical significance (p>0.05). Nevertheless, the survival of patients with LGG showed no relationship with PIM-1 expression levels (Median survival time of PIM-1-high group: 9.58 months and median survival time of PIM-1-low group: 11.02 months) (Fig. 3C-F).

Table 1. Correlations between PIM-1 expression and clinicopathology in TMA Cohort.

Clinicopathological features	Number of cases	PIM-1 expression		
		Low	High	*p* value
All patients	180	66	114	
Age(years)				p=0.908
≤60	130	48	82	
>60	50	18	32	
Gender				p=0.096
Male	115	37	78	
Female	65	29	36	
WHO grade				p=0.047
I~II	55	26	29	
III	27	12	15	
IV	98	28	70	
IDH1 mutations				p=0.020
MUT	16	10	6	
WT	76	24	52	

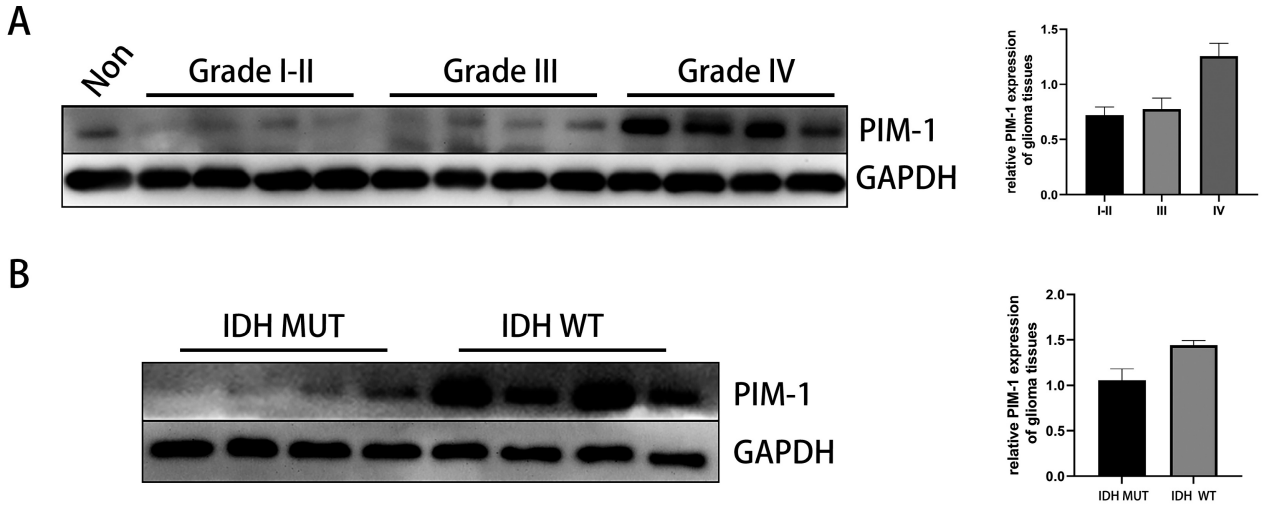

Fig. 2. Evaluation of PIM-1 expression in glioma tissues. **A.** Expression of PIM-1 in glioma specimens detected by western blot analysis. **B.** Expression of PIM-1 in IDH1-mutant and wild-type glioma specimens. GAPDH was used as a loading control.

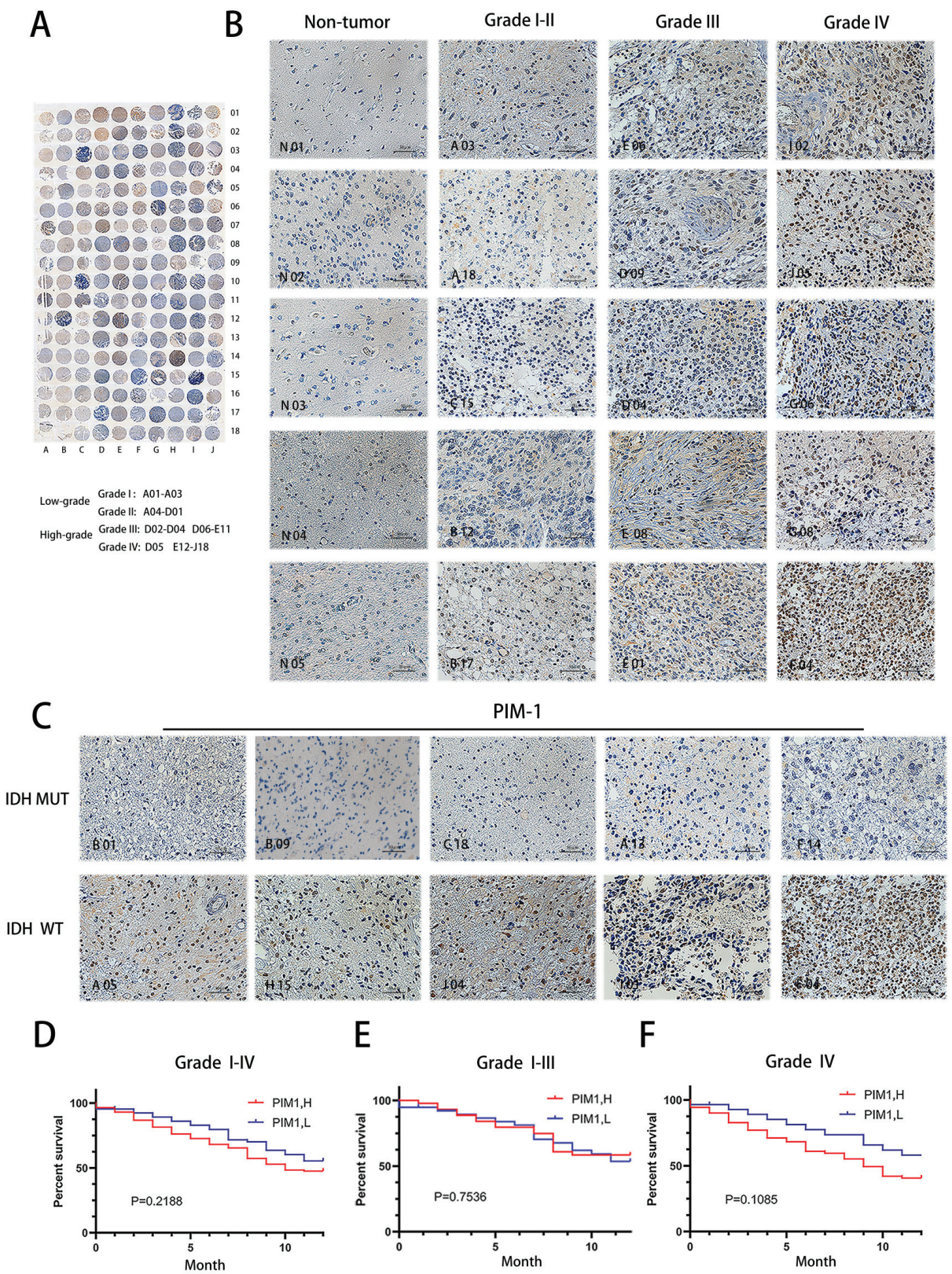

Fig. 3. Evaluation of *PIM-1* gene expression in tumour tissue microarray. **A.** Immunohistochemical staining of PIM-1 in glioma tissues of different WHO grades. **B.** Immunohistochemical staining of PIM-1 in IDH1- mutant and IDH1-wild-type gliomas. Scale bar represents 50 μm. **C.** Kaplan-Meier survival curves in glioma patients with different WHO grades for 12-month survival. **D.** The survival data for glioma patients of WHO grades I-III. **E, F.** The survival data for glioma patients of WHO grades I-IV.

Suppressing PIM-1 decreased glioma cell proliferation

To investigate the effect of PIM-1 on glioma cell proliferation, PIM-1 expression was knocked down via PIM-1 siRNA in the U87MG cell line; a notable decrease in PIM-1 expression was detected by western blot analysis after PIM-1 siRNA transfection (Fig. 4A). The viability was also decreased in U87MG glioma cells following transfection with PIM-1 siRNA (Fig. 4B). The inhibitory effect was obvious on the second day after transfection ($p<0.05$), and the most pronounced effect was seen on the sixth day ($p<0.01$).

PIM-1 expression in glioma analysed with the GEPIA and GENT2 databases

The pan-cancer expression of PIM-1 was analysed using data from the GENT2 database. PIM-1 was upregulated in brain, lung, bladder, and testis cancers, while it was downregulated in blood, skin, breast, endometrium, stomach, head, and neck cancers (Fig. 5A). Similar results were also obtained from the GEPIA database (Fig. 5B). The results from the GENT2 online tool revealed that PIM-1 was upregulated in gliomas, and high expression of PIM-1 was associated significantly with high WHO grades and IDH1 wild-type (Fig. 5C, Table 2). The result was consistent with the experimental results. The epidermal growth factor receptor (EGFR) status is also important and related to proliferation in GBM. After the GEPIA database analysis, a positive correlation was found between EGFR and PIM-1 expression (Fig. 5D). According to the CGGA database, we found that high PIM-1 expression in GBM patients was significantly correlated with poor OS and DFS ($p<0.001$) (Fig. 5E). In the WHO grade III group, patients with high PIM-1 expression tended to have shorter OS and DFS than those with low PIM-1 expression, however, this difference was not statistically significant ($p>0.05$). In the WHO grade II group, the high *PIM-1* gene expression group tended to have longer OS and DFS than patients with low *PIM-1* gene expression ($p>0.05$).

The expression of PIM-1 in gliomas and their clinical characteristics were analysed with the TCGA database. It was also revealed that PIM-1 upregulation was positively correlated with tumour grades and IDH1 wild-type ($p<0.001$), as well as age. The ratio of high PIM-1 expression in the >60 years age group was higher than in the ≤60 years age group ($p<0.05$), while its expression had no significant relevance to gender (Table 2).

Co-expression gene network and binding proteins of PIM-1 in glioma

In the study, 5,012 genes were positively correlated with *PIM-1* gene expression and 3,651 genes were negatively correlated (Fig. 6A). Further heat map analysis revealed the top 50 genes that were positively

Table 2. Correlations between PIM-1 expression and clinicopathology in TCGA-LGG and TCGA-GBM Cohort.

Clinicopathological features	Number of cases	PIM-1 expression		*p* value
		Low	High	
All patients	696	348	348	
Age(years)				$p<0.001$
≤60	553	303	250	
>60	143	45	98	
Gender				$p=0.818$
Male	298	147	151	
Female	398	201	197	
WHO grade				$p<0.001$
I-II	224	171	53	
III	243	111	132	
IV	168	28	140	
IDH1 mutations				$p<0.001$
MUT	440	290	150	
WT	256	65	191	

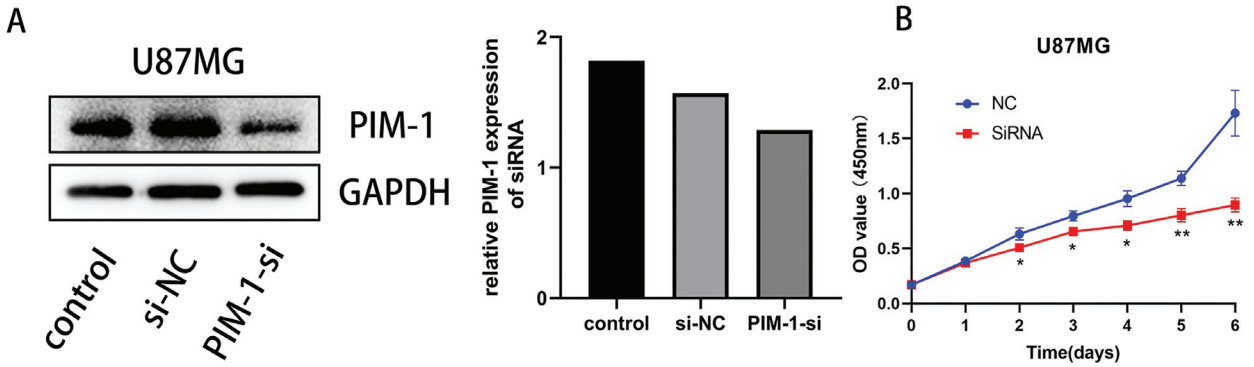

Fig. 4. The effect of PIM-1 on U87MG cell line proliferation. **A.** The expression of PIM-1 in U87MG after PIM-1-specific siRNA transfection. **B.** The proliferation of GBM cells evaluated by the CCK-8 assay. *$p<0.05$, **$p<0.01$.

and negatively correlated with *PIM-1* expression (Fig. 6B,C). Through the annotation of GO terms, we found that co-expressed genes of *PIM-1* play an important role in the adaptive immune response, lymphocyte-mediated immune response, acute inflammatory response, and neutrophil-mediated immune response (Fig. 6D). The results of KEGG pathway analysis showed that these co-expressed genes were enriched in complement and coagulation cascades, intestinal immune networks generated by immunoglobulin A, and haematopoietic

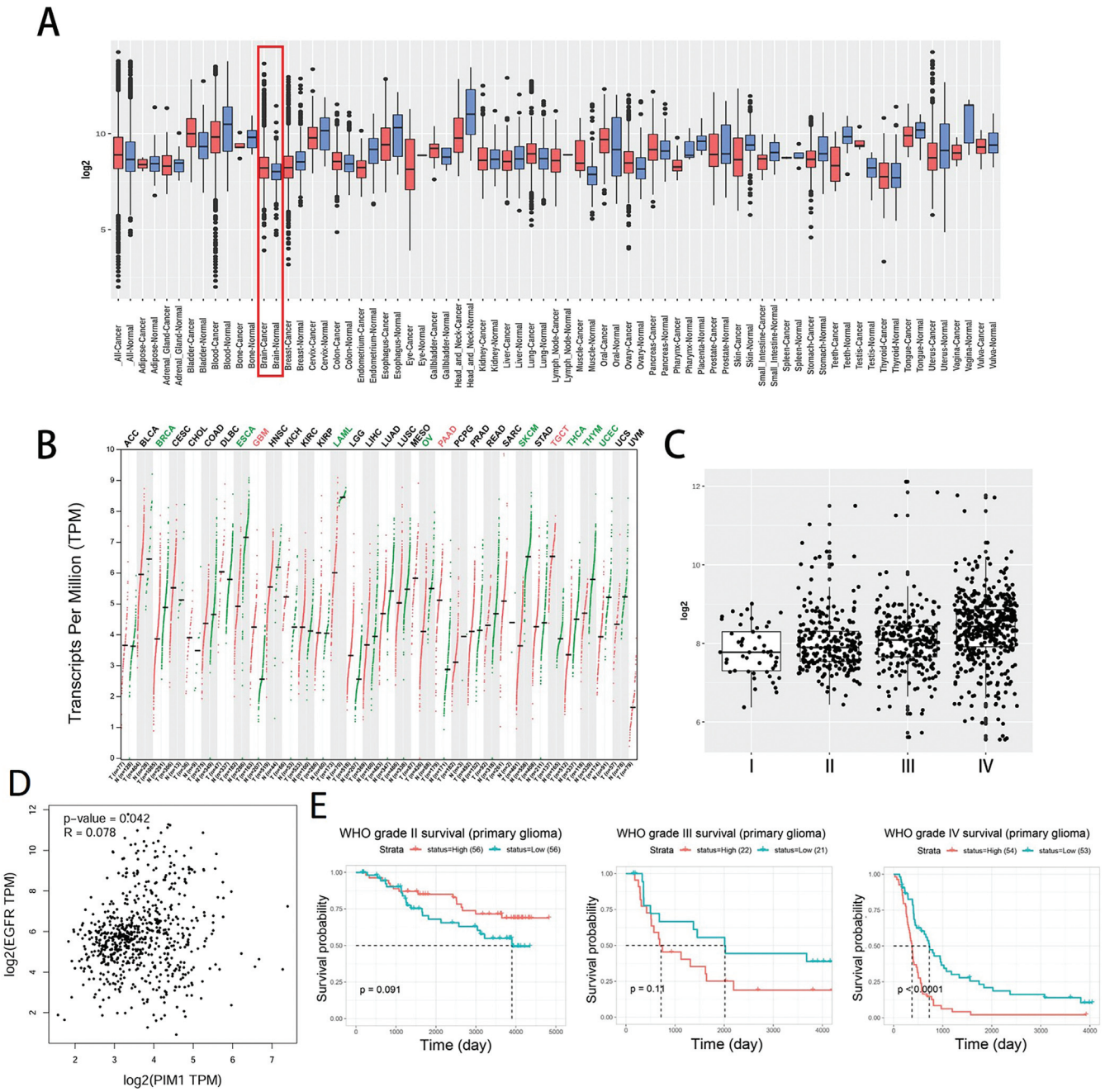

Fig. 5. Evaluation of *PIM-1* gene expression in pan-cancer tissues via public databases. **A.** Boxplot shows the tissue-wide gene expression pattern of *PIM-1* across paired tissue samples from the GPL570 platform (HG-U133_Plus_2) of the Affymetrix mRNA gene array GENT2 database. The red boxplot indicates cancer samples and the blue boxplot denotes normal samples. **B.** Profile shows the tissue-wide gene expression pattern of *PIM-1* across paired tissue samples from the GEPIA database. **C.** Expression level of PIM-1 from glioma patients with different WHO Grades in the GENT2 database. **D.** A positive correlation between EGFR and PIM-1 expression in the GENT2 database. **E.** Kaplan-Meier overall survival and disease-free survival curve of glioma patients with low or high levels of PIM-1 from the mRNA_ array_301 CGGA database.

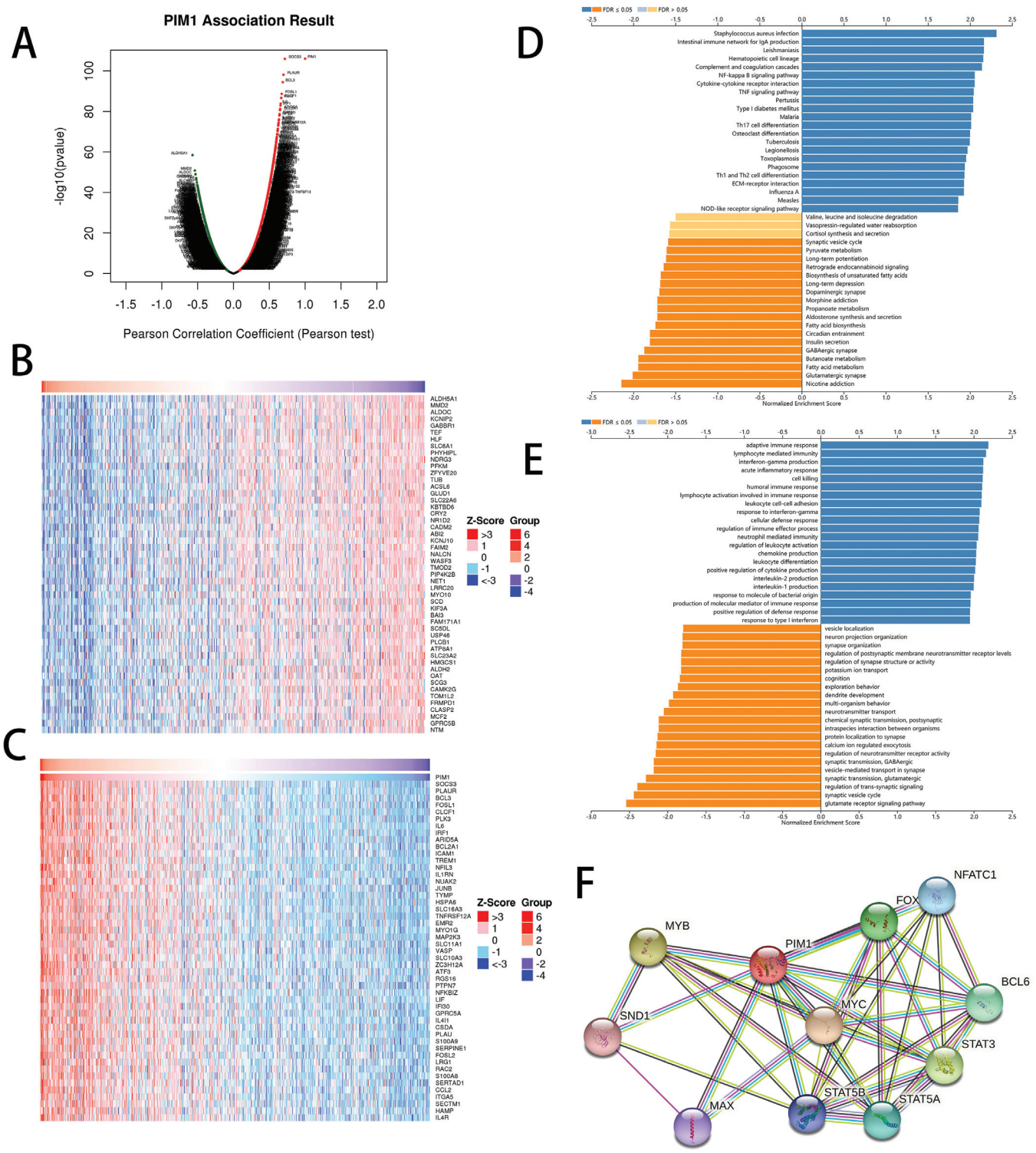

Fig. 6. *PIM-1* co-expressed genes and functional enrichment analysis. **A.** Volcano map of co-expressed profiling of PIM-1 in glioma by the LinkedOmics database. **B.** Heat map of top 50 genes positively correlated with *PIM-1*. **C.** Heat map of top 50 genes negatively correlated with *PIM-1*. **D.** *PIM-1* co-expression genes were annotated by GO analysis. **E.** *PIM-1* co-expression genes were annotated by KEGG pathway analysis. **F.** PIM-1 binding proteins obtained from the STRING database.

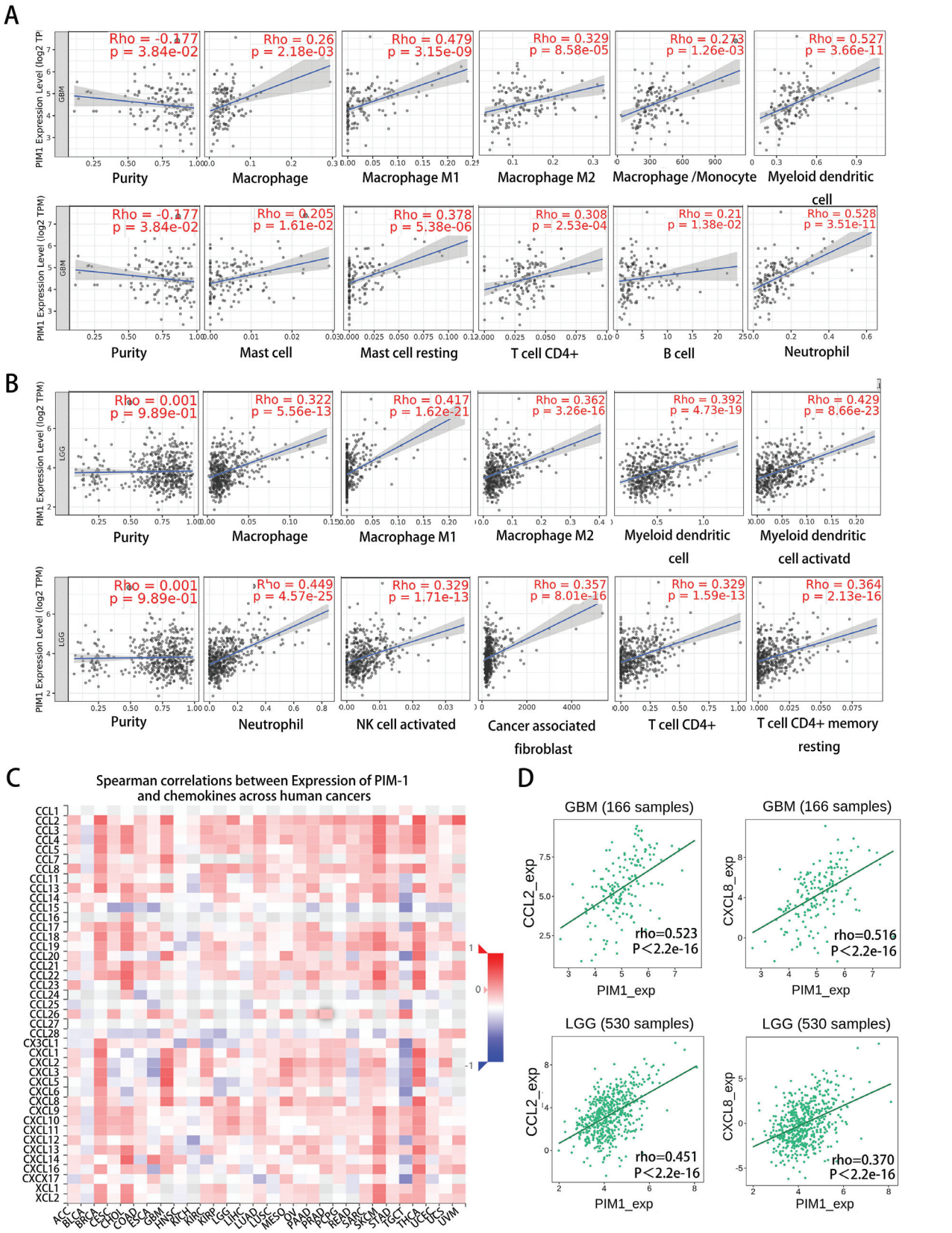

Fig. 7. Correlation between PIM-1 with immune cell infiltration and chemokines in gliomas. **A.** Correlation between PIM-1 with immune cell infiltration in GBM. **B.** Correlation between PIM-1 with immune cell infiltration in LGG. **C.** Correlation between PIM-1 and chemokines in tumours by heatmap analysis. **D.** PIM-1 expression is positively correlated with CCL2 and CXCL8 chemokine levels in GBM and LGG.

cell lineages (Fig. 6E). These findings imply that PIM-1 may play a critical role in shaping the immune microenvironment and have a significant impact on the immune response of gliomas. In addition, proteins interacting with PIM-1 were screened using the STRING database to explore the potential function of PIM-1 in tumorigenesis. Based on experimental data and database information, 10 proteins that interact with PIM-1 were identified, namely MYC, MYB, STAT3, FOXO3, BCL6, STAT5A, NFATC1, STAT5B, MAX, and SND1; the interaction network of these proteins with PIM-1, shown in Fig. 6F, provides further insights into the role of PIM-1 in tumour biology.

PIM-1 was correlated with immune response in glioma

The results reveal the association of *PIM-1* co-expressed genes with multiple immune processes, which prompted the authors to hypothesise that PIM-1 may play a role in the regulation of tumour immune responses. To further interrogate this, differences in immune cell infiltration in GBM and LGG tumours at different PIM-1 expression levels were analysed using the TIMER2.0 database. This approach aimed to uncover a potential link between PIM-1 expression and the glioma immune microenvironment.

The results showed that the expression level of PIM-1 was positively correlated with the level of infiltrating macrophages (r=0.26, p<0.001), myeloid dendritic cells (r=0.527, p<0.001), mast cells (r=0.205, p<0.001), CD4+ T cells (r=0.308, p<0.001), B cells (r=0.21, p<0.001), and neutrophils (r=0.528, p<0.001) in GBM (Fig. 7A). In LGG, the expression level of PIM-1 was positively correlated with the levels of activated macrophages (r=0.322, p<0.001), myeloid dendritic cells (r=0.392, p<0.001), NK cells (r=0.329, p<0.001), CD4 T cells (r=0.329, p<0.001), cancer-associated fibroblasts (r=0.357, p<0.001), and neutrophils (r=0.449, p<0.001) (Fig. 7B).

The study focused on investigating the role of chemokines and their receptors in the tumour immune microenvironment, particularly their critical role in attracting immune cells and promoting tumour cell growth and spread. To gain insight into the function of these molecules in glioma, TISIDB was specifically utilised to analyse the association between *PIM-1* gene expression and the expression of several chemokines in patients with GBM and LGG (Fig. 7C). The results showed that PIM-1 expression levels were positively correlated with those of several chemokines, i.e., CCL2 (r=0.523, p<0.001), CXCL8 (r=0.516, p<0.001) in GBM, and CCL2 (r=0.451, p<0.001) and CXCL8 (r=0.37, p<0.001) in LGG (Fig. 7D). Therefore, these results suggested that PIM-1 may participate in the regulation of tumour immunity.

Discussion

In recent years, the standard of treatment generally used for gliomas included maximal safety resection, radiotherapy, and chemotherapy. However, despite the multimodal and aggressive treatments, glioma prognosis, especially GBM, is still poor. Therefore, it is urgent to search for novel strategies to fight against GBM progression and prolong the survival of patients. In our study, we identified PIM-1 expression in glioma cell lines and specimens by western blot analysis and immunocytochemical staining. We found that PIM-1 was upregulated in most glioma cell lines compared with astrocytes. In glioma specimens, PIM-1 expression was significantly higher than in non-neoplastic brain tissues, and its expression level increased with ascending WHO grades. The results of TMA immunochemical staining showed that high PIM-1 expression was found in 62.8% of TMA glioma samples. Among them, 71.4% of high-expression samples were WHO grade IV glioma.

In glioma specimens and TMA, the PIM-1 expression level was higher in IDH1-wild-type samples than in IDH1-mutant ones. However, IDH mutation can be found in 70% of low-grade gliomas but in only 6% of primary GBM (Ducray et al., 2009; van den Bent et al., 2017). Some studies have shown that patients with IDH-mutant tumours had more favourable outcomes than those with IDH-wild-type (Ducray et al., 2009; van den Bent et al., 2017); therefore, we further studied if PIM-1 expression could be a marker of prognosis in patients with glioma.

According to Kaplan-Meier survival analysis of TMA data, patients with high PIM-1 expression tended to be correlated with poor prognosis only in the GBM group for the first year. Herzog also reported that patients with GBM with high PIM-1 expression had shorter survivals in the first year (Herzog et al., 2015). The proliferation of U87MG cells was suppressed by transfection with PIM-1 siRNA as determined by the CCK-8 assay. This result suggested that PIM-1 may participate in glioma progression. Yan et al. (2022) reported that PIM-1 could promote glioma progression, while melatonin inhibited malignant GBM progression by regulating miR-16-5p-mediated PIM-1. The above study is generally consistent with our results, that PIM-1 can promote glioma progression, showing that targeting PIM-1 in GBM is a potential therapeutic target. In addition, the design and development of PIM-1 inhibitors have the potential to provide new approaches toward the treatment of GBM. Dib et al. (2024) identified a variety of potential PIM-1 inhibitors through screening, and the study of these inhibitors will provide new ideas for the treatment of GBM. Currently, in GBM treatment, an increasing number of approaches have been developed due to a deeper exploration of the molecular mechanisms. For example, (Hacioglu et al., 2023; Hacioglu and Tuncer 2024; Tuncer and Hacioglu, 2024) found that targeting the ferroptosis pathway was effective in GBM treatment, and borax could have potential as an anticancer therapy for GBM by targeting the ferroptosis signalling pathway.

We further demonstrated our results by bio-

informatic analysis with data from bioinformatic platforms. We found that PIM-1 expression was upregulated in a variety of tumours, such as brain, lung, and bladder. Therefore, PIM-1 has been identified to play a certain role in cancers. PIM-1 expression was further identified to be upregulated in GBM and LGG. In addition, the PIM-1 expression level was positively correlated with WHO grades and was higher in IDH1-wild-type than in IDH1-mutant gliomas; these results are consistent with what we observed in glioma specimens. Based on data from the CCGA database, patients with glioma with high *PIM-1* gene expression had shorter OS and DFS both in the GBM and WHO grade III groups; this finding was significant in GBM ($p<0.001$) but not in grade III groups ($p>0.05$). Furthermore, our results from TMA data indicated that high PIM-1 expression was correlated with poor prognosis only in the GBM group for the first year. Therefore, the correlation between PIM-1 expression and survival of patients with glioma should be further observed in a larger number of glioma samples and followed up with more patients.

To explore the underlying molecular mechanism of PIM-1 involved in the pathogenesis of glioma, we utilised GO functional enrichment and GSEA and KEGG pathway analysis to find PIM-1 co-expression genes in gliomas; we found that the relevant genes were involved in diverse pathophysiological processes. Data from TIMER2.0 and TISIDB also revealed a correlation between PIM-1 expression and the level of immune cell infiltration in GBM and LGG. The results showed that high expression of PIM-1 was positively correlated with the infiltration level of macrophages, CD4+ T cells, and neutrophils. Chemokines, a subfamily of cytokines, are involved in cell migration and immune cell infiltration. Based on the data from TISIDB, PIM-1 expression was also positively correlated with the expression of some chemokines, such as CCL2 and CXCL8. These findings indicated that PIM-1 may be associated with tumour immunity response and plays an essential regulatory role in the tumour immune microenvironment.

Conclusion

In summary, by combining bioinformatic analyses and experimental results, we preliminary demonstrated that PIM-1 expression increased positively with glioma grades and was upregulated in IDH1-wild-type glioma. In GBM, high PIM-1 tended to be correlated with worse outcomes in the first year. Patients with high PIM-1 levels had significantly shorter survival in GBM according to bioinformatic analyses. Suppressing PIM-1 inhibited GBM cell growth. In this study, by bio-informatic analyses, we also demonstrated that PIM-1-relevant genes and PIM-1 may participate in regulating immune responses in glioma, which provided potential clues for the mechanism of PIM-1's effect on the progression of glioma; this is worthy of further study.

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Ethics approval and consent to participate. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Tianjin Huanhu Hospital (approval number: ZL-XP201402).

Competing Interest. The authors declare that they have no competing interests.

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Availability of data and materials. The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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