GPR27 expression correlates with prognosis and tumor progression in gliomas

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Backgrounds: Glioma is a highly aggressive type of brain tumor, and its prognosis is still poor despite recent progress in treatment strategies. G protein-coupled receptor 27 (GPR27) is a member of the G protein-coupled receptor family and has been reported to be involved in various cellular processes, including tumor progression. Nevertheless, the clinical potential and tumor-related role of GPR27 in glioma remain unknown. Here we aimed to explore the function and role of GPR27 in gliomas.

Methods: In the current study, we evaluated the expression and clinical significance of GPR27 in gliomas using data from TCGA datasets. We also conducted cellular experiments to evaluate the functional role of GPR27 in glioma cell growth.

Results: We found that GPR27 expression level was closely associated with disease status of glioma. Of note, GPR27 was negatively correlated with WHO grade, with grade IV samples showing the lowest GPR27 levels, while grade II samples showed the highest levels. Patients with IDH mutation or 1p/19q co-deletion exhibited higher GPR27 levels. In addition, lower GPR27 levels were correlated with higher death possibilities. In cellular experiments, we confirmed that GPR27 inhibited glioma cell growth.

Conclusions: Our results indicate that GPR27 may function as a potential prognostic biomarker and therapeutic target in gliomas. Further studies are needed to illustrate the signaling mechanism and clinical implications of GPR27 in gliomas.

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Ethics

The Ethics Committee of Suining Central Hospital requires no ethic approval and grants an exemption from informed consent for this public-database study.

Abstract

Backgrounds: Glioma is a highly aggressive type of brain tumor, and its prognosis is still poor despite recent progress in treatment strategies. G protein-coupled receptor 27 (GPR27) is a member of the G protein-coupled receptor family and has been reported to be involved in various cellular processes, including tumor progression. Nevertheless, the clinical potential and tumor-related role of GPR27 in glioma remain unknown. Here we aimed to explore the function and role of GPR27 in gliomas.

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Keywords

Biomarkers, Cell proliferation, Glioblastoma, Neuroscience, Molecular biology, Survival analysis.

Introduction

Gliomas and glioblastomas are types of primary brain tumors that originate from glial cell, which are the supportive cells of the central nervous system [1]. Glioma is the most common form of brain malignancies, accounting for about 80% of brain tumors, and they can vary in grade from slow-growing low-grade tumor to aggressive high-grade tumor. Among gliomas, glioblastoma, also known as glioblastoma multiforme (GBM), represents the most aggressive subtype, representing the highest grade of glioma [2]. Glioblastoma is characterized by its rapid growth, infiltrative nature, and resistance to treatment, making it one of the most challenging cancers to manage. Understanding the basic features and biology of gliomas and glioblastomas is crucial for advancing diagnosis, prognosis, and treatment strategies for these complex brain tumors [3].

GPR27, a member of the G protein-coupled receptor family, is a protein-coding gene located on human chromosome 3q25.1 [4]. It encodes a 7-transmembrane receptor that expresses in various tissues and is involved in multiple cellular functions including neurotransmission, immune activation, and cellular growth [5, 6]. Recent research has implied that GPR27 might play critical roles during cancer progression, particularly in hepatocellular carcinoma (HCC) [7] and breast cancer [8]. Abnormal expression of GPR27 has been observed in these cancers, and it has been recognized to contribute to tumor growth and angiogenesis. For example, Wang et al. discussed function of GPR27 in HCC progression and its potential mechanism of action through MAPK-ERK signaling pathway [7]. The study highlights the importance of GPR27 in HCC, a type of liver cancer, and proposes that GPR27 may promote tumor progression by activating the MAPK/ERK pathway. The paper provides insights into the potential molecular mechanisms underlying HCC development and indicates that GPR27 could be a valuable therapeutic target in HCC treatment. However, further study will be needed to fully understand the exact mechanisms by which GPR27 affects cancer development and to explore its potential as a treatment target for cancers.

This study sheds light on expression and clinical relevance of GPR27 in gliomas and provides evidence for its oncogenic role in glioma growth. The findings presented here have the potential to enhance our understanding of underlying mechanism of glioma progression and could contribute to the development of novel therapeutic strategies for this devastating disease.

Methods

In silico data collection and analysis

RNA expression information in the Fragments Per Kilobase per Million (FPKM) formatof glioma samples was obtained as previously published by M Ceccarelli et al. [9]. The clinic-pathological information of glioma patients was also extracted (n=1122). The difference of various clinicopathological parameters were compared between the high-GPR27 and low-GPR27 expression groups. Regression analyses were used to assess the relationships between GPR27 expression and clinicpathological variables of glioma cases.

Survival information of glioma cases in TCGA-glioma was analyzed. Kaplan-Meier survival as well as multivariate Cox regression analyses was conducted to identify the patients' prognoses according to GPR27 level as well as other clinic-pathological characteristics.

Establishment of nomogram and calibration curves

We employed the "RMS" package in R to construct the nomogram for predicting possibility of individual survival. The calibration of the nomogram was evaluated through calibration curves.

Immune infiltration analyses

We evaluated the association between GPR27 expression and immune cell infiltration in gliomas using ssGSEA algorithm available in the "GSVA" R package [10]. This algorithm helped us assess infiltration status of 24 kinds of immune cell types [11]. We performed Spearman correlation analysis to clarify correlation between GPR27 expression and immune cell infiltration status.

Cell culture and transfection

U87 and U251 human glioblastoma cells will be obtained from ATCC and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin

at 37°C with 5% CO2. Culturing medium was replaced every two days, and cells were sub-seeded once they reached 70-80% confluence. Transient transfection of pcDNA3.1-GPR27 plasmids (Cat. #66349, Addgene, Watertown, MA, USA). or control pcDNA3.1-vector plasmids (Cat. #138209, Addgene). was achieved with Lipofectamine 3000 based on manufacturer's instructions. Afterwards, cells were incubated to allow for transfection. After the incubation period, the transfection medium will be replaced with fresh complete growth medium to allow for further cell culture or further experimental tests. Each experiment was repeated three times independently.

Western blotting

Protein expression levels were assessed through Western blot analysis. Proteins were extracted from the U87 and U251 cell lines utilizing RIPA lysis buffer, and their concentrations were subsequently semi-quantified using a BCA protein assay kit provided by Santa Cruz Biotechnology. Proteins, in the amount of 20 μg per lane, were resolved via 12% SDS-PAGE and subsequently transferred onto PVDF membranes. These membranes were then blocked using 5% BSA sourced from Sigma-Aldrich for 1 hour at ambient temperature before the blocking solution was discarded. Subsequently, the membranes were incubated overnight at 4°C with primary antibodies: anti-GPR27 (PA5-110977, Invitrogen, Cambridge, MA, USA) and anti-GAPDH (MA1-16757, Invitrogen, Cambridge, MA, USA), both diluted at a ratio of 1:2000. Following the primary antibody incubation, the membranes were washed with TBST containing 0.1% Tween. Then, an HRP-conjugated secondary antibody was applied to incubate at room temperature for 1 hour. Detection of the proteins of interest was achieved using the ECL Western Blotting substrate kit (Cat.#ab65623; Abcam). Each experiment was repeated three times independently.

Cell proliferation assay

Cell viability was gauged using the Cell Counting Kit-8 (CCK-8), which estimates the quantity of live cells in culture based on the production of a colored formazan product. Briefly, transfected cells were seeded at 5000 cells/well. Then CCK-8 experiment was done according to the

manufacturer's instruction. Briefly, the medium was replaced with CCK-8 reagent, and the cells were incubated for 2 hours. The absorbance of the formazan dye, which is proportional to the number of viable cells, was measured spectrophotometrically at 450 nm. The percentage of cell viability or proliferation was calculated by normalizing to the control group. Results were expressed as mean ± SD to determine statistical significance. Each experiment was repeated three times independently.

Statistics

All statistical analyses and plots were performed using R (version 4.1.3). Statistical significance was set at $P<0.05$. * indicates $P<0.05$, ** indicates $P<0.01$, *** indicates $P<0.001$.

Ethics

The Ethics Committee of Suining Central Hospital requires no ethic approval and grants an exemption from informed consent for this public-database study.

Results

Aberrant GRP27 expression in gliomas

RNA expression data from TCGA datasets suggested that GPR27 expression was closely correlated with disease status of glioma. For example, GPR27 was negatively correlated with WHO grade, on that grade IV samples showed the lowest GPR27 level while grade II samples showed the highest GPR27 level (Figure 1A, $P<0.001$). In contrast, patients with IDH mutation (Figure 1B) or 1p/19q co-deletion (Figure 1C) exhibited higher GPR27 levels ($P<0.001$).

Correlations between GPR27 expression and clinic-pathological characteristics of gliomas

Among the 1122 cases from TCGA cohort, we excluded those without sufficient matched clinical information and therefore 699 cases were enrolled for further analysis (Table 1). By dividing TCGA cohorts into high-GPR27 group (n=350) and low-GPR27 group (n=349) based on the

median value of log2(FPKM+1) of GPR27, we further analyzed its correlation with clinic-pathological variables (Table 1). Accordingly, lower GPR27 expression was observed in elder patients, higher-grade gliomas, as well as in the specimens with wild-type IDH status or non-codeletion of 1p/19q (all P<0.001). Considering all the above-mentioned variables had been reported to be correlated with patients' prognosis, we were engaged to further explore whether GPR27 can affect the overall survival of glioma patients.

GPR27 is an independent survival predictor of gliomas

Among the 699 cases, 1 case was further excluded due to unavailable survival information. Therefore, the other 698 patients were subjected for survival analyses. Kaplan-Meier survival analysis showed that patients with lower GPR27 level had significantly worse prognosis (Figure 2A, P<0.001). Briefly, the median overall survival time of low-GPR27 group was 26.3 months (95% CI 23.6-34.1 months), while was up to 99.6 months (95% CI 68.4-135.6 months) of high-GPR27 group. In addition to GPR27, several other variables were identified with prognostic significances in univariate analyses. For example, comparing with WHO grade II patients, WHO grade III patients showed a death hazard ratio as 2.967 (95% CI 1.986 - 4.433, P<0.001) and WHO grade IV patients showed a death hazard ratio as 18.6 (95% CI 12.448 - 27.794, P<0.001). Consistent with our previous data, mutated IDH status indicated a better survival with a death hazard ratio as 0.116 (95% CI 0.089 - 0.151, P<0.001) comparing to those with wild-type IDH status. Similarly, co-deletion of 1p/19q was a favorable prognostic factor with a death hazard ratio as 0.225 (95% CI 0.147 - 0.346, P<0.001).

To figure out the independent prognostic factors, we further subjected the variables into a Cox multivariate regression model for survival analysis (Table 2). As a result, elder age and higher WHO grade were confirmed as independent unfavorable prognosis factors. In contrast, mutated IDH status was identified as an independent favorable prognostic factor (hazard ratio 0.265, 95% CI 0.176 - 0.399, P<0.001). Importantly, our data, for the first time, showed that higher GPR27

expression was an independent benefit biomarker for glioma prognosis (hazard ratio 0.679, 95% CI 0.486 - 0.947, P=0.023).

Based on the multivariate survival analysis, we also established a nomogram to help predict overall survival of glioma patients (Figure 2B); the variables in the nomogram included patients' age, gender, WHO grade, IDH status, 1p/19q co-deletion, and GPR27 expression level.

GPR27 inhibits proliferation of glioma cells

We next conducted western blot experiments to confirm the efficiency of overexpressing GPR27 in U87 and U251 cell lines. Western blot analysis revealed a notable increase in GPR27 levels in cells transfected with pcDNA3.1-GPR27 plasmids compared to cells transfected with the pcDNA3.1-vector. This suggests that the overexpression of GPR27 was successfully achieved (Figure 3A). To further investigate the role of GPR27 in cell proliferation, we conducted CCK-8 experiments. The results showed that overexpressing GPR27 significantly inhibited the proliferation (Figure 3B- 3C) capacities of both U87 and U251 cell lines. These findings suggested that GPR27 plays a tumor-inhibiting role in glioma cells. Modulation of GPR27 expression may be a promising therapeutic approach for treating glioma. Overall, the results of this study provide important insights into the molecular mechanisms underlying glioma development and progression, and may have significant implications for the development of novel therapeutic strategies for this devastating disease.

GPR27 is correlated with the immune cell infiltration in gliomas

The results of GSEA showed that the GPR27 has a negative association with macrophages (R=-0.551, P < 0.001), neutrophils (R=-0.473, P<0.001), aDC (R=-0.405, P<0.001), eosinophils (R=-0.397, P<0.001), iDC cells (R=-0.324, P<0.001), cytotoxic cells (R=-0.286, P<0.001). etc. These findings indicate that GPR27 may play a crucial role in modulating the immune response in the tumor microenvironment. Oppositely, GPR27 shows positive associations with pDC cells (R=0.325, P<0.001), TFH cells (R=0.281, P=0.003), NK CD56bright cells (R=0.251, P<0.001), Tcm cells (R=0.214, P<0.001), TReg cells (R=0.190, P<0.001), etc (Figure 4A-C). These results

199 suggest that GPR27 may play a significant role in promoting the differentiation and activation of
200 these immune cells.

201

202 **Discussions**

203 The results presented in this study suggest that GPR27 expression levels are closely correlated
204 with the disease status of glioma patients. Specifically, GPR27 expression was negatively
205 correlated with WHO grade, and patients with IDH mutation or 1p/19q co-deletion exhibited
206 higher GPR27 levels. Additionally, in silico analysis showed that lower GPR27 expression was
207 correlated with higher death rates in glioma patients. In line with these findings, the authors
208 performed further analyses and showed that low GPR27 expression was associated with elder
209 patients, higher-grade gliomas, and wild-type IDH status or non-codeletion of 1p/19q. Importantly,
210 Kaplan-Meier survival analysis indicated that lower GPR27 expression was a significant
211 independent predictor of poor overall survival in glioma patients. Taken together, clinical data
212 analyses suggest that GPR27 may serve as a useful prognostic biomarker for glioma and may
213 have implications for the development of novel therapeutic strategies.

214 Consistently, cellular experiments suggest that GPR27 plays a crucial role in the development
215 and progression of glioma, as overexpressing GPR27 expression significantly inhibited the
216 proliferation capacities of U87 and U251 cell lines. These findings are distinct with previous
217 reports that have demonstrated that GPR27 is overexpressed in several types of cancer and
218 promotes tumor growth and metastasis. For example, Wang et al. confirmed that GPR27
219 expression was upregulated in HCC tissues and cell lines. They then used cellular experiments
220 to show that knockdown of GPR27 inhibited HCC cell proliferation, migration, and invasion.
221 Further analysis revealed that GPR27 acted through the MAPK/ERK pathway to promote HCC
222 progression [7]. Besides the classical downstream G-protein signaling, GPR27 can also
223 activate beta-arrestin-biased signaling pathways [6]. Meanwhile, beta-arrestins had been well-
224 acknowledged to be involved in malignancies including glioblastoma [12, 13]. Therefore, it's high

likely that GPR27 may modulate glioma progression via distinct signaling pathways in different cancer types. Furthermore, the identification of GPR27 as a potential therapeutic target for glioma treatment is of significant clinical relevance, as there is a dire need for novel therapeutic strategies for this devastating disease. Further studies will be necessary to further explore its functional mechanisms.

The present study provides important insights underlying glioma development and progression. The observed negative correlation between GPR27 and various immune cell types suggests that GPR27 may play a role in immune evasion by glioma cells, and further investigations into the mechanism underlying this association could help to develop effective immunotherapeutic strategies [14, 15]. Moreover, the identification of GPR27 as a potential therapeutic target could pave the way for the development of new drugs, which could offer a promising avenue for glioma treatment. However, more research is needed to fully understand the mechanisms underlying the role of GPR27 in glioma development and progression, as well as the potential therapeutic implications of targeting GPR27.

Our research has revealed notable connections between GPR27 and various immune cells, including pDC and macrophages. Specifically, data from Serena Pellegatta et al. indicate that administering pDC directly into tumors considerably extends survival in a murine model of glioma [16]. This study indicates that pDC within the tumor can enhance anti-cancer immune activity, primarily through modulating pro-immune cytokines, reducing Treg cells, and directly curbing tumor growth via TNF-α. Given the observed positive link between GPR27 and pDC presence, it's plausible that the interaction between GPR27 and pDC might contribute, at least in part, to GPR27's tumor-fighting properties. Conversely, our findings indicate an inverse relationship between GPR27 and macrophage presence, suggesting that lower GPR27 levels could be associated with increased macrophage presence within the tumor environment. This is supported by findings from Candice C. Poon, who observed that glioblastoma-associated macrophages, when co-cultured alongside glioblastoma stem cells, enhanced the growth of

glioblastoma cells [17]. This underscores the potential of macrophages within the glioma environment to facilitate tumor growth.

The implications of these findings could be far-reaching in the field of cancer immunotherapy. Given the crucial role that the tumor microenvironment plays in cancer progression [18], GPR27 could serve as a potential target for the development of novel cancer immunotherapies. By moduclating the expression of GPR27, it may be possible to adjust the immune response in the tumor microenvironment to promote an anti-tumor immune response. Alternatively, targeting GPR27 could be used to promote the differentiation and activation of immune cells that are positively associated with GPR27, such as pDC cells and TReg cells. Overall, these findings provide important insights into the role of GPR27 in the modulation of the immune response in the tumor microenvironment and could have significant implications for the development of novel cancer immunotherapies.

The study has several limitations, such as a restricted sample size, which may not be representative of the entire population. Moreover, the study's outcomes may not be generalized to other regions due to its geographic confinement. The self-reported data may also be influenced by potential biases. Furthermore, the study's cross-sectional design prevents establishing a cause-and-effect relationship between variables.

Conclusions

Taken together, our data highlights the potential clinical significance of GPR27 in gliomas. The findings indicate that GPR27 expression is negatively correlated with WHO grade. Additionally, lower GPR27 expression is associated with elder patients, higher-grade gliomas, and specimens with wild-type IDH status or non-codeletion of 1p/19q, and predicts worse prognosis. These results suggest that GPR27 may be a potential prognostic biomarker for glioma patients. However, further research is needed to fully understand the role of GPR27 in gliomas and to explore its potential as a therapeutic target.

Acknowledgement

None.

Figure legends

Figure 1. The correlation between GPR27 expression and disease status in glioma.

A) GPR27 expression was negatively correlated with WHO grade, with grade IV samples exhibiting the lowest GPR27 levels and grade II samples showing the highest levels (P<0.001).

B) Patients with IDH mutation showed higher GPR27 levels (P<0.001).

C) Similarly, patients with 1p/19q co-deletion exhibited higher GPR27 levels (P<0.001).

Figure 2. Prognostic significance of GPR27 on glioma patients.

A) Kaplan-Meier survival analysis of glioma patients based on GPR27 expression level. Patients with lower GPR27 level had significantly worse prognosis compared to those with higher GPR27 level (P<0.001).

B) A nomogram established based on the multivariate survival analysis. The variables in the nomogram included patients' age, gender, WHO grade, IDH status, 1p/19q co-deletion, and GPR27 expression level.

Figure 3. GPR27-overexpression inhibit glioma progression.

A) Western blot analysis confirmed the successful overexpression of GPR27 expression in U87 and U251 cell lines.

B) CCK-8 assay results indicated that overexpressing GPR27 significantly inhibited the proliferation of U87 cells compared to the control group.

C) CCK-8 assay results indicated that overexpressing GPR27 significantly inhibited the

proliferation of U251 cells compared to the control group.

Each experiment was repeated three times independently.

Figure 4. The correlation between immune cell infiltration and GPR27 expression in glioma.

A) Spearman analysis result showed the correlation between the infiltration of 24 types of immune cells and GPR27 expression in glioma tissues.

B) and C) showed representative infiltration level of pDC and macrophages in cells with different GPR27 level.

DC, dendritic cells; pDC, plasmacytoid DC; aDC, activated DC; iDCs, immature DCs; NK, natural killer cells; Tgd, T gamma delta; TReg, regulatory T cells; Tem, T effector memory; Tcm, T central memory; Th1 cells, type 1 Th cells; Th2 cells, type 2 Th cells; Th17 cells, type 17 Th cells; TFH, T follicular helper.

References

1. Omuro A, DeAngelis LM. Glioblastoma and other malignant gliomas: a clinical review. Jama. 2013;310(17):1842-50.
2. Wen PY, Reardon DA. Progress in glioma diagnosis, classification and treatment. Nature Reviews Neurology. 2016;12(2):69-70.
3. Melin BS, Barnholtz-Sloan JS, Wrensch MR, Johansen C, Il'Yasova D, Kinnersley B, et al. Genome-wide association study of glioma subtypes identifies specific differences in genetic susceptibility to glioblastoma and non-glioblastoma tumors. Nature genetics. 2017;49(5):789-94.
4. Pillaiyar T, Rosato F, Wozniak M, Blavier J, Charles M, Laschet C, et al. Structure-activity relationships of agonists for the orphan G protein-coupled receptor GPR27. European Journal of Medicinal Chemistry. 2021;225:113777.
5. Chopra DG, Yiv N, Hennings TG, Zhang Y, Ku GM. Deletion of Gpr27 in vivo reduces insulin mRNA but does not result in diabetes. Scientific reports. 2020;10(1):1-9.
6. Dupuis N, Laschet C, Franssen D, Szpakowska M, Gilissen J, Geubelle P, et al. Activation of the orphan G protein–coupled receptor GPR27 by surrogate ligands promotes β-arrestin 2 recruitment. Molecular Pharmacology. 2017;91(6):595-608.
7. Wang H, Du D, Huang J, Wang S, He X, Yuan S, et al. GPR27 Regulates Hepatocellular Carcinoma Progression via MAPK/ERK Pathway. Cancer Management and Research. 2022:1165-77.
8. Milioli HH, Tishchenko I, Riveros C, Berretta R, Moscato P. Basal-like breast cancer: molecular profiles, clinical features and survival outcomes. BMC medical genomics. 2017;10:1-17.
9. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular Profiling Reveals Biologically Discrete Subsets and Pathways of Progression in Diffuse Glioma. Cell. 2016;164(3):550-63. doi: 10.1016/j.cell.2015.12.028.
10. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC bioinformatics. 2013;14:1-15.
11. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782-95.
12. Lan T, Wang H, Zhang Z, Zhang M, Qu Y, Zhao Z, et al. Downregulation of β-arrestin 1 suppresses glioblastoma cell malignant progression vis inhibition of Src signaling. Experimental cell research. 2017;357(1):51-8.
13. Bae W-Y, Choi J-S, Nam S, Jeong J-W. β-arrestin 2 stimulates degradation of HIF-1α and modulates tumor progression of glioblastoma. Cell Death & Differentiation. 2021;28(11):3092-104.
14. Njonkou R, Jackson CM, Woodworth GF, Hersh DS. Pediatric glioblastoma: mechanisms of immune evasion and potential therapeutic opportunities. Cancer Immunology, Immunotherapy. 2022;71(8):1813-22.
15. Razavi S-M, Lee KE, Jin BE, Aujla PS, Gholamin S, Li G. Immune evasion strategies of glioblastoma. Frontiers in surgery. 2016;3:11.
16. Pellegatta S, Poliani PL, Stucchi E, Corno D, Colombo CA, Orzan F, et al. Intra-tumoral dendritic cells increase efficacy of peripheral vaccination by modulation of glioma microenvironment. Neuro-Oncology. 2010;12(4):377-88. doi: 10.1093/neuonc/nop024.
17. Poon CC, Sarkar S, Yong VW, Kelly JJP. Glioblastoma-associated microglia and macrophages: targets for therapies to improve prognosis. Brain. 2017;140(6):1548-60. doi: 10.1093/brain/aww355.
18. Barthel L, Hadamitzky M, Dammann P, Schedlowski M, Sure U, Thakur BK, et al. Glioma: molecular signature and crossroads with tumor microenvironment. Cancer and Metastasis Reviews. 2022:1-23.

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Table 1(on next page)

Table 1. Basic information of enrolled glioma patients.

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Characteristics	Low GPR27	High GPR27	P value
Total cases, n	349	350	
Age, n (%)			< 0.001***
≤ 60 years old	245 (35.1%)	311 (44.5%)	
> 60 years old	104 (14.9%)	39 (5.6%)	
Gender, n (%)			0.134
Female	139 (19.9%)	159 (22.7%)	
Male	210 (30%)	191 (27.3%)	
WHO grade, n (%)			< 0.001***
G2	67 (10.5%)	157 (24.6%)	
G3	119 (18.7%)	126 (19.8%)	
G4	141 (22.1%)	27 (4.2%)	
IDH status, n (%)			< 0.001***
WT	189 (27.4%)	57 (8.3%)	
Mutation	152 (22.1%)	291 (42.2%)	
1p/19q codeletion, n (%)			< 0.001***
Non-codel	328 (47.4%)	192 (27.7%)	
Codel	14 (2%)	158 (22.8%)	
OS event, n (%)			< 0.001***
Alive	162 (23.2%)	265 (37.9%)	
Dead	187 (26.8%)	85 (12.2%)	

Table 2(on next page)

Table 2. Univariate and multivariate Cox regression analyses of the overall survival of glioma patients.

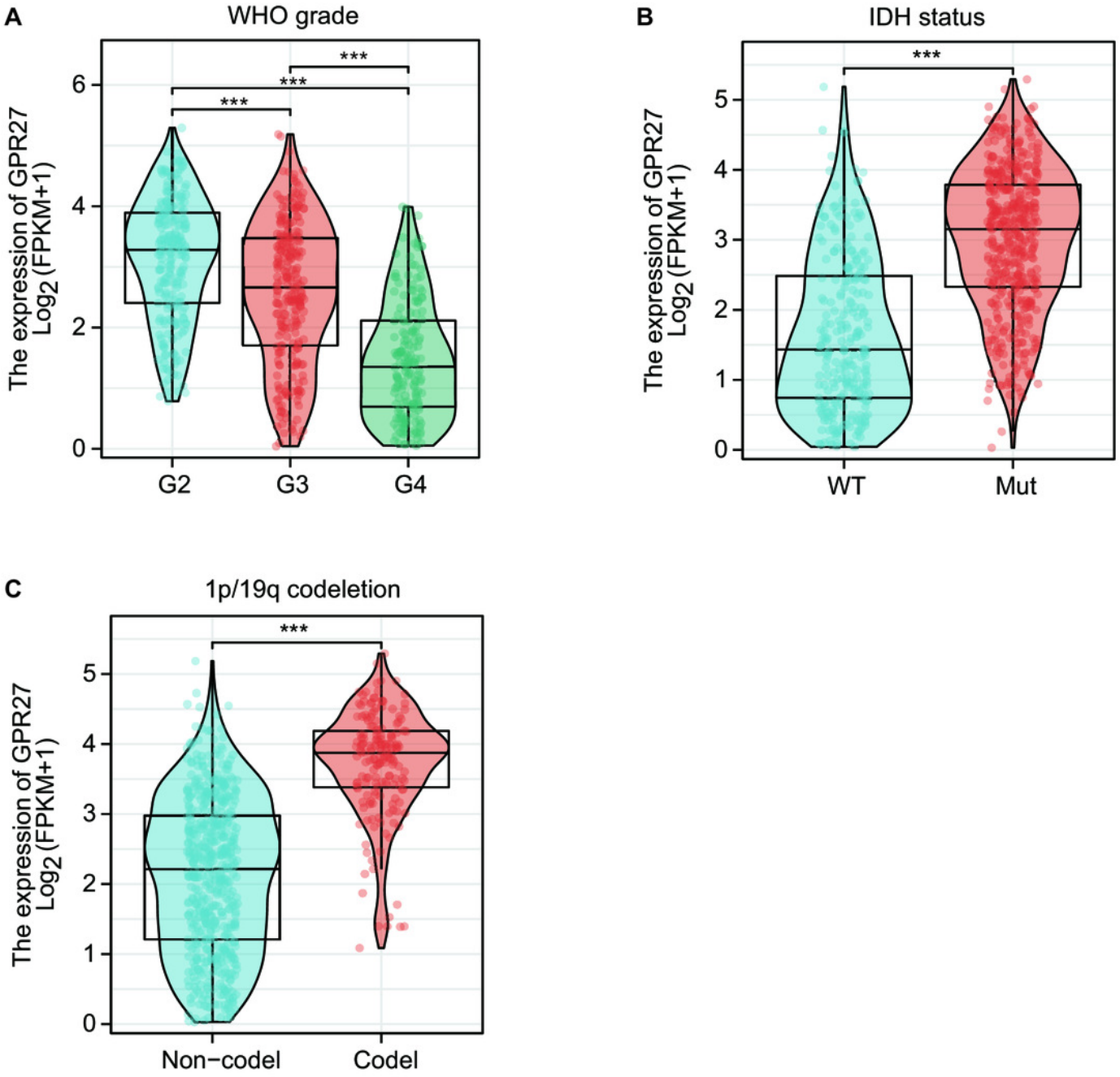
Table 2. Univariate and multivariate Cox regression analyses of the overall survival of glioma patients.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age	698				
≤ 60 years old	555	Reference		Reference	
> 60 years old	143	4.696 (3.620 - 6.093)	< 0.001***	1.429 (1.046 - 1.952)	0.025*
Gender	698				
Female	297	Reference		Reference	
Male	401	1.250 (0.979 - 1.595)	0.073	1.185 (0.903 - 1.553)	0.220
WHO grade	636				
G2	223	Reference		Reference	
G3	245	2.967 (1.986 - 4.433)	< 0.001***	1.873 (1.223 - 2.868)	0.004**
G4	168	18.600 (12.448 - 27.794)	< 0.001***	4.253 (2.525 - 7.162)	< 0.001***
IDH status	688				
WT	246	Reference		Reference	
Mutation	442	0.116 (0.089 - 0.151)	< 0.001***	0.265 (0.176 - 0.399)	< 0.001***
1p/19q codeletion	691				
Non-codel	520	Reference		Reference	
Codel	171	0.225 (0.147 - 0.346)	< 0.001***	0.843 (0.494 - 1.440)	0.533
GPR27	698				
Low	349	Reference		Reference	
High	349	0.326 (0.251 - 0.423)	< 0.001***	0.679 (0.486 - 0.947)	0.023*

Figure 1

Figure 1. The correlation between GPR27 expression and disease status in glioma.

A) GPR27 expression was negatively correlated with WHO grade, with grade IV samples exhibiting the lowest GPR27 levels and grade II samples showing the highest levels ($P<0.001$). B) Patients with IDH mutation showed higher GPR27 levels ($P<0.001$). C) Similarly, patients with 1p/19q co-deletion exhibited higher GPR27 levels ($P<0.001$).


A
WHO grade
The expression of GPR27
Log2 (FPKM+1)
***
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IDH status
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1p/19q codeletion
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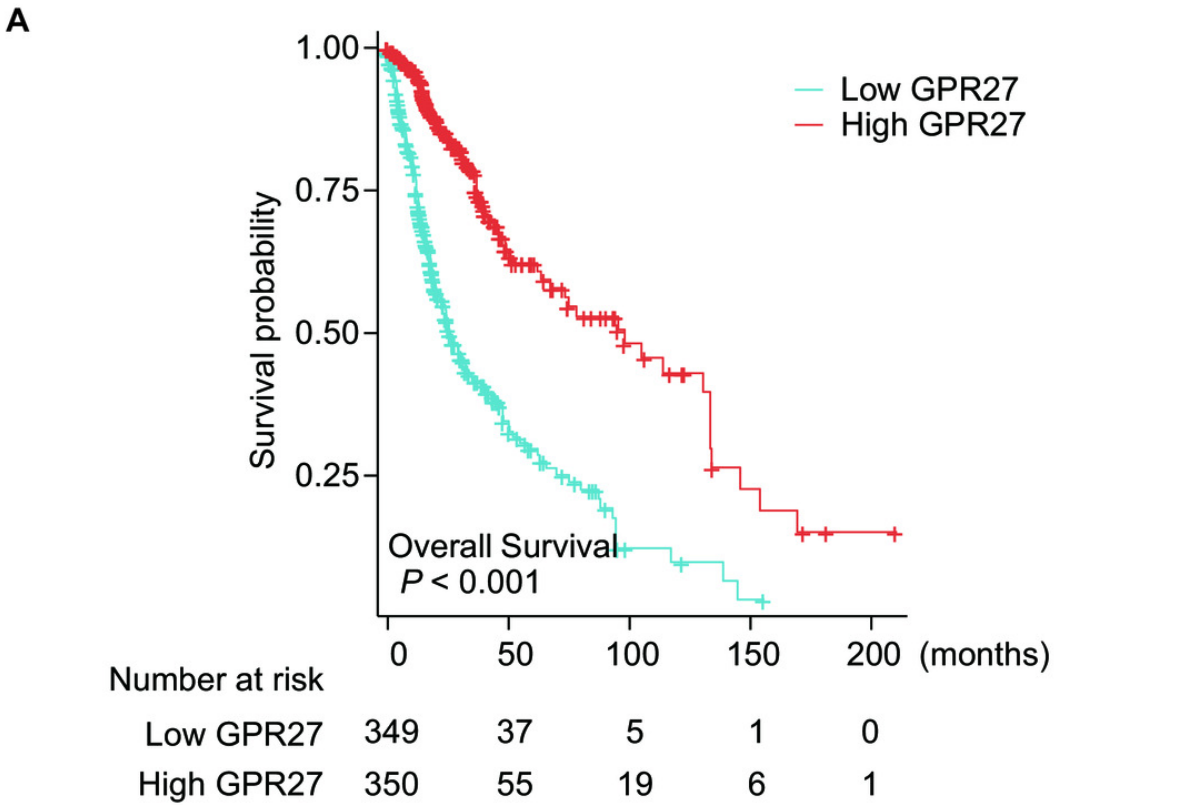
Non−codel
Codel

Figure 2

Figure 2. Prognostic significance of GPR27 on glioma patients.

A) Kaplan-Meier survival analysis of glioma patients based on GPR27 expression level. Patients with lower GPR27 level had significantly worse prognosis compared to those with higher GPR27 level ($P<0.001$). B) A nomogram established based on the multivariate survival analysis. The variables in the nomogram included patients' age, gender, WHO grade, IDH status, 1p/19q co-deletion, and GPR27 expression level.


A
1.00
0.75
0.50
0.25
Survival probability
Low GPR27
High GPR27
Overall Survival
P < 0.001
0
50
100
150
200
(months)
Number at risk
Low GPR27 349 37 5 1 0
High GPR27 350 55 19 6 1

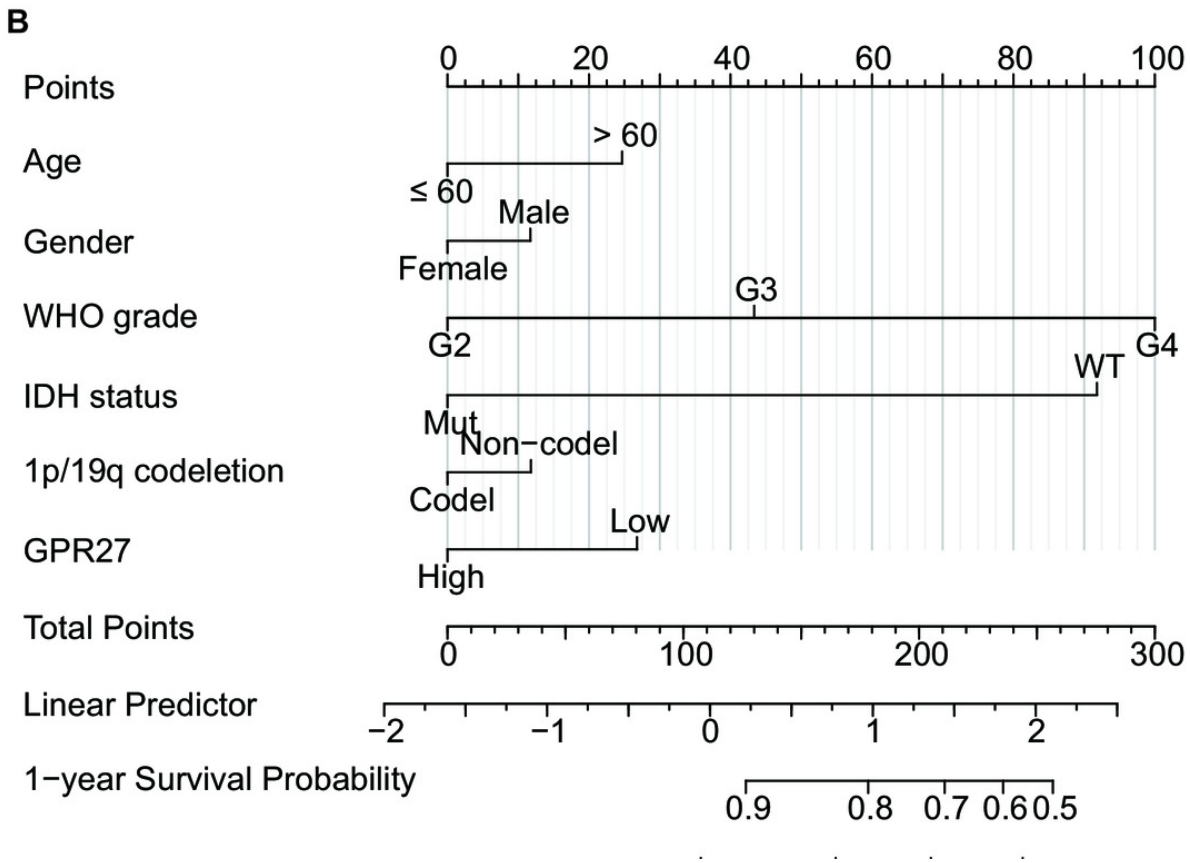

B
Points
0
20
40
60
80
100
Age
> 60
≤ 60
Gender
Male
Female
WHO grade
G3
G2
G4
IDH status
WT
Mut
1p/19q codeletion
Non−codel
Codel
GPR27
Low
High
Total Points
0
100
200
300
Linear Predictor
−2
−1
0
1
2
1−year Survival Probability
0.9
0.8
0.7
0.6
0.5

Figure 3

Figure 3. GPR27-overexpression inhibit glioma progression.

A) Western blot analysis confirmed the successful overexpression of GPR27 expression in U87 and U251 cell lines. B) CCK-8 assay results indicated that overexpressing GPR27 significantly inhibited the proliferation of U87 cells compared to the control group. C) CCK-8 assay results indicated that overexpressing GPR27 significantly inhibited the proliferation of U251 cells compared to the control group. Each experiment was repeated three times independently.

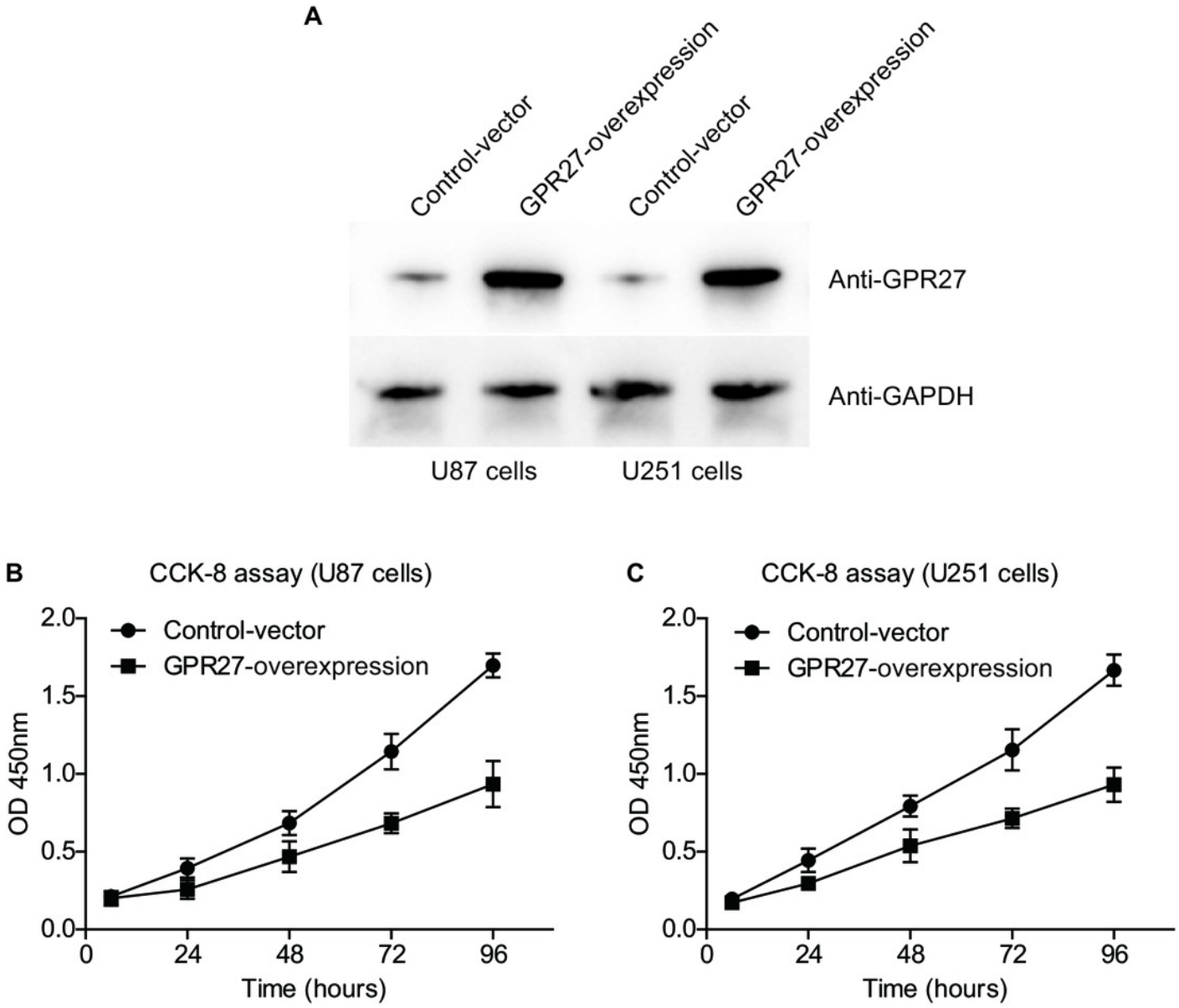
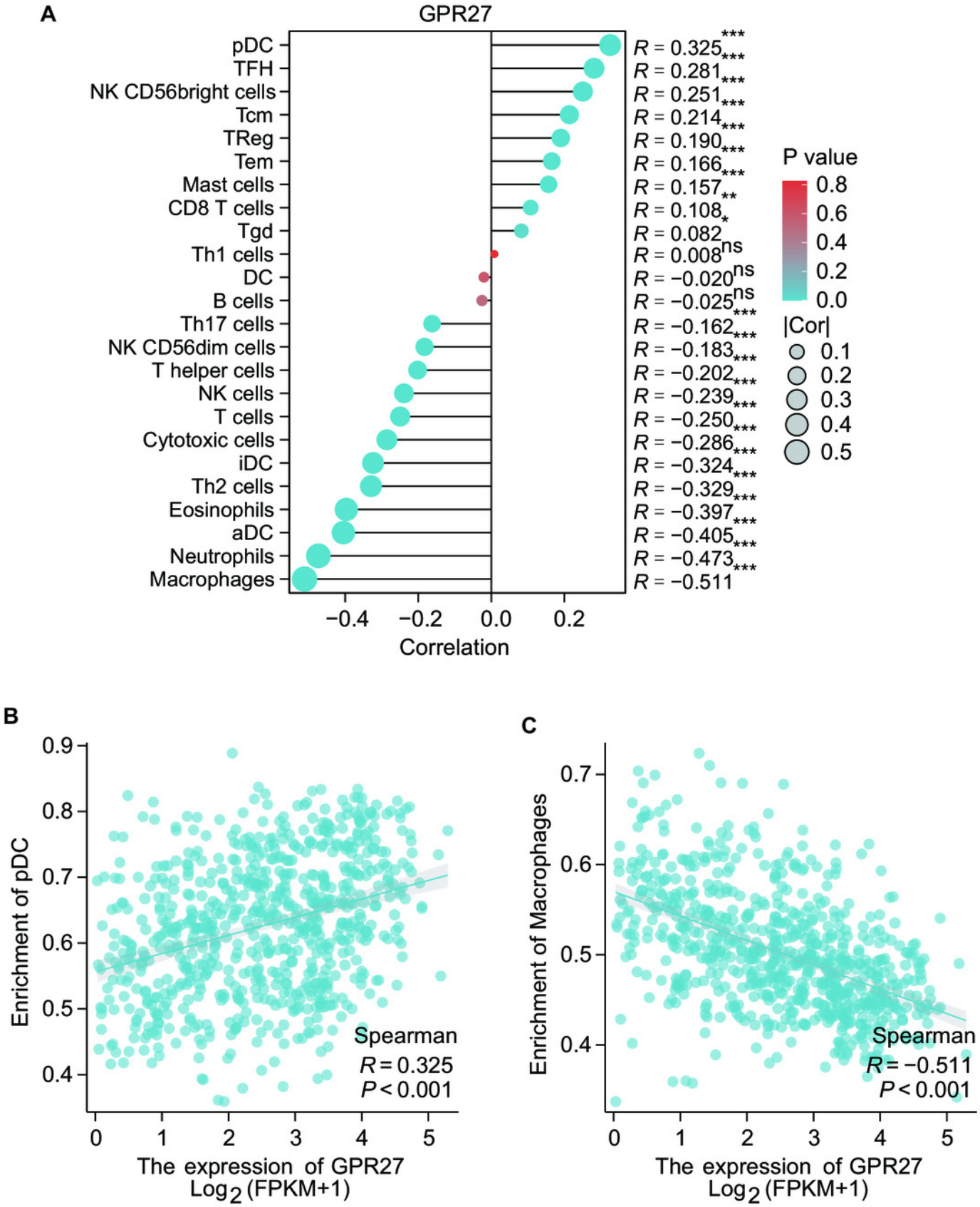

A
Control-vector
GPR27-overexpression
Control-vector
GPR27-overexpression
Anti-GPR27
Anti-GAPDH
U87 cells
U251 cells
B
CCK-8 assay (U87 cells)
Control-vector
GPR27-overexpression
OD 450nm
Time (hours)
C
CCK-8 assay (U251 cells)
Control-vector
GPR27-overexpression
OD 450nm
Time (hours)

Figure 4

Figure 4. The correlation between immune cell infiltration and GPR27 expression in glioma.

A)Spearman analysis result showed the correlation between the infiltration of 24 types of immune cells and GPR27 expression in glioma tissues. B) and C) showed representative infiltration level ofpDC and macrophages in cells with different GPR27 level. DC, dendritic cells; pDC, plasmacytoid DC; aDC, activated DC; iDCs, immature DCs; NK, natural killer cells; Tgd, T gamma delta; TReg, regulatory T cells; Tem, T effector memory; Tcm, T central memory; Th1 cells, type 1 Th cells; Th2 cells, type 2 Th cells; Th17 cells, type 17 Th cells; TFH, T follicular helper.


A
GPR27
pDC
TFH
NK CD56bright cells
Tcm
TReg
Tem
Mast cells
CD8 T cells
Tgd
Th1 cells
DC
B cells
Th17 cells
NK CD56dim cells
T helper cells
NK cells
T cells
Cytotoxic cells
iDC
Th2 cells
Eosinophils
aDC
Neutrophils
Macrophages
R = 0.325***
R = 0.281***
R = 0.251***
R = 0.214***
R = 0.190***
R = 0.166***
R = 0.157**
R = 0.108*
R = 0.082ns
R = 0.008ns
R = −0.020ns
R = −0.025***
R = −0.162***
R = −0.183***
R = −0.202***
R = −0.239***
R = −0.250***
R = −0.286***
R = −0.324***
R = −0.329***
R = −0.397***
R = −0.405***
R = −0.473***
R = −0.511
−0.4
−0.2
0.0
0.2
Correlation
P value
0.8
0.6
0.4
0.2
0.0
|Cor|
0.1
0.2
0.3
0.4
0.5
B
Enrichment of pDC
0.9
0.8
0.7
0.6
0.5
0.4
Spearman
R = 0.325
P < 0.001
0
1
2
3
4
5
The expression of GPR27
Log2 (FPKM+1)
C
Enrichment of Macrophages
0.7
0.6
0.5
0.4
Spearman
R = −0.511
P < 0.001
0
1
2
3
4
5
The expression of GPR27
Log2 (FPKM+1)