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GPR116 overexpression correlates with poor prognosis in gastric cancer

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Abstract

The G protein-coupled receptor 116 (GPR116) is an adhesion subtype of the G protein-coupled receptor family and has been reported to be involved in tumorigenesis and cancer progression. Moreover, it has been shown to predict poor prognosis in breast and colorectal cancers. However, little is known about the role of GPR116 in gastric cancer (GC). In this study, we aimed to investigate the expression and clinical prognostic significance of GPR116 in GC.The mRNA expression levels of GPR116 in GC were analyzed using Gene Expression Omnibus and UALCAN databases, and GPR116 protein expression in GC tissues was detected using immunohistochemistry. The relationship between GPR116 expression and prognosis in patients with GC was analyzed and further validated using the Kaplan–Meier Plotter database. The correlation between GPR116 and the differentially expressed genes identified was analyzed using the LinkedOmics database. Gene set enrichment analysis was performed using WebGestalt. The results revealed that GPR116 expression was significantly upregulated in GC tissues, which was positively correlated with tumor node metastasis (TNM) staging and tumor invasion. Prognostic analysis suggested that high GPR116 expression contributed to poor overall survival in GC patients. Multivariate Cox analysis indicated that GPR116 overexpression was an independent prognostic indicator in patients with GC (HR = 1.855, 95% CI 1.021–3.370, $P = .043$). Enrichment analysis showed that GPR116 co-expression genes were mainly involved in extracellular matrix-receptor interaction, focal adhesion, cell adhesion, PI3K-Akt signaling, DNA replication, and cell cycle pathways. In conclusion, GPR116 was highly expressed in GC tissues and associated with poor prognosis in patients with GC, Thus GPR116 may be a novel prognostic marker and a potential therapeutic target for GC treatment.

Abbreviations: GC = gastric cancer, GEO = Gene Expression Omnibus, GPR116 = G protein-coupled receptor 116, KEGG = Kyoto encyclopedia of genes and genome, STAD = stomach adenocarcinoma, TNM = tumor-node-metastasis.

Keywords: bioinformatics analysis, gastric cancer, GPR116, immunohistochemistry, prognosis, signal pathway

1. Introduction

Gastric cancer (GC) is one of the most common malignancies, and it has a poor prognosis.[1] Despite improvements in treatment techniques, the 5-year survival of patients with GC is still <30%, with most patients experiencing metastases at the time of diagnosis.[2,3] Therefore, identifying reliable prognostic biomarkers for early diagnosis and prognostic assessment is the need of the hour.

G protein-coupled receptors (GPCRs) are the largest superfamily of proteins in biological cell membranes;they are coupled with G proteins to produce biological effects. Due to the involvement of GPCRs in processes, such as cell adhesion and motility, a large part of their function in tumors is related to the movement and migration of tumor cells, which is closely related to tumor progression and metastasis.[4–7] G protein-coupled receptor 116 (GPR116) is a member of the adhesion subtype of the G protein-coupled receptor family and is widely distributed in embryonic cells, germ cells, leukocytes, neurons, and tumor cells.[8,9] As its extracellular terminal contains an adhesive protein domain, GPR116 is considered to be associated with cell motility and cell recognition.[10] Some studies have revealed that GPR116 is highly expressed in breast and colorectal cancers and closely related to tumor invasion and metastasis.[5,10] However, little is known about the role of GPR116 in GC. Thus, in this study, we used bioinformatics analysis combined with clinical data to explore the relationship between GPR116 expression and GC progression.

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2. Methods

2.1. Patients and tissue specimens

Human GC tissue microarrays containing 80 pairs of primary GC and corresponding para-cancerous tissues were collected from Fujian Provincial Hospital from July 2010 to April 2011. Specimens were included in this study if

1. primary focus specimens were GC and all specimens were confirmed by two pathologists;
2. no radiotherapy/chemotherapy was performed before the operation;
3. complete clinical/pathological and follow-up data were available;
4. the cause of death was tumor recurrence or metastasis only.

The study was approved by the Ethics Committee of Fujian Provincial Hospital, and written informed consent was obtained from all patients. The follow-up time was from the operation date to GC-related death time, while the last follow-up time (July 31, 2019) was recorded for non-deceased patients. Histological grading and clinical staging were based on the tumor-node-metastasis (TNM) staging criteria defined by the American Joint Committee on Cancer (version 7).[11]

2.2. Immunohistochemical staining and scoring

Immunohistochemical staining was performed on 80 paired GC tissues and corresponding para-cancerous tissues. Tissue microarray sections were de-waxed in xylene and rehydrated with different concentrations of ethanol. The sections were later treated with 3% hydrogen peroxide, followed by retrieval of antigen with 10 mmol/L citric acid buffer (pH 6.0) using a microwave. After blocking with 10% goat serum for 30 min, the sections were incubated overnight at 4°C with rabbit anti-human GPR116 antibodies (1:100, Proteintech Group, Rosemont, IL), followed by peroxidase-labeled secondary antibody. Positive cells were identified by the presence of brownish granules in the cell pulp.

The scoring was based on the percentage of positive cells (0–5%: 0; 6–35%: 1; 36–70%: 2; >70%: 3). Staining intensity was scored as follows: negative staining, 0 points; weak staining, 1 point; medium staining, 2 points; and strong staining, 3 points. The final score was the percentage of positive cells × staining intensity score[5] (score 0–1: "−"; score 2–4: "+"; score 5–6: "++"; score >6: "+++"). A final score <6 was designated as low GPR116 expression, and a final score ≥6 was designated as high expression. Two pathologists estimated the results in a double-blind manner.

2.3. Gene expression omnibus (GEO) database analysis

Three GC gene expression profile datasets (GSE54129,[12] GSE65801,[13] and GSE63089[14]) containing 188 GC tissues and 98 normal gastric tissues were downloaded from GEO.[15] GPR116 mRNA expression was analyzed using the online tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/).

2.4. UALCAN analysis

The UALCAN database (http://ualcan.path.uab.edu)[16] was used to analyze GPR116 mRNA expression in normal gastric tissues and GC tissues, as well as in different subgroups according to sex, age, race, tumor stage, tumor grade, and lymph node metastasis.

2.5. Kaplan–Meier plotter analysis

The relationship between GPR116 expression and GC prognosis was assessed using the Kaplan–Meier Plotter database (https://kmplot.com/).[17,18]

2.6. LinkedOmics analysis

Differentially expressed genes from TCGA co-expressed with GPR116 in stomach adenocarcinoma (STAD) were analyzed using LinkFinder, an analysis module of the LinkedOmics database (http://www.linkedomics.org/login).[19] Gene set enrichment analysis was performed using the online tool WebGestalt in the LinkInterpreter module to obtain the gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of co-expression genes. The rank standard referred to a false detection rate <0.05 among 1000 simulations.

2.7. Statistical analysis

Statistical analysis was performed using SPSS (version 17.0; SPSS Inc, Chicago, IL). Correlation analysis and comparison between groups were performed using the Chi-square test or Fisher's exact test. The relationship between GPR116 and co-expression genes was evaluated using the Pearson correlation analysis. The Kaplan–Meier method was used to analyze the overall and subgroup survival. Differences between survival curves were calculated using

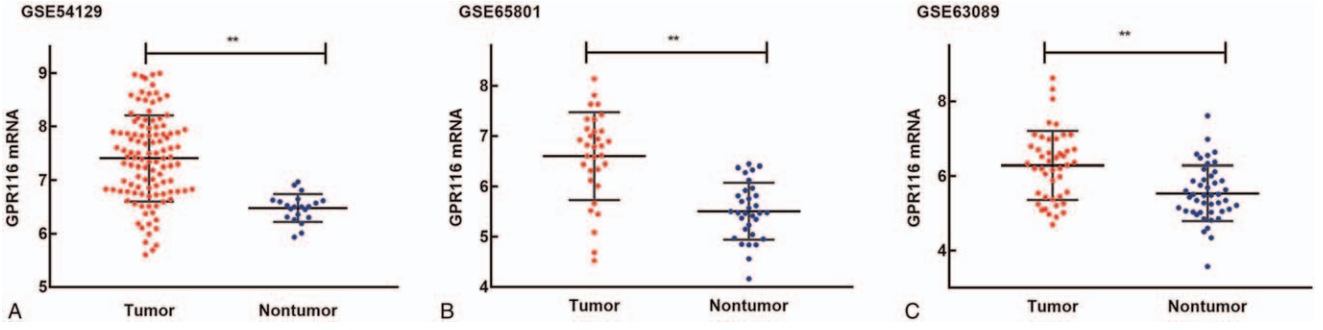

Figure 1. GPR116 mRNA expression levels between tumor and nontumor tissues in gastric cancer patients based on the Gene Expression Omnibus database. The expression of GPR116 in three datasets including GSE54129 (A), GSE65801 (B), and GSE63089 (C) are shown. GPR116: G protein-coupled receptor 116. $^{**}P < .01$.

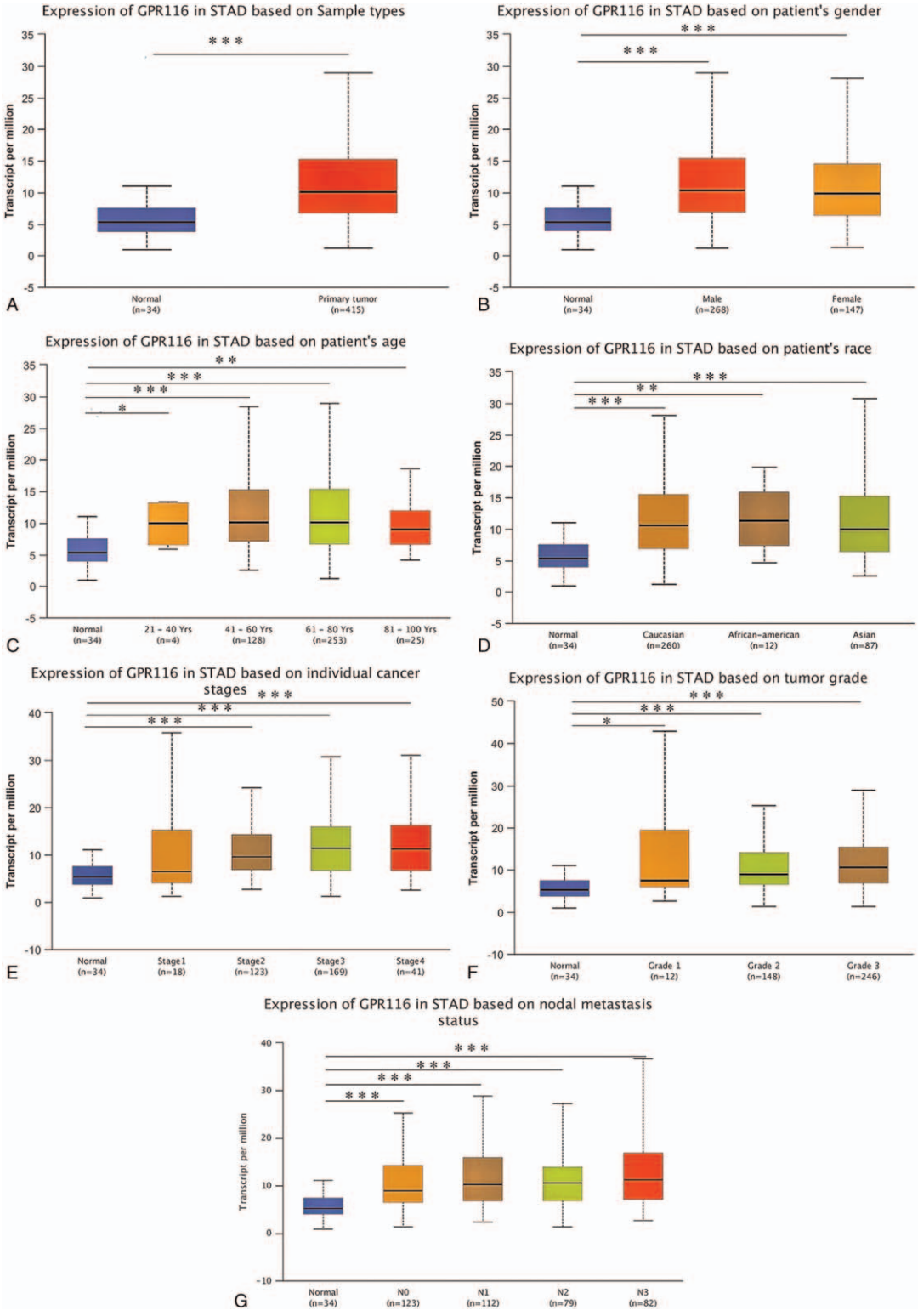

Figure 2. GPR116 transcription in subgroups of patients with STAD based on the UALCAN database. The boxplot shows the relative expression of GPR116 (A) in normal and STAD samples; (B) in normal individuals of either gender or in male or female STAD patients; (C) in normal individuals of any age or in STAD patients aged 21–40, 41–60, 61–80, or 81–100 years, respectively; (D) in normal individuals of any ethnicity or in STAD patients of Caucasian, African American, or Asian ethnicity, respectively; (E) in normal individuals or in STAD patients in stages 1, 2, 3, or 4, respectively; (F) in normal individuals or in STAD patients with grade 1, 2, or 3 tumors, respectively; (G) in normal individuals or in STAD patients with nodal metastasis status N0, N1, N2, or N3, respectively. Data are presented as mean ± standard error. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. GPR116: G protein-coupled receptor 116; STAD: stomach adenocarcinoma.

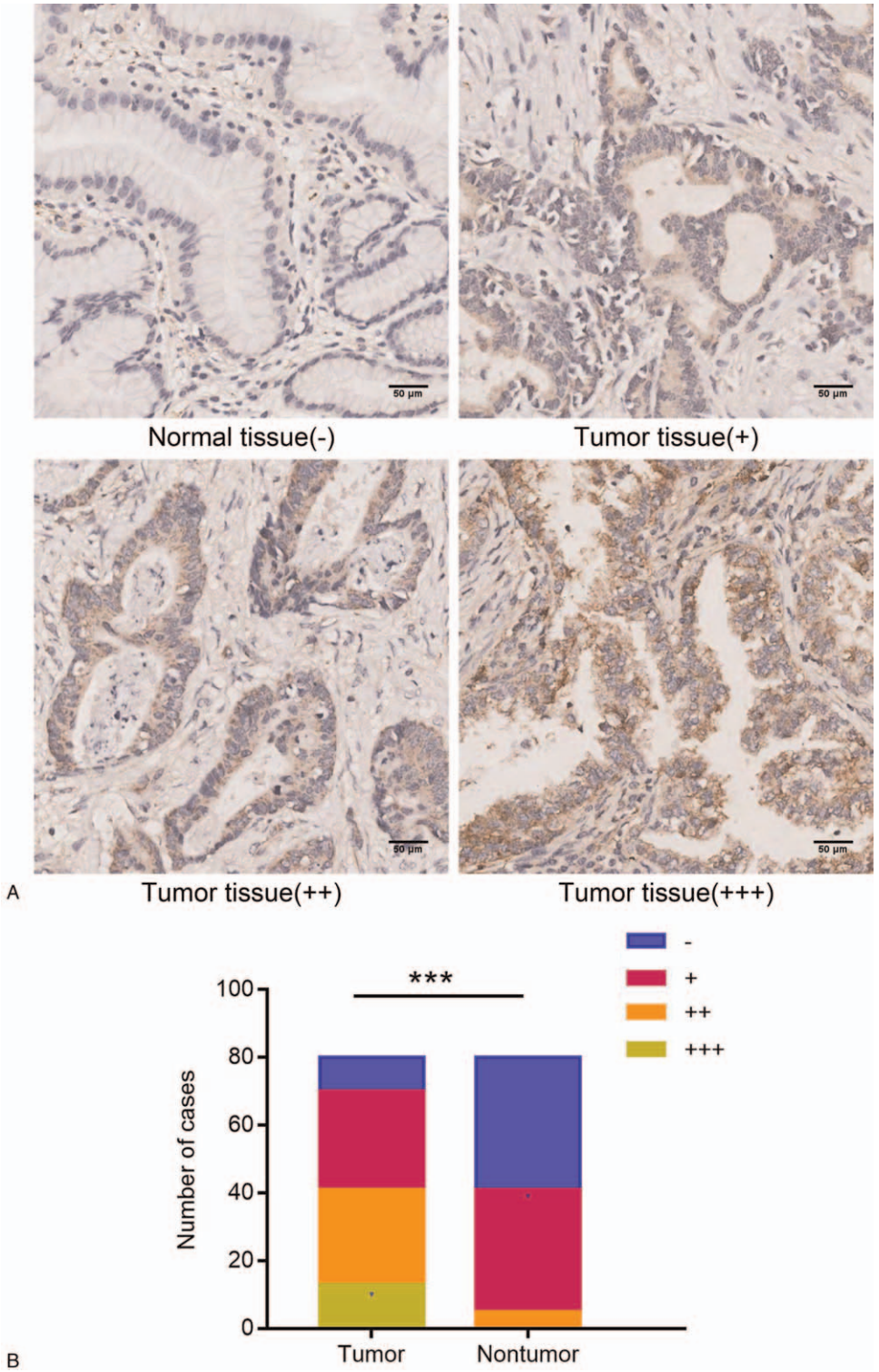

Figure 3. GPR116 expression in gastric cancer tissue samples. (A) GPR116 protein level was measured by immunohistochemical analysis in normal gastric and gastric cancer tissues. Original magnification × 100 (bar = 200 μm), 200 (bar = 100 μm), 400 (bar = 50 μm). (B) The numbers of different immunohistochemical grade expression in gastric cancer tissue and normal gastric tissue. GPR116: G protein-coupled receptor 116. $^{***}P < .001$.

the log-rank test. Prognostic indicators were identified with univariate and multivariate analyses using a Cox proportional hazard regression model. $P < .05$ was considered statistically significant.

3. Results

3.1. GEO database analysis suggested significantly higher expression of GPR116 in GC tissues

Analysis of three microarray datasets from the GEO database suggested that GPR116 expression was significantly higher in GC tissues than that in normal tissues (Fig. 1A–C). Based on the UALCAN analysis results, we also observed GPR116 upregulation in GC tissues (Fig. 2A). Furthermore, subgroup analyses found that GPR116 expression in GC tissues of each subgroup was higher than that in normal tissues (Fig. 2B–G).

3.2. Immunohistochemistry showed significant GPR116 upregulation in GC tissues

Immunohistochemical staining showed that GPR116 was mainly expressed in the cytoplasm. In GC tissues, 41 of 80 (51.25%) specimens showed higher GPR116 expression (GPR116 ++ or +++), while the remaining 39 (48.75%) displayed lower GPR116 expression (GPR116 – or +).Only 5 of 80 (6.25%) specimens showeda "++" GPR116 expression in para-cancerous tissues. GPR116 protein expression was significantly higher in GC tissues than that in para-cancerous tissues ($P < .001$, Fig. 3A and B).

3.3. Correlation between GPR116 protein expression and clinicopathological characteristics of patients with GC

The clinicopathological characteristics of the 80 patients with GC are shown in Table 1. Based on the median value of 6 as the final scoring for GPR116, 41 (51.25%) out of 80 patients with GC were assigned to the high GPR116 expression group and 39 patients (48.75%) were assigned to the low GPR116 expression group. Furthermore, GPR116 protein overexpression was positively correlated with the TNM stage ($P = .045$) and tumor invasion ($P = .007$). However, there was no significant association between GPR116 expression and other clinical factors, such as age, sex, tumor location, tumor size, differentiation, lymph node metastasis, distant metastasis, and vascular invasion ($P > .05$, Table 1).

3.4. The relationship between GPR116 expression and GC prognosis

Overall survival time in the high GPR116 expression group was 42.31 ± 37.82 months, shorter than the 64.11 ± 35.48 months in the low expression group ($P = .038$, Fig. 4A). Further subgroup analysis revealed that high GPR116 expression also indicated a shorter overall survival in patients who were younger than 65 years ($P = .028$), with tumor invasion of T1-2 ($P = .001$), lymph node metastasis ($P = .018$), no distant metastasis ($P = .011$), and no vascular invasion ($P = .011$) (Fig. 4B–F). To enhance the reliability of this result, we analyzed the Kaplan–Meier Plotter database and obtained consistent results (Fig. 5A). The results suggested that high GPR116 expression was a prognostic factor for 1-, 3-, and 5-year overall survival in patients with GC (HR = 1.74, log-rank $P = .041$; HR = 1.51, log-rank $P = .039$; HR = 1.53, log-rank $P = .021$, respectively, Fig. 5B–D).

Independent prognostic factors in patients with GC were identified using the Cox regression proportional hazard model. Univariate analysis showed that GPR116 expression level ($P = .01$), age ($P = .015$), TNM stage ($P = .006$), tumor invasion ($P = .043$), lymph node metastasis ($P = .034$), distant metastasis ($P = .004$), and vascular invasion ($P = .002$) were significantly correlated with overall survival. Furthermore, multivariate Cox analysis confirmed that GPR116 expression level (HR = 1.855, $P = .043$), age (HR = 2.370, $P = .007$), TNM stage (HR = 2.460, $P = .048$), distant metastasis (HR = 4.055, $P = .007$), and vascular invasion (HR = 2.547, $P = .01$) were independent prognostic factors in patients with GC (Table 2).

3.5. Differentially expressed genes that co-expressed with GPR116 in patients with GC

Genes co-expressed with GPR116 were analyzed using LinkedOmics in 415 patients with GG from TCGA database. The volcano plot (Fig. 6A) revealed all genes related to GPR116. The top 50 significant genes positively or negatively correlated with GPR116 expression are shown in B and C, respectively. ELTD1, CD93, and CDH5 were the top 3 genes positively related to GPR116 expression (Fig. 7A-C), while PGAP2, POLD2, and

Table 1

Correlation between GPR116 expression and clinicopathologic features in patients with gastric carcinoma.

Characteristics	Total (n = 80)	GPR116 expression		P
		High (n = 41)	Low (n = 39)	
Gender				.059
Male	49	21	28	
Female	31	20	11	
Age (years)				.273
<65	34	15	19	
≥65	46	26	20	
Tumor location				.882
Cardia/gastric fundus	11	5	6	
Gastric body	26	13	13	
Gastric angle/antrum	43	23	20	
Size				.987
<5 cm	43	22	21	
≥5 cm	37	19	18	
Differentiation				.383
Well/moderate	23	10	13	
Not/poor	57	31	26	
TNM stage				.045
I–II	34	13	21	
III–IV	46	28	18	
Tumor invasion				.007
T1-2	15	3	12	
T3-4	65	38	27	
Lymph node metastasis				.160
N0	21	8	13	
N1-3	59	33	26	
Distant metastasis				.686
M0	75	38	37	
M1	5	3	2	
Vascular invasion				.838
Absent	67	34	33	
Present	13	7	6	

GPR116 = G protein-coupled receptor116, TNM = tumor-node-metastasis.

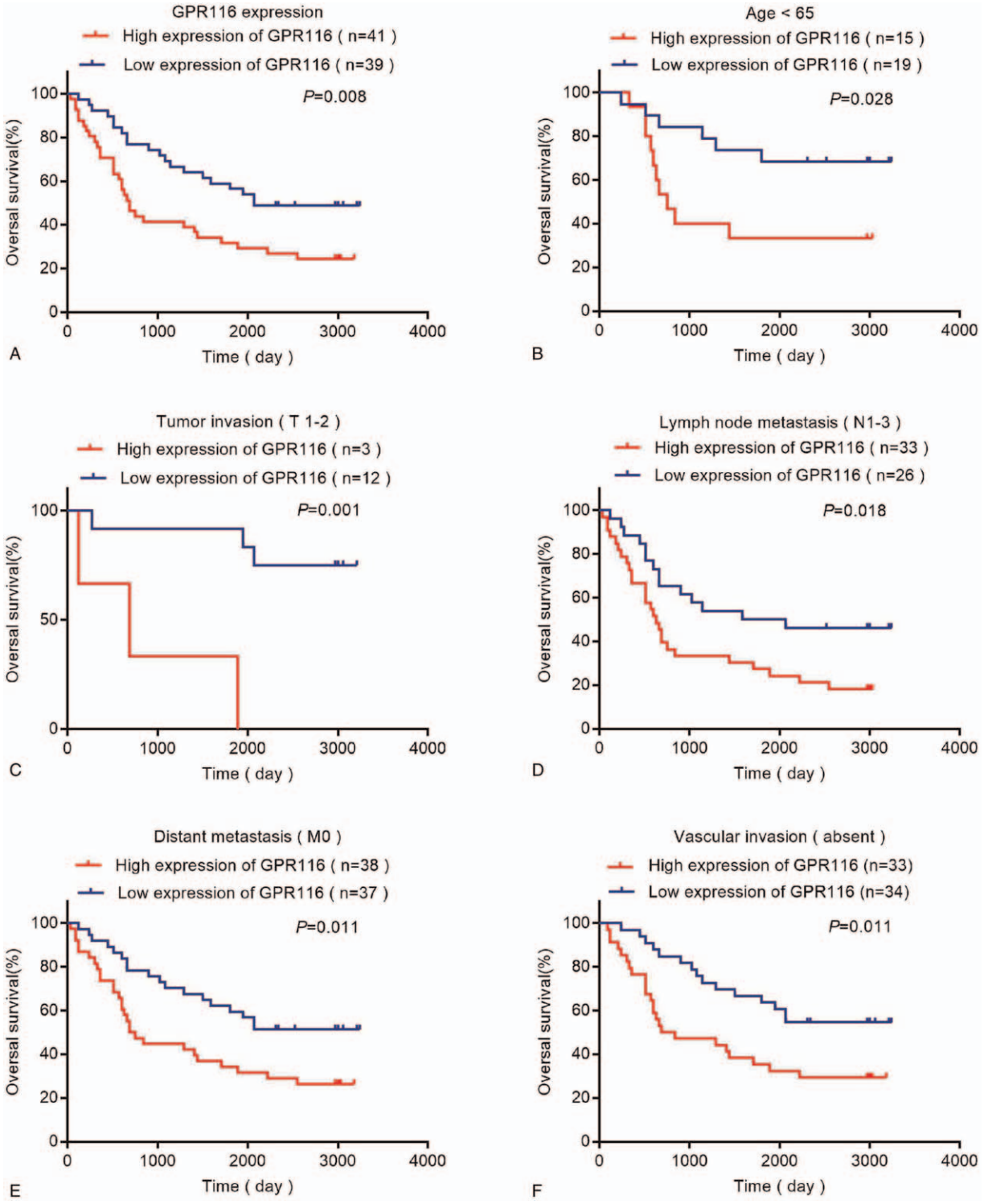

Figure 4. Kaplan-Meier survival analysis.Comparisons of overall survival between the high GPR116 expression group and low expression group in 80 gastric cancer patients (A); with age <65 years old (B); with tumor invasion I–II (C); with lymph node metastasis (D); without distant metastasis (E); and without vascular invasion (F). *P*-values were calculated by Log-rank test. GPR116: G protein-coupled receptor 116.

SNRPA were the top 3 genes negatively related to GPR116 expression (Fig. 7D–F).

Gene set enrichment analysis was used to analyze significantly enriched gene ontology annotation. Differentially expressed genes associated with GPR116 were mainly involved in biological processes, such as biological regulation, metabolic processes, responses to stimuli, and cellular communication (Fig. 8A). These genes were located mainly in the cell membrane, nucleus,

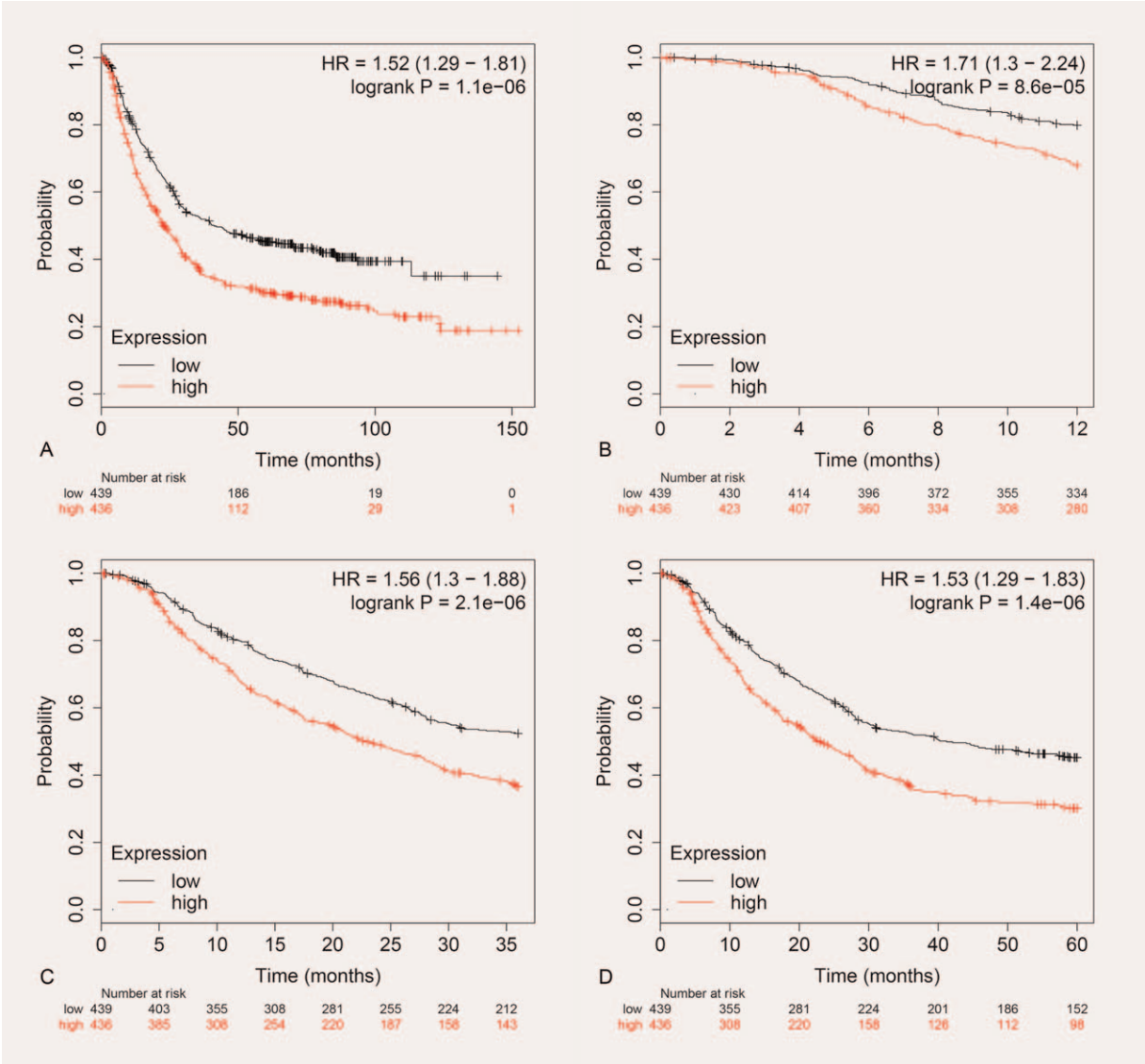

Figure 5. Overall survival analysis in gastric cancer patients with different GPR116 protein expression assessed by Kaplan–Meier plotter. Total (A), 1-year (B), 3-year (C) and 5-year (D) overall survival was compared between high and low GPR116 expression groups. *P*-values were calculated by Log-rank test. GPR116: G protein-coupled receptor 116.

endomembrane system, cytoplasm, and protein-containing complexes (Fig. 8B), participating primarily in protein and ion binding, nucleic acid binding, and hydrolase and transferase activities (Fig. 8C). KEGG pathway enrichment analysis revealed enrichment in the extracellular matrix-receptor interaction, adhesion plaque, cell adhesion, PI3K-Akt signaling, DNA replication, and cell cycle pathways (Fig. 8D).

4. Discussion

This study was the first to report the role of GPR116 in GC progression. Our study demonstrated that GPR116 was significantly upregulated in GC tissues based on the GEO and TCGA databases, and these results were further validated using immunohistochemistry analysis. In addition, subgroup analysis based on sex, age, race, tumor grade, disease stage, and lymph node metastasis indicated that GPR116 transcription levels were significantly higher in patients with GC than in healthy individuals. This suggested that GPR116 may be a candidate marker for the early diagnosis of GC.

Further analysis revealed that GPR116 protein expression levels were positively correlated with TNM stage and tumor invasion, suggesting that GPR116 may promote the invasion of tumor cells and cancer progression. Prognostic analysis revealed that patients with GC and high GPR116 expression had significantly shorter overall survival than those with low GPR116 expression. These results were consistent with the prognostic prediction using the Kaplan–Meier Plotter database. In addition, Cox regression analysis further indicated that GPR116 was an independent risk factor for poor prognosis in patients with GC. To study the potential mechanism of GPR116, we performed a gene enrichment analysis for genes co-expressed

Table 2

Univariate and multivariate analysis of prognostic parameters for survival in patients with gastric carcinoma.

	Univariate analysis			Multivariate analysis		
Prognostic parameter	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
Expression of GPR116 (vs low)	2.107	1.198, 3.707	.010	1.855	1.021, 3.370	.043
Gender (vs male)	0.646	0.0372, 1.120	.120			
Age (vs <65)	2.090	1.156, 3.781	.015	2.370	1.269, 4.423	.007
Tumor location (vs Cardiac/gastric fundus)	0.856	0.576, 1.272	.441			
Tumor size (vs <5 cm)	1.446	0.834, 2.508	.189			
Differentiation (vs poor)	1.653	0.084, 3.226	.141			
TNM stage (vs I–II)	2.291	1.275, 4.116	.006	2.460	1.009, 6.002	.048
Tumor invasion (vs T1-2)	2.416	1.028, 5.681	.043	0.980	0.378, 2.544	.967
Lymph node metastasis (vs N0)	2.118	1.058, 4.238	.034	0.871	0.313, 2.423	.791
Distant metastasis (vs M0)	4.064	1.563, 10.572	.004	4.055	1.463, 11.243	.007
Vascular invasion (vs absent)	2.872	1.43, 5.525	.002	2.547	1.249, 5.194	.010

GPR116 = G protein-coupled receptor 116.

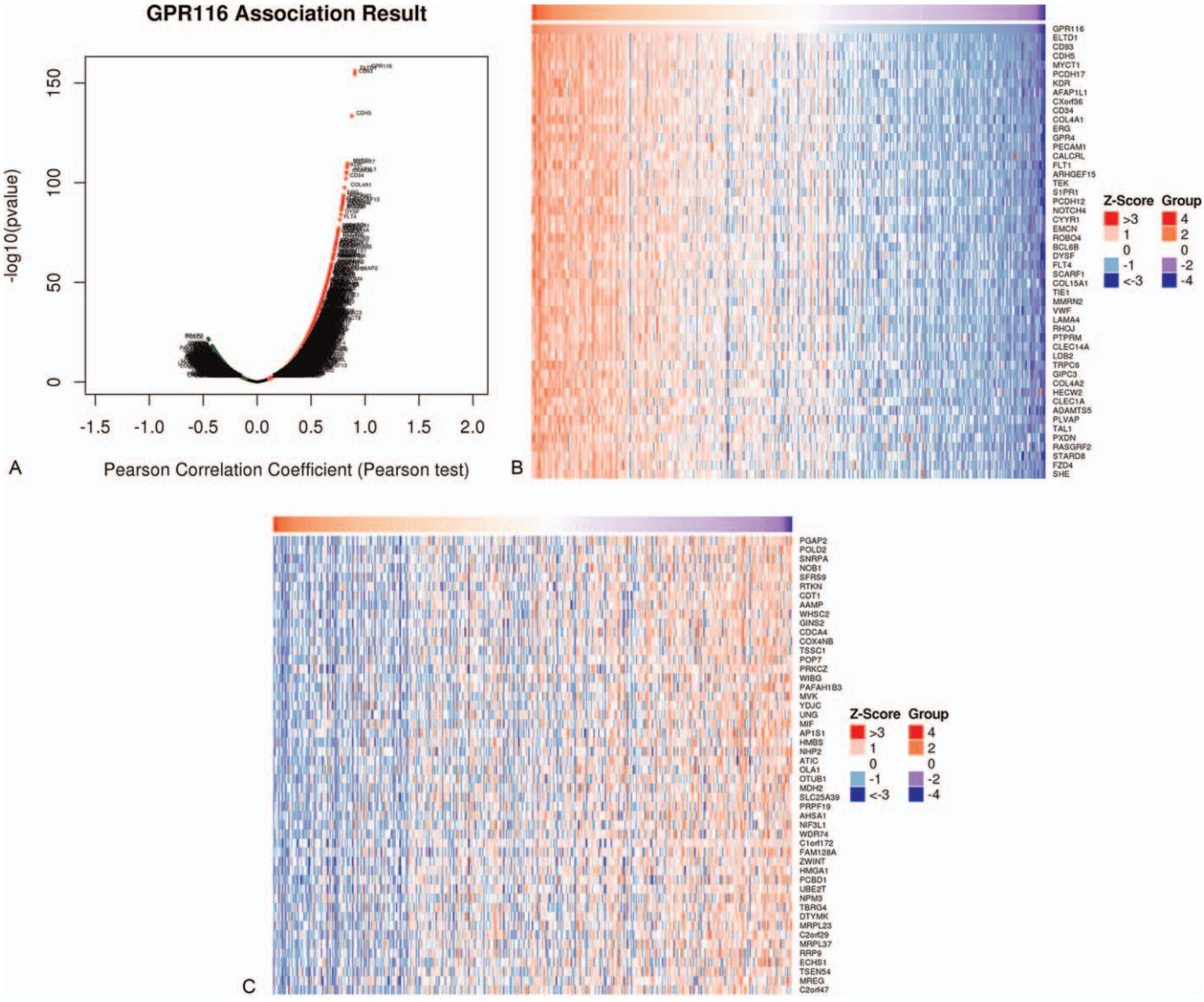

Figure 6. Genes differentially expressed in correlation with GPR116 in STAD (LinkedOmics). Pearson test was used to analyze correlations between GPR116 and genes differentially expressed in STAD (A). Heat maps showed genes positively (B) and negatively (C) correlated with GPR116 in STAD (TOP 50). GPR116: G protein-coupled receptor 116; STAD: stomach adenocarcinoma.

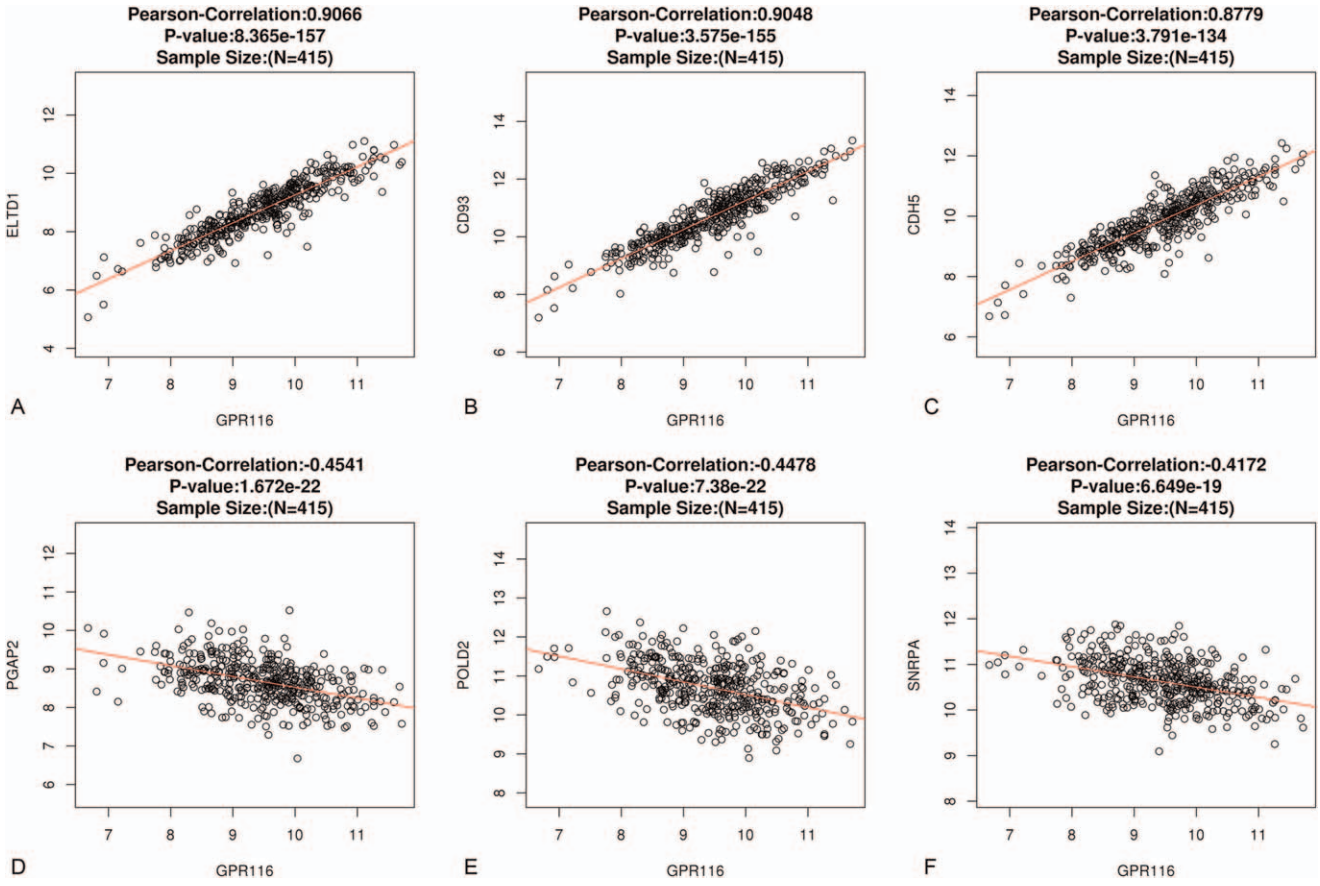

Figure 7. Verification of the correlation of GPR116 and the top three differentially expressed genes using LinkedOmics. The positive correlation between GPR116 and the top three genes (ELTD1, CD93, and CDH5) are shown (A–C). The negative correlation between GPR116 and the top three genes (PGAP2, POLD2, and SNRPA) are shown (D–F). GPR116: G protein-coupled receptor 116.

with GPR116. The results revealed that genes positively related to GPR116 expression participated primarily in protein and ion binding, nucleic acid binding, and hydrolase and transferase activities, which are involved in pathways such as the extracellular matrix-receptor interaction, cell adhesion, and PI3K-Akt signaling pathways. These pathways are important regulatory networks that mediate epithelial-mesenchymal transition[20,21] and are closely associated with tumorigenesis, invasion, metastasis, and resistance to therapy.[22] These results further confirmed that GPR116 is involved in GC metastasis and invasion, suggesting that GPR116 has a pro-tumorigenic effect and could potentially be a prognostic marker and therapeutic target for patients with GC.

In this study, ELTD1, CD93, and CDH5 were the top 3 genes upregulated simultaneously with GPR116. Eltd1, a rare G protein-coupled receptor, is a new regulatory factor of tumor angiogenesis[23] that has been found to be upregulated in colorectal, ovarian, and renal cancers, promoting tumor cell invasion and metastasis.[24] CD93, a transmembrane receptor, is one of the top 20 genes that characterize human tumor angiogenesis. It is highly expressed in tumor vascular endothelial cells and has become a potential target for therapy.[25] CDH5, also known as vascular endothelial cadherin, plays an important role in cell adhesion, inhibition of endothelial cell migration, and apoptosis.[26] CDH5 is highly expressed in various malignant tumor cells and has been shown to play a key role in GC progression, metastasis, and recurrence[26,27]. Thus, we inferred that GPR116 may contribute to tumor invasion and migration by promoting tumor angiogenesis.

PGAP2, POLD2, and SNRPA were the top 3 genes negatively related to GPR116 expression. PGAP2 is involved in the synthesis of glycosylphosphatidylinositol-anchored proteins, which are associated with developmental delays and intellectual disability in humans.[28] However, there has been no study on PGAP2 in tumors. POLD2 is a subunit of the DNA polymerase complex, which is involved in DNA replication and repair. It has also been reported to be associated with ovarian carcinogenesis.[29] SNRPA is a 282-amino-acid protein containing two RNA-binding domains, which play a vital role in shear body formation and mRNA cleavage. SNRPA in GC has been reported to promote tumor cell proliferation by regulating nerve growth factors.[30] Therefore, our analysis indicates that low levels of PGAP2, POLD2, and SNRPA expression may be involved in the metastasis and invasion of GC, which requires further validation.

There are several limitations to this study. First, this was a single-center retrospective study with a relatively small sample size; hence, selection bias cannot be ruled out. Second, due to the long follow-up time, the degradation of tumor tissue cell proteins

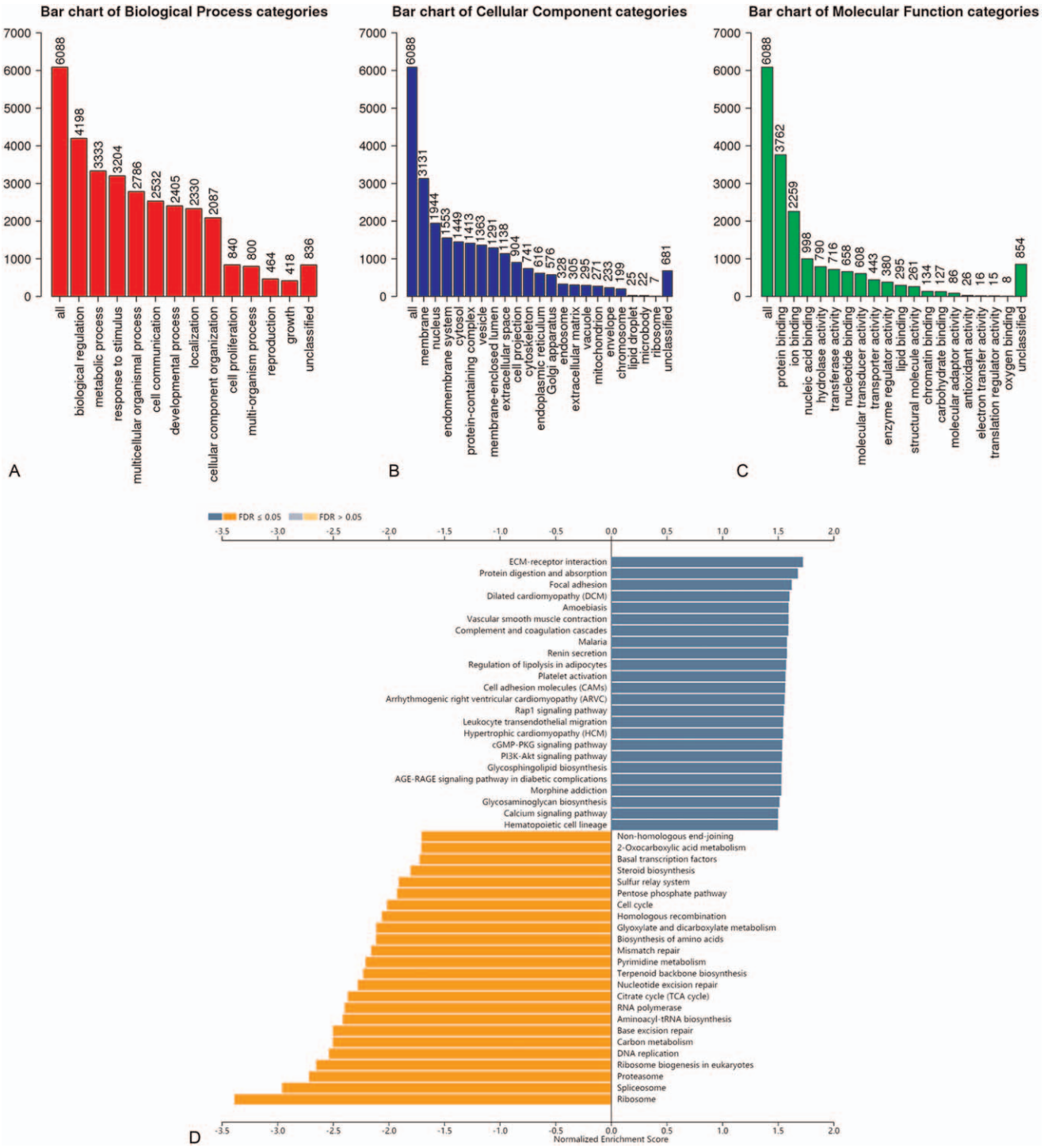

Figure 8. Analysis of gene ontology enrichment and KEGG pathways of GPR116 co-expression genes in stomach adenocarcinoma using WebGestalt via LinkedOmics. (A) Cellular components. (B) Biological processes. (C) Molecular functions. (D) KEGG pathway analysis. KEGG: Kyoto encyclopedia of genes and genomes, GPR116: G protein-coupled receptor 116.

may affect the experimental results. Third, the biological function analysis findings need to be further validated using experimental and clinical data.

In conclusion, we revealed that GPR116 is upregulated in GC, which was linked to poor prognosis in patients with GC. Therefore, GPR116 could potentially serve as a prognostic marker and therapeutic target when treating patients with GC. Further experiments and clinical trials are needed to validate the value of GPR116 in GC and other cancers.

Author contributions

All authors contributed to drafting or revising the article, gave final approval of the version to be published.

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