Increased expression of GIPC2 in colon adenocarcinoma is associated with a favorable prognosis and high levels of immune cell infiltration

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Abstract. Gα-interacting protein C-terminus PDZ-domain-containing family member 2 (GIPC2) serves an important role in the development of digestive tract tumors; however, its role in colon adenocarcinoma (COAD) has yet to be elucidated. In the present study, data were retrieved from The Cancer Genome Atlas database to investigate the association between GIPC2 expression and prognosis, as well as the levels of tumor-infiltrating immune cells. Immunohistochemical analysis was subsequently performed on 22 pairs of COAD and adjacent normal colon tissues, which were collected during surgery, to verify GIPC2 protein expression. The results showed that the positive rate in the normal intestinal mucosa group (18/22, 81.82%) was significantly higher than that in the COAD group (3/22, 13.64%, χ^2=20.497, P<0.001). Gene set enrichment analysis was used to predict the signaling pathways regulated by GIPC2 in COAD, whereas the CIBERSORT algorithm was used to analyze the association between GIPC2 expression and immune cell infiltration. The expression levels of GIPC2 were revealed to be significantly downregulated in COAD compared with in normal colon tissues (P<0.05). Notably, the overall survival (P=0.004), disease-specific survival (P=0.003) and progression-free interval (P=0.011) rates of the group with high GIPC2 expression were higher compared with those in the group with low GIPC2 expression. In addition, the results of the regression analysis suggested that GIPC2 was an independent prognostic factor for COAD (P=0.007). The expression levels of GIPC2 were significantly associated with tumor stage, lymph node status and lymphatic invasion, and GIPC2 expression was enriched in 'cell cycle checkpoints', 'DNA replication' and 'mitosis-associated signaling pathways'. In addition, a positive association was observed between high GIPC2 expression and levels of infiltrating immune cells. Moreover, the expression of immune checkpoint-associated genes was significantly higher in the group with low GIPC2 expression. Taken together, the findings of the present study demonstrated that high expression levels of GIPC2 were associated with a favorable prognosis and increased infiltration of immune cells in COAD; therefore, GIPC2 may serve as a biomarker to assess prognosis and the level of immune cell infiltration in patients with COAD.

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Abbreviations: GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2; COAD, colon adenocarcinoma; IHC, immunohistochemistry; TCGA, The Cancer Genome Atlas; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; GEPIA2, Gene Expression Profiling Interactive Analysis 2

Key words: COAD, GIPC2, immune cell infiltration, biomarker, prognosis

Introduction

Colon adenocarcinoma (COAD) is the third most common type of cancer worldwide (1). Although great progress has been made in terms of the treatment of COAD, the high recurrence rates associated with this type of cancer remain a major clinical challenge (2,3). There are only limited methods available to effectively inhibit COAD metastasis and there are no effective therapies for patients with distant metastases (4). Numerous studies have investigated the underlying molecular mechanisms of COAD, which may solve clinical problems associated with this type of cancer (5,6). The complex function and regulation of the immune system offers more diverse strategies for cancer treatment. These strategies include adoptive T-cell transfer, cytokine therapy, and administration of ligands and monoclonal antibodies. Immunotherapy provides important leads for potential therapies for the treatment of patients with advanced colon cancer; therefore, further scientific research is required to improve the efficacy of immunotherapy against colon cancer (7).

Gα-interacting protein (GAIP) C-terminus (GIPC) PDZ-domain-containing family member 2 (GIPC2) is a member of the GIPC family of proteins, which can activate the Wnt signaling pathway by binding to the GTP-coupled

proteins, RCSI9 and RCSUGAIP, and TGF-β type 3 receptor proteins through their PDZ domain (8). The expression levels of GIPC2 vary among human tissues, with high expression levels being reported in digestive organs, such as the small intestine, colon, stomach, esophagus, liver and other tissues, whereas tissues such as the bone marrow, thymus, retina, smooth muscle and placenta have negligible levels of expression (9). In addition, GIPC2 is expressed in certain glands, such as breast, adrenal, salivary and thyroid glands (10). Previous studies have demonstrated an important role for GIPC2 in embryonic development (11,12) and the occurrence of digestive tract tumors (13,14); however, the role of GIPC2 in COAD has yet to be elucidated.

In the present study, database analysis was used to explore the expression levels of GIPC2 in different types of cancer tissue, and to determine the association between its expression and prognosis, the level of infiltrating immune cells and expression of immune checkpoint-associated genes in COAD.

Materials and methods

GIPC2 expression analyses. The Xiantao bioinformatics analysis tool (https://www.xiantao.love/products) is an online comprehensive bioinformatics tool platform based on visual R language programming (15). This tool was first employed to analyze the mRNA expression levels of GIPC2 in 11,093 samples of 33 types of cancer based on data retrieved from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/). The expression levels of GIPC2 were then compared between normal and tumor tissues obtained from 478 patients with COAD, which included tumor tissues from all patients, and 41 normal tissues adjacent to the cancer from 41 patients.

Patient samples. A total of 22 pairs of COAD samples and adjacent normal colon tissues were collected from surgical samples at the People's Hospital of Tongling City (Tongling, China). Among the 22 patients who underwent surgery between July 2019 and June 2021, 15 were male and seven were female, with a mean age of 69 years. Of these patients, 16 underwent laparoscopic right hemicolectomy, five underwent laparoscopic sigmoidectomy and one underwent laparoscopic left hemicolectomy. None of the patients were treated with preoperative therapy. All patients provided written informed consent. The present study was approved by the Ethics Committee of the People's Hospital of Tongling City (approval no. 2022002).

Immunohistochemistry (IHC). GIPC2 protein detection was performed using IHC with horseradish peroxidase. The concentrated rabbit polyclonal antibody against human GIPC2 protein was purchased from BIOSS (cat no. KT22301; 1:200). Normal adult kidney tissue was used as a positive control and PBS was used as a negative control. The normal adult kidney tissue was derived from the same patients with kidney cancer, with normal tissue taken >5 cm away from cancer tissue. IHC kits were purchased from Fuzhou Maixin Biotech Co., Ltd. and were performed according to the manufacturer's protocol. Briefly, the *ex vivo* tissue was immediately fixed in 10% neutral formalin fixative for 24 h at 20-25˚C, embedded in paraffin, continuously sectioned (4 μm) and mounted on slides at 60˚C for 2 h. Subsequently, the tissue was conventionally dewaxed using xylene and hydrated in a gradient series of alcohol. The endogenous peroxidase was blocked with 3% hydrogen peroxide for 15 min at room temperature and antigen retrieval was performed in a pressure cooker with 1% citric acid antigen repair solution (pH 6.0) for 2 min. Tissues were then incubated with 50 μl primary antibody at 4˚C for ~12 h, and with 50 μl secondary antibody (ready to use; cat. no. KIT-5010; Fuzhou Maixin Biotech Co., Ltd.) at room temperature for 30 min. All sections were counterstained with hematoxylin after the reaction. Two senior pathologists performed double-blind evaluations by examining the sections under a light microscope. According to a previous study (16), 10 high-power fields were randomly selected from each section. A semi-quantitative score based on the intensity of positively stained cells and the staining area was used to evaluate the results of IHC; the comprehensive score of staining intensity was multiplied by the staining area. Staining intensity was scored as follows: 0, colorless areas (no staining); 1, light yellow staining; 2, brown-yellow staining; 3, brown staining. The numbers of positive cells were evaluated as follows: <5%, 0; 5-25%, 1; 26-50%, 2; 51-75%, 3; >75%, 4. The staining intensity score was subsequently multiplied by the positive cell number score; a score of 0-3 was classified as negative, whereas a score >3 was classified as positive.

Association between GIPC2 and clinicopathological features. Differential expression of GIPC2 according to pathological stage, tumor stage, lymph node status, metastasis, sex, age, lymphatic invasion and perineural invasion in patients with COAD based on TCGA database was assessed using box plots. The same variables were used in multiple logistic regression analyses to determine the factors associated with GIPC2 expression.

Survival analyses based on GIPC2 expression. The effect of GIPC2 expression on the survival of patients with COAD was determined by performing a Kaplan-Meier analysis and log-rank test, based on data retrieved from TCGA database. Data for the patients with COAD for whom the relevant prognostic information was known were used to analyze the association between GIPC2 expression (based on median levels) and overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI). Cox regression analysis was subsequently used to determine the risk factors for OS.

Analyses of genes co-expressed with GIPC2. The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) server (http://gepia.cancer-pku.cn/index.html) is an online server for the analysis of TCGA data. GEPIA2 was used to estimate the top 100 genes co-expressed with GIPC2. Enrichment analysis was subsequently performed using the clusterProfiler package in R (version 4.0.3) on the Xiantao bioinformatics analysis tool (https://www.xiantao.love/) to perform Gene Ontology (GO) biological process and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Finally, Spearman rank correlation test was used to determine the

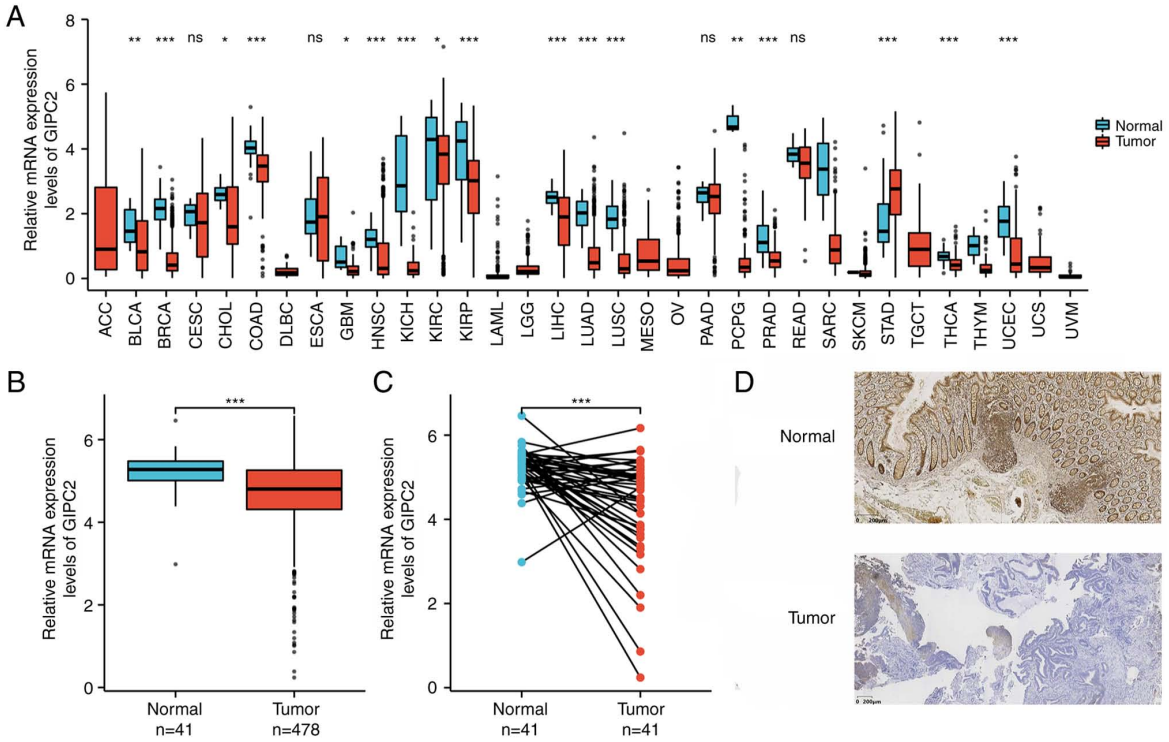

Figure 1. Expression levels of GIPC2 in COAD. (A) Expression levels of GIPC2 (mean ± SD) in 33 types of cancer based on The Cancer Genome Atlas data. (B) Unpaired analysis of GIPC2 expression (mean ± SD) between tumor and normal tissues in 478 patients with COAD. (C) Paired analysis of GIPC2 expression (scatter points represent expression levels of individual samples) between tumor and normal tissues (n=41). (D) Comparison of GIPC2 protein expression levels between normal and tumor tissues using immunohistochemistry (100x magnification). *P<0.05, **P<0.01, ***P<0.001 vs. normal. COAD, colon adenocarcinoma; GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2; ns, not significant.

correlation between GIPC2 and the top five co-expressed genes.

Gene set enrichment analysis (GSEA). To explore the potential pathological processes associated with GIPC2, GSEA was conducted for TCGA-COAD data using GSEA v4.3.0 software (https://www.gsea-msigdb.org/gsea/index.jsp). The gene set c2.cp.kegg.v7.1.symbols.gmt was selected for further analysis. The number of permutations was set as 5,000. Normalized enrichment scores >1, false discovery rate q-values <0.05 and adjusted P-values <0.05 were set as the cut-off values for significant enrichment.

Evaluation of tumor-infiltrating immune cells. The immunedeconv package in R (https://www.aclbi.com/static/index.html#/immunoassay), which integrates CIBERSORT (17), is a deconvolution algorithm based on gene expression that is able to evaluate changes in the expression of one set of genes relative to all other genes in the sample. This package was used to analyze the levels of tumor-infiltrating immune cells. Among 478 COAD samples based on TCGA-COAD data, samples with the top 25% and the lowest 25% levels of GIPC2 expression were classified into the high- and low-expression groups, respectively. The abundance of 22 types of immune cells [naïve B cells, memory B cells, plasma B cells, $CD8^+$ T cells, naïve $CD4^+$ T cells, resting $CD4^+$ memory T cells, activated $CD4^+$ memory T cells, follicular helper T cells, regulatory T cells, γδ T cells, resting natural killer (NK) cells, activated NK cells, monocytes, M0 macrophages, M1 macrophages, M2 macrophages, resting myeloid dendritic cells, activated myeloid dendritic cells, activated mast cells, resting mast cells, eosinophils and neutrophils] were estimated using the CIBERSORT algorithm. Briefly, gene expression datasets from TCGA were uploaded to the Xiantao bioinformatics analysis tool, and after standard annotation, the immunedeconv R package was used to estimate the P-values for deconvolution via the CIBERSORT algorithm. This tool was then used to compare the expression of immune checkpoint-associated genes, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, TIGIT and SIGLEC15, between patients with COAD in the high and low GIPC2 expression groups, respectively. The aforementioned analyses and R package were implemented using R foundation for statistical computing (2020) version 4.0.3 (18) and the software packages ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) and pheatmap (https://cran.r-project.org/web/packages/pheatmap/index.html) were used for generating images.

Statistical analysis. SPSS 19.0 software (IBM Corp.) was used to perform the statistical analyses. Comparisons between or among groups were performed using unpaired χ^2 test, Student's t-test, paired Student's t-test, Mann-Whitney U-test or one-way ANOVA. Tukey's HSD was used as a post hoc test following ANOVA. As aforementioned, a Kaplan-Meier analysis and log-rank test was performed for survival analysis using R language package (version 4.0.3). Spearman rank correlation test was used for the correlation analysis between GIPC2 and co-expressed genes. Logistic multiple regression analysis was used to determine the risk factors associated with GIPC2

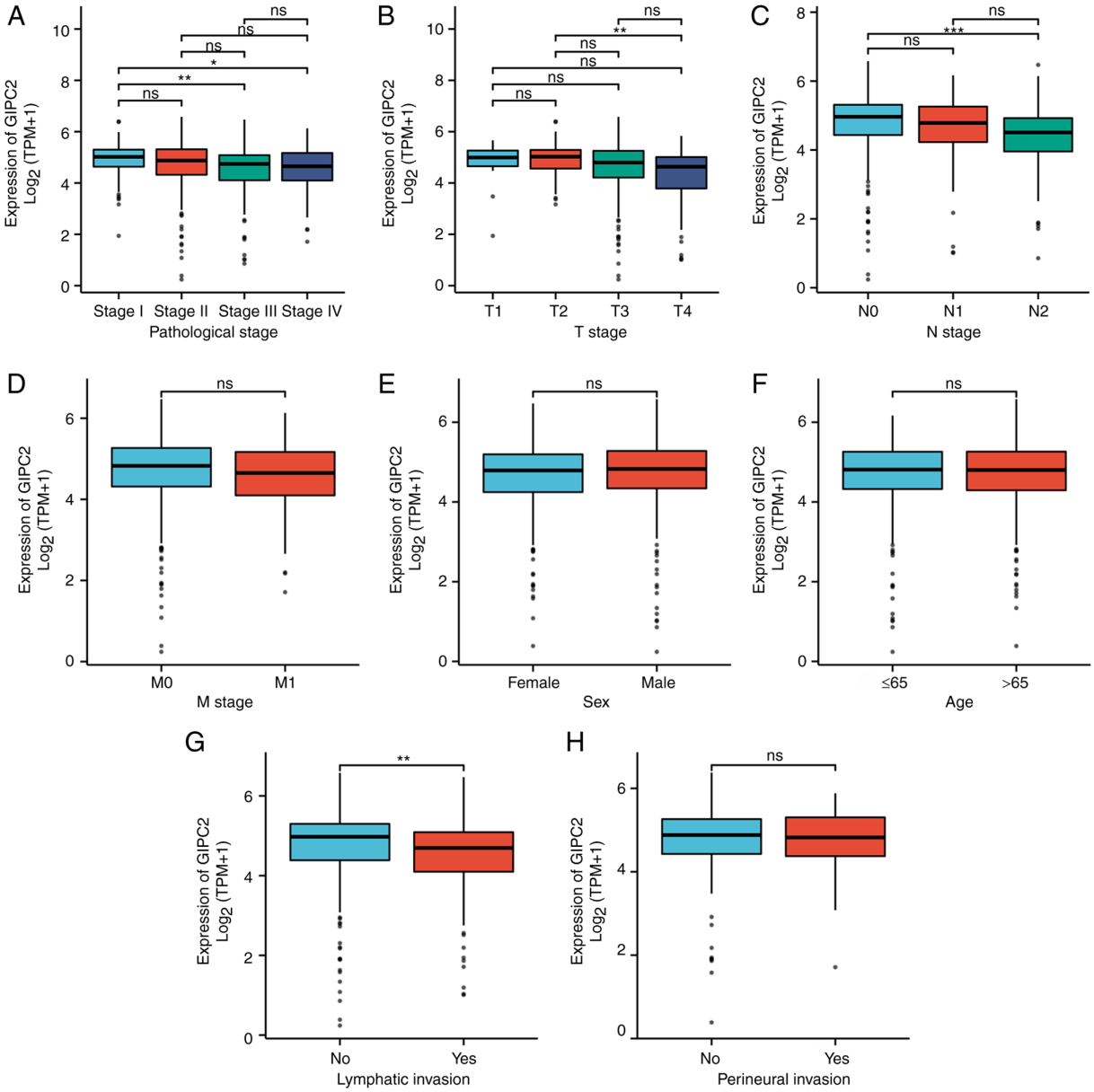

Figure 2. Association between GIPC2 expression levels and clinicopathological variables. Association of GIPC2 with (A) pathological stage, (B) T stage, (C) N stage, (D) M stage, (E) sex, (F) age, (G) lymphatic invasion and (H) perineural invasion. Data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2; ns, not significant.

expression, and Cox regression analysis was performed to determine the risk factors for OS. P<0.05 was considered to indicate a statistically significant difference.

Results

Pan-cancer analysis of the expression levels of GIPC2. The expression levels of GIPC2 in 33 types of cancer based on TCGA data were analyzed. GIPC2 expression was revealed to be low in bladder cancer, breast cancer, bile duct cancer, COAD, glioblastoma, head and neck cancer, chromophobe renal cell carcinoma, kidney clear cell carcinoma, kidney papillary cell carcinoma, liver cancer, lung adenocarcinoma, lung squamous cell carcinoma, pheochromocytoma and paraganglioma, prostate cancer, thyroid cancer, thymoma and endometrioid cancer, whereas it was high in stomach cancer compared with the tissue adjacent to the cancer (Fig. 1A). The results of unpaired (Fig. 1B) and paired (Fig. 1C) analyses of COAD confirmed that the expression levels of GIPC2 were significantly higher in normal tissues compared with those in tumor tissues. The results of IHC also confirmed that the protein expression levels of GIPC2 were lower in COAD tissues compared with those in normal tissue samples (Fig. 1D). The positive rate in the normal intestinal mucosa group (18/22,81.82%) was significantly higher than that in the COAD group (3/22, 13.64%; χ^2=20.497; P<0.001) (data not shown).

Association between GIPC2 expression and clinicopathological variables. R version 4.0.3 was used to assess the association of GIPC2 with the relevant clinical information from 478 cases of COAD obtained from TCGA. Differential expression of GIPC2 according to the pathological stage

Table I. Relationship between Gα-interacting protein C-terminus PDZ-domain-containing family member 2 expression and clinicopathological variables.

Characteristic	Odds ratio (95% CI)	P-value
T stage (T3 and T4 vs. T1 and T2)	0.439 (0.271-0.698)	<0.001[a]
N stage (N1 and N2 vs. N0)	0.496 (0.341-0.717)	<0.001[a]
M stage (M1 vs. M0)	0.530 (0.304-0.907)	0.022
Sex (Male vs. Female)	1.106 (0.772-1.585)	0.583
Age (>65 vs. ≤65 years)	0.966 (0.670-1.392)	0.852
Perineural invasion (Yes vs. No)	1.011 (0.517-1.991)	0.974
Lymphatic invasion (Yes vs. No)	0.452 (0.304-0.669)	<0.001[a]

[a]P<0.05.

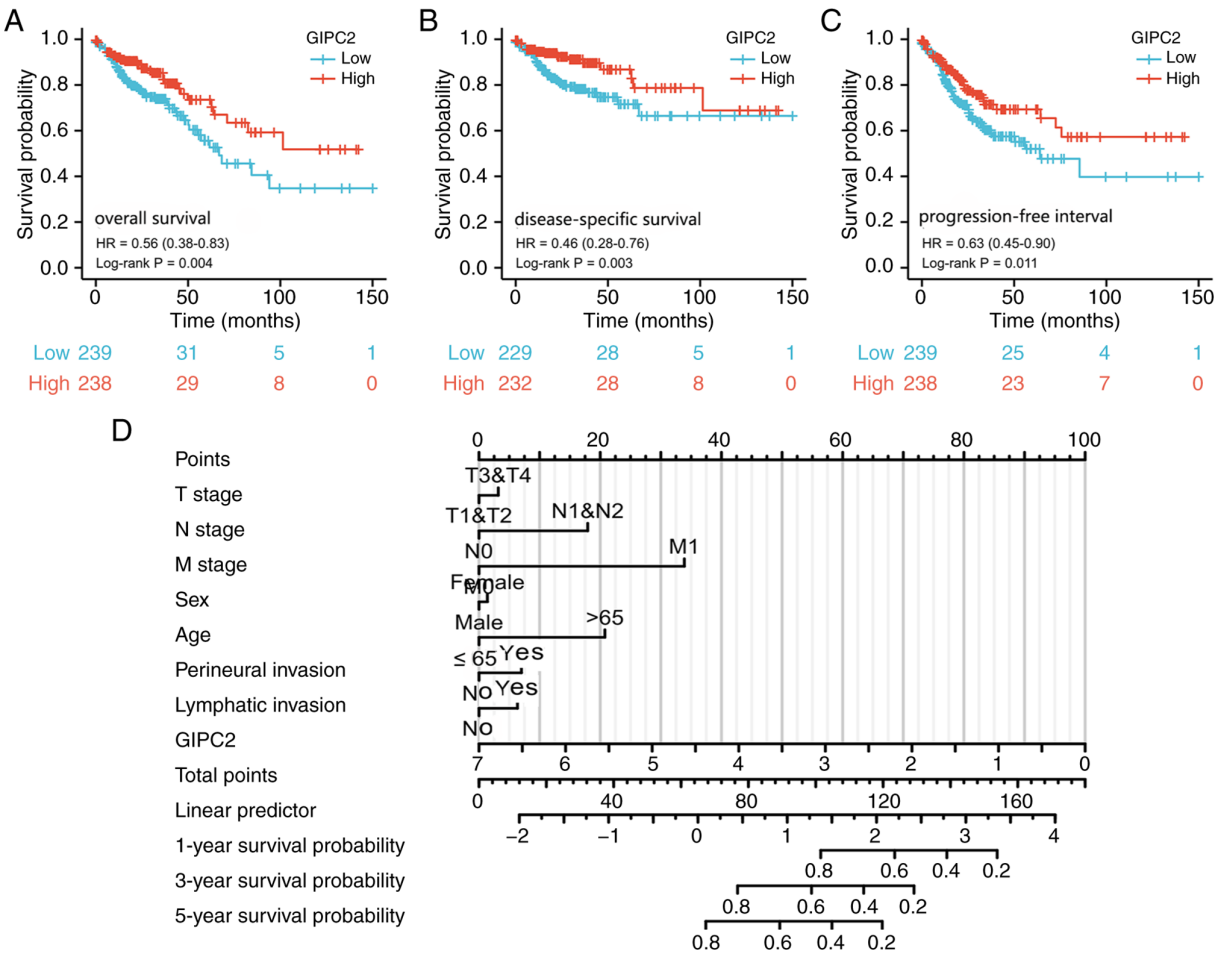

Figure 3. Association between GIPC2 expression levels and prognosis. Increased GIPC2 expression in COAD was associated with favorable (A) overall survival, (B) disease-specific survival and (C) progression-free interval. (D) Multivariate Cox regression analysis between GIPC2 expression and clinicopathological factors. Visualization of 1, 3 and 5-year survival probabilities and risk coefficients for each variable of the Cox survival model using forest plots. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2.

(Fig. 2A), tumor stage (Fig. 2B), lymph node status (Fig. 2C), metastasis status (Fig. 2D), sex (Fig. 2E), age (Fig. 2F), lymphatic invasion (Fig. 2G) and perineural invasion (Fig. 2H) was analyzed. The results of univariate analysis revealed that GIPC2 expression (based on the median expression value) was markedly associated with pathological stage, tumor stage, lymph node status and lymphatic invasion. Multivariate analysis using logistic regression showed that tumor stage, lymph node status and lymphatic invasion were significantly associated with GIPC2 expression (Table I).

Low GIPC2 expression is associated with poor prognosis in patients with COAD. As shown in Fig. 3, low expression levels of GIPC2 (based on the median expression value) was

Table II. Cox regression analysis for overall survival.

	Univariate analysis		Multivariate analysis	
Characteristic	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
T stage				
T1 and T2	Reference			
T3 and T4	3.072 (1.423-6.631)	0.004[a]	1.120 (0.218-5.747)	0.892
N stage				
N0	Reference			
N1 and N2	2.592 (1.743-3.855)	<0.001[a]	1.973 (0.600-6.491)	0.263
M stage				
M0	Reference			
M1	4.193 (2.683-6.554)	<0.001[a]	3.593 (1.300-9.930)	0.014[a]
Sex				
Female	Reference			
Male	1.101 (0.746-1.625)	0.627		
Age				
≤65 years	Reference			
>65 years	1.610 (1.052-2.463)	0.028[a]	2.157 (0.947-4.913)	0.067
Perineural invasion				
No	Reference			
Yes	1.940 (0.982-3.832)	0.056	1.286 (0.502-3.294)	0.600
Lymphatic invasion				
No	Reference			
Yes	2.450 (1.614-3.720)	<0.001	1.269 (0.523-3.079)	0.598
GIPC2, log2+1	0.832 (0.695-0.997)	0.046[a]	0.582 (0.393-0.862)	0.007[a]

[a]P<0.05. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2.

significantly associated with poor OS (Fig. 3A), DSS (Fig. 3B) and PFI (Fig. 3C). Furthermore, the results of the Cox multivariate analysis revealed that low expression levels of GIPC2 and positive distant metastasis were independent prognostic factors (Fig. 3D; Table II).

Analysis of genes co-expressed with GIPC2 in COAD. The top five genes that exhibited a significant positive correlation with GIPC2 were EPCAM (Fig. 4A), LRRC8D (Fig. 4B), EPB41L4B (Fig. 4C), ACSL5 (Fig. 4D) and CDS1 (Fig. 4E). The top 100 genes co-expressed with GIPC2 were subsequently selected for enrichment analysis. The terms 'epithelial cell signaling in *Helicobacter pylori* infection', 'tight junction' and 'peroxisome' were significantly enriched in the GO biological process analysis (Fig. 4F). The terms 'ligase activity, forming carbon-sulfur bonds', 'actin filament binding' and 'acid-thiol ligase activity' were significantly enriched in the GO cellular component analysis (Fig. 4F). In the GO molecular function analysis, the terms 'brush border', 'apical junction complex' and 'tight junction' were highly enriched (Fig. 4F). Finally, the KEGG pathway analysis indicated that the pathways 'intestinal absorption', 'regulation of microvillus organization' and 'microvillus organization' were significantly enriched (Fig. 4F).

Identification of GIPC2-associated pathways by GSEA. GSEA was performed using COAD data from TCGA, and the results were compared between tissues with high and low GIPC2 expression to identify the possible biological pathways regulated by GIPC2. A total of 316 pathways were significantly enriched in the GIPC2 high expression group. The results showed that the top nine significantly enriched terms comprised 'cell cycle checkpoints' (Fig. 5A), 'DNA replication' (Fig. 5B), 'mitotic G1 phase and G1-S transition' (Fig. 5C), 'cell cycle mitotic' (Fig. 5D), 'mitotic metaphase and anaphase' (Fig. 5E), 'G2 M checkpoints' (Fig. 5F), 'DNA replication pre-initiation' (Fig. 5G), 'retinoblastoma gene in cancer' (Fig. 5H) and 'separation of sister chromatids' (Fig. 5I).

Association between GIPC2 expression and tumor-infiltrating immune cells. The results showed that the numbers of plasma B cells (P=0.018), resting $CD4^+$ memory T cells (P=0.015), activated $CD4^+$ memory T cells (P=0.023), activated myeloid dendritic cells (P=0.005) and activated mast cells (P=0.023) were significantly higher, whereas the numbers of regulatory T cells (P=0.021), M0 macrophages (P=0.038) and neutrophils (P=0.029) were significantly lower in the high GIPC2 expression group compared with the low expression group (Fig. 6A and B). The expression levels of immune

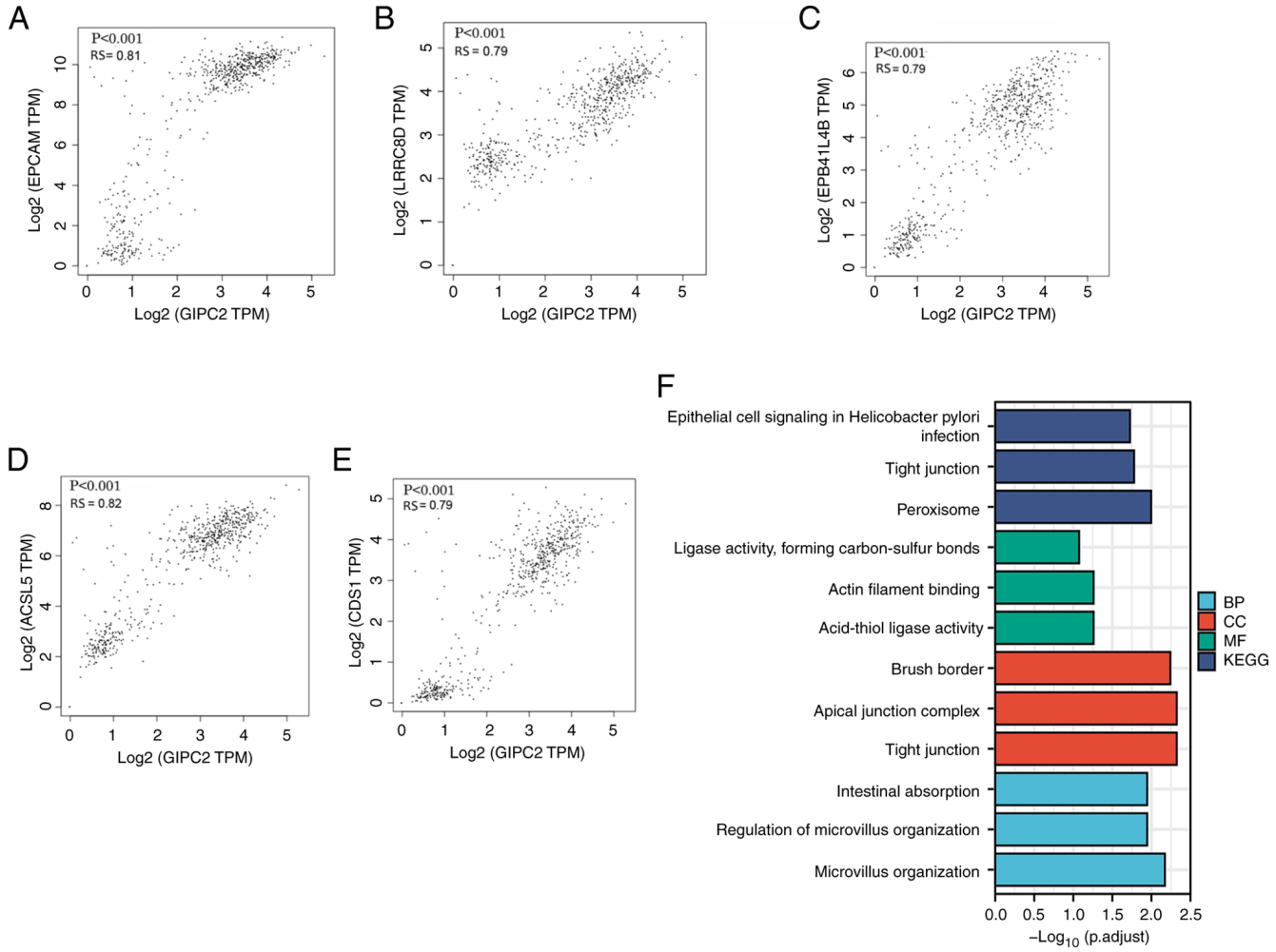

Figure 4. Analysis of genes co-expressed with GIPC2 in COAD. Relationship between GIPC2 and the top five co-expressed genes: (A) EPCAM, (B) LRRC8D, (C) EPB41L4B, (D) ACSL5 and (E) CDS1. (F) Top 100 co-expressed genes of GIPC2 were selected to conduct the enrichment analysis. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2.

checkpoint-associated genes, including HAVCR2, LAG3, PDCD1 and SIGLEC15 were significantly higher in the low GIPC2 expression group compared with in the GIPC2 high expression group (Fig. 7).

Discussion

GIPC2 is an important member of the PDZ domain family, and its abnormal expression has previously been reported to be associated with the development of tumors and abnormal embryonic development (19-22). Notably, GIPC2 expression is significantly increased in diffuse gastric cancer cell lines, including the OKAJIMA, TMK1, MKN45 and KATO-I cell lines; however, the expression of GIPC2 has been shown to be negligible in the HL-60 leukemia cell line, HeLaS3 cervical cancer cell line, K-562 chronic myeloid leukemia cell line, Burkitt lymphoma, SW480 colon cancer cell line and A549 lung cancer cell line (19).

The PDZ domain is the main functional domain of the GIPC2 protein, which interacts with FZD3-type Wnt receptor, insulin-like growth factor receptor, receptor tyrosine kinase A receptor, TGF-β R type II receptor and the RGS19 protein of the RGS family (23-25). The RGS19 protein is an important protein that regulates heterotrimers in the G-protein signaling pathway. Therefore, GIPC2 may have an important role in tumorigenesis and embryonic development through promoting the interaction between G-protein heterotrimers and Wnt receptors or receptor tyrosine kinases (26,27). Somatic mutations of GIPC2 in different types of cancer have been detected by whole-genome or whole-exome sequencing. Cancer genomic testing of ovarian cancer cases identified a G102E missense mutation in GIPC2 (28). D125N and E288K missense mutations of GIPC2 have also been identified in malignant melanoma (9). Furthermore, the F74Y and R312Q missense mutations, and E216X nonsense mutation of GIPC2 have been identified upon performing a colorectal cancer genome-level analysis (29). The E216X nonsense mutation is a deleterious mutation that causes the loss of the GH2 domain, which enables GIPC2 to bind to MY06 (30). Collectively, these data suggested that GIPC2 serves certain biological functional roles in different diseases.

To the best of our knowledge, the present study is the first to investigate the role of GIPC2 in COAD. The results demonstrated that the expression of GIPC2 was reduced in COAD tissues compared with that in normal tissues. Moreover, the

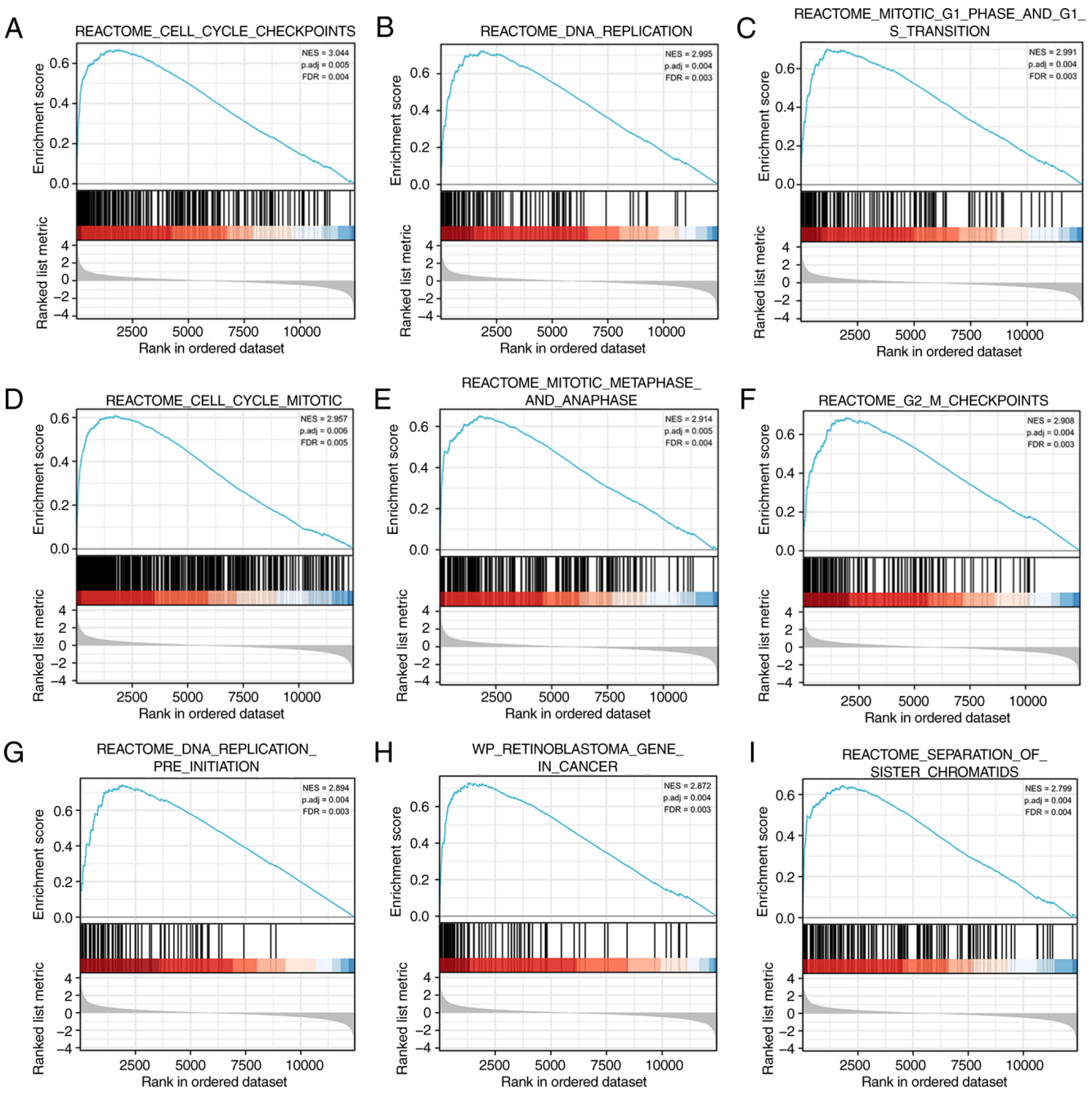

Figure 5. Gene set enrichment analysis of Gα-interacting protein C-terminus PDZ-domain-containing family member 2 in colon adenocarcinoma. (A) 'Cell cycle checkpoints', (B) 'DNA replication', (C) 'mitotic G1 phase and G1-S transition', (D) 'cell cycle mitotic', (E) 'mitotic metaphase and anaphase', (F) 'G2 M checkpoints', (G) 'DNA replication pre-initiation', (H) 'retinoblastoma gene in cancer' and (I) 'separation of sister chromatids'.

expression levels of GIPC2 were negatively associated with COAD tumor stage, lymph node status and lymphatic invasion. Additionally, the survival analysis revealed that high expression of GIPC2 was significantly associated with favorable OS, DSS and PFI in patients with COAD. The results of the regression analysis also suggested that GIPC2 was an independent prognostic factor for COAD. Taken together, these findings suggested that GIPC2 may act as a prognostic biomarker for COAD.

Co-expressed genes often have similar functions. The results of the co-expression analysis performed in the present study identified a significant positive correlation between GIPC2 and EPCAM, LRRC8D, EPB41L4B, ACSL5 and CDS1. Several of these genes have been reported to serve important roles in maintaining normal intestinal mucosal function and cancer resistance (31-35), thus indicating that GIPC2 and its co-expressed genes may serve as potential prognostic markers for COAD. At present, a large number of studies have shown that members of the GIPC family are able to fully exert their role as adaptors and interact with a variety of proteins, including RGS19/GAIP, MY06 and type III TGF-P receptors, and subsequently participate in the regulation of a variety of biological processes, including cell signaling, transmembrane protein transport, cell movement and endocytosis (36,37). To explore the underlying biological mechanism of GIPC2, GO and KEGG analyses, and

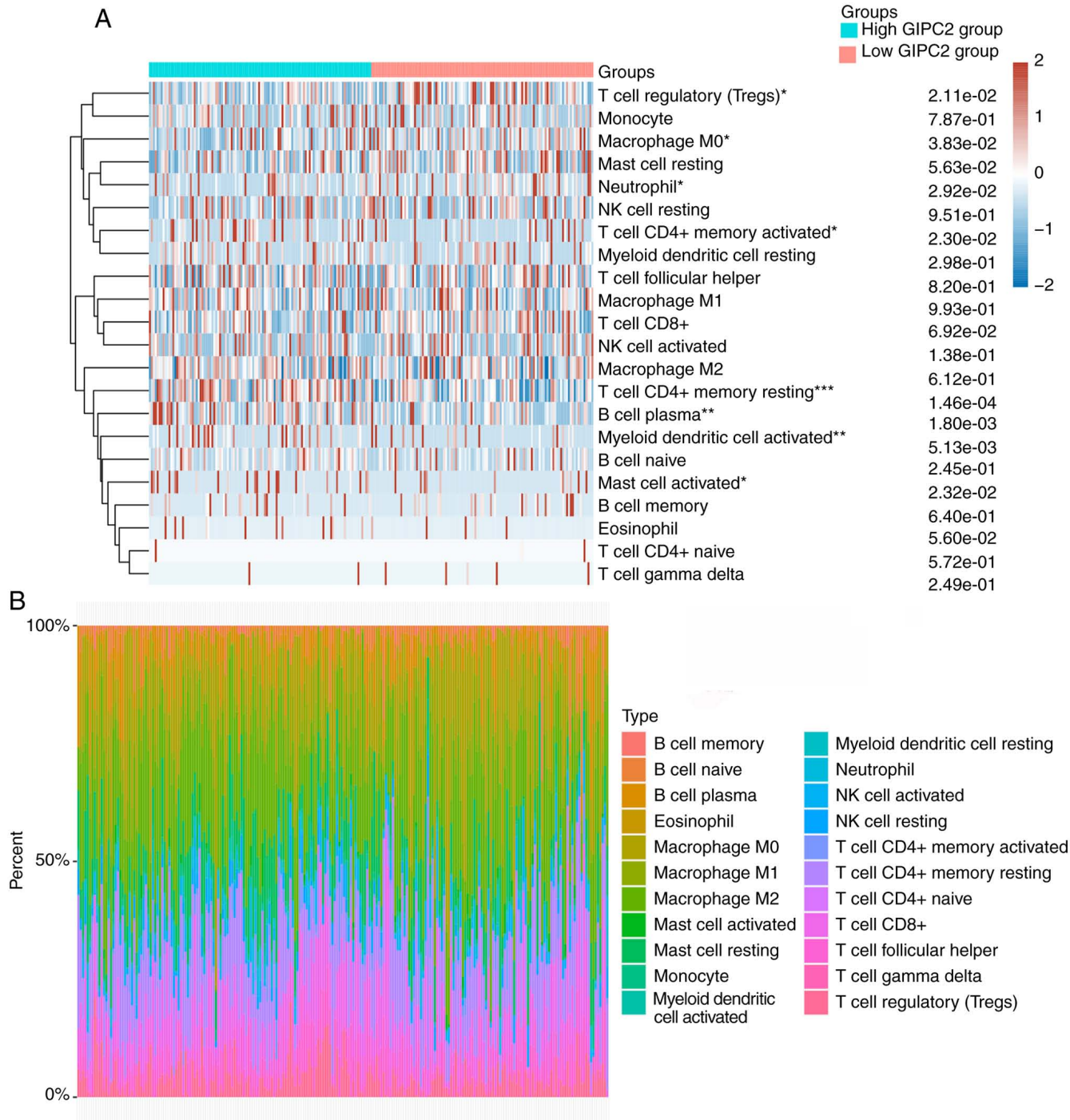

Figure 6. Relationship between GIPC2 expression levels and tumor-infiltrating immune cells. (A) Immune cell score heat map; different colors represent the expression trend in different samples according to the grouping of GIPC2 expression levels (high GIPC2 expression vs. low GIPC2 expression group). *P<0.05, **P<0.01, ***P<0.001. The significance between the two groups was determined using the Mann-Whitney U test. (B) Abundance of tumor-infiltrating immune cells in each sample, with different colors referring to different types of immune cells. The abscissa represents the sample, and the ordinate represents the percentage of immune cells in a single sample. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2; NK, natural killer.

GSEA were performed on genes co-expressed with GIPC2 in the present study. The enrichment analysis of the top 100 co-expressed genes and the GSEA revealed that 'intestinal absorption', 'regulation of microvillus organization', 'microvillus organization', 'cell cycle checkpoints', 'DNA replication' and 'mitosis-associated' pathways were significantly enriched. Although certain pathways, including cell cycle checkpoints, DNA replication and mitosis, have been verified in the occurrence and development of cancer (38), further mechanistic studies are required to fully elucidate their roles in the association between GIPC2 and COAD.

An important finding in the present study was identifying the association between GIPC2 expression and the level of immune cell infiltration in COAD. Analysis of the results obtained using the CIBERSORT algorithm demonstrated that plasma B cells, resting $CD4^+$ memory T cells, activated $CD4^+$ memory T cells, activated myeloid dendritic cells and activated mast cells were present in significantly

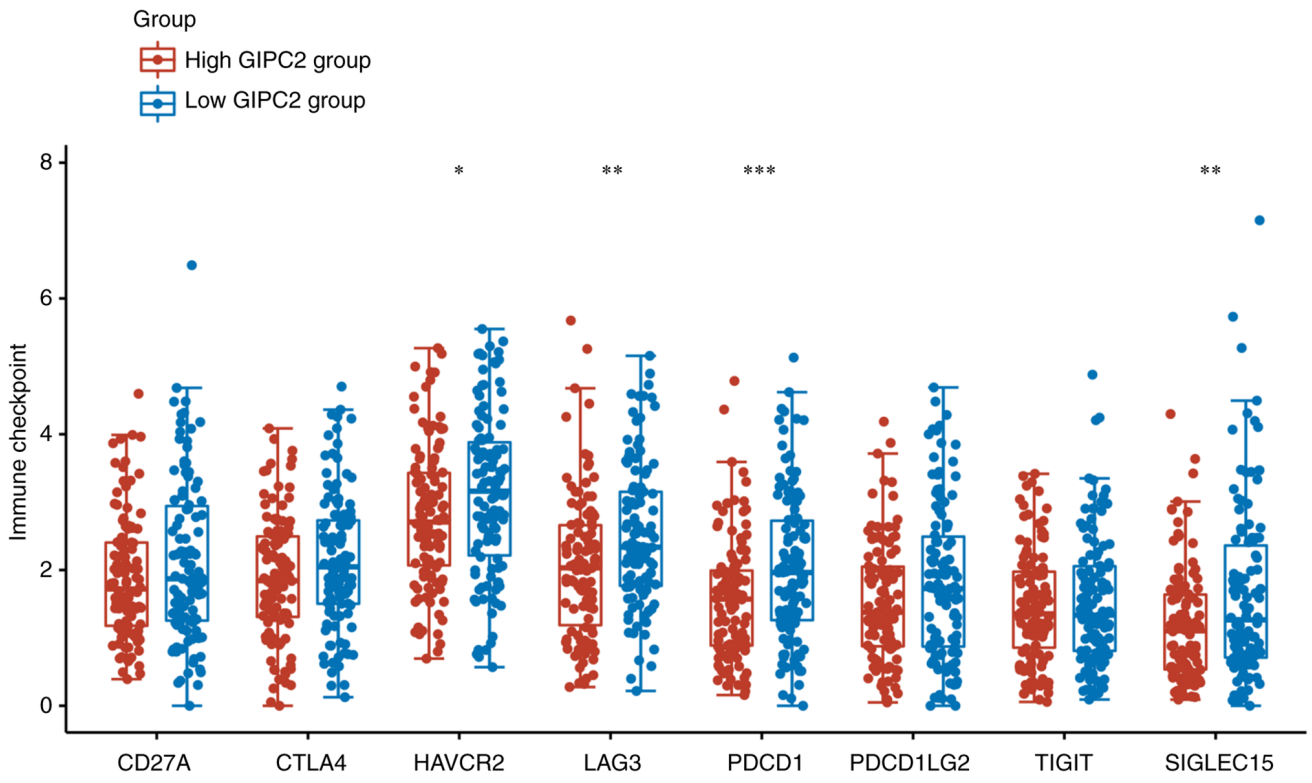

Figure 7. Relationship between GIPC2 expression and the expression levels of immune checkpoint-related genes. Data are presented as the mean ± SD. *P<0.05, **P<0.01, ***P<0.001 vs. high GIPC2 expression. GIPC2, Gα-interacting protein C-terminus PDZ-domain-containing family member 2.

higher proportions in the GIPC2 high expression group in COAD. Immune checkpoints are a class of immunosuppressive molecules that are expressed on immune cells and are able to regulate the degree of immune activation (39,40). These checkpoints have an important role in preventing the occurrence of autoimmunity. Immunotherapy through immune checkpoints is a treatment method that modulates T-cell activity to kill tumor cells through a series of pathways, including co-suppression or co-stimulatory signaling (41,42). The present study also revealed that there were significant differences in the expression levels of immune checkpoint-associated genes, including HAVCR2, LAG3, PDCD1 and SIGLEC15, between the high and low GIPC2 expression groups. Taken together, these findings indicated that GIPC2 may have an important role in regulating tumor-infiltrating immune cells in COAD, and may be considered a biomarker for immune therapy.

In conclusion, the present study revealed that GIPC2 expression was significantly downregulated in COAD and that it was associated with malignant progression in patients with COAD. Furthermore, increased expression levels of GIPC2 may regulate the level of infiltrating immune cells and proteins involved in various pathways during COAD progression. With a deeper understanding of its function, GIPC2 may serve as an independent prognostic factor for COAD, and therefore may be a target for the diagnosis and treatment of COAD.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SZ and KM designed the study, collected data and performed the analysis. SZ drafted the manuscript. KM performed immunohistochemistry. Both authors read and approved the final manuscript. SZ and KM confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All patients provided written informed consent. The present study was approved by the Ethics Committee of the People's Hospital of Tongling City (approval no. 2022002).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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