Elevated G-Protein Receptor 125 (GPR125) Expression Predicts Good Outcomes in Colorectal Cancer and Inhibits Wnt/β-Catenin Signaling Pathway

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Background: G-protein receptor 125 (GPR125), as a transmembrane signal transducer, is involved in regulating cancer development. Although GPR125 is related with several cancers, its role in colorectal cancer (CRC) and the underlying mechanism are still unknown. Here, we investigated the clinical significance of GPR125 in CRC.

Material/Methods: We assessed the expression level of GPR125 in CRC tissues by analyzing 3 datasets in the Gene Expression Omnibus (GEO) database and in human samples. The correlation between GPR125 expression and clinicopathological features was further analyzed. Survival analysis was performed to assess the association between GPR125 expression and recurrence-free survival (RFS). Cox logistic regression analysis was used to analyze the role of GPR125 expression in overall survival (OS). Moreover, we activated the Wnt pathway in HCT116 cells to investigate their potential mechanism.

Results: Analysis of the GEO database showed that the expression of GPR125 was down-regulated in CRC tissues, consistent with our human samples experiments, and patients with higher GPR125 expression had a longer RFS. Also, we found that high GPR125 expression was associated with better tumor outcomes in clinical stage, metastasis, and KRAS status. Cox logistic regression analysis demonstrated that GPR125 was an independent prognostic factor for favorable outcome. Mechanistically, GPR125 overexpression inhibited the β-catenin transcriptional activity, and down-regulated the expression levels of the Wnt downstream proteins-Axin2, c-Myc, cyclinD1, and lef-1.

Conclusions: GPR125 may be a potential prognosis-related anti-oncogene and its effects on inactivating Wnt/β-catenin signaling pathway might be a key link to inhibiting CRC formation.

MeSH Keywords: Colorectal Neoplasms • Genes, Suppressor • Prognosis • Receptors, G-Protein-Coupled • Wnt Signaling Pathway

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Background

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-associated death worldwide [1,2], but therapeutic strategies, especially for advanced stages, have evolved relatively little.

The molecular pathogenesis of CRC is closely related with successive acquisition of genetic alterations that lead to aberrant activation of proto-oncogenes and inactivation of tumor suppressor genes in the Wnt/β-catenin signaling pathway [3,4]. For example, adenomatous polyposis coli (APC), whose mutation causes 85% of colorectal cancer formation, is involved in adenoma formation followed by oncogenic activation of KRAS, which promotes the transition from intermediate adenomas to carcinomas with TP53 inactivation as a late event [5]. Modification for mutation genes in the Wnt pathway is an important research focus in developing CRC therapies [6]. Recently, we found that silencing G-protein receptor 125 (GPR125) can up-regulate the canonical Wnt pathway [7]. Also, GPR125 was demonstrated to affect embryonic development via modulating Wnt signaling [8]. The Wnt pathway appears to be the most relevant pathway in CRC progression; therefore, we speculated that GPR125 might suppress CRC formation by interacting with the Wnt pathway.

GPR125 is a 57-KDa transmembrane signal transducer and belongs to the family of adhesion G-protein-coupled receptors (aGPCRs) [9]. Owing to its unique structure, GPR125 is postulated to play a crucial role in cell adhesion and signal transduction. Most studies on GPR125 are focussed on embryonic development [8,10]. In spermatogonial stem cells (SSCs), GPR125 was found to be a marker for SSCs, with great potential value in cell-based, autologous organ regeneration therapy for various diseases [11]. An increasing body of evidence shows the role of GPCRs and their ligands in various aspects of tumor biology [12,13]. Recent data indicate that GPCRs are associated with cancer initiation and progression, including aberrant cell proliferation, invasion, metastasis, adhesion, and angiogenesis [14,15]. GPR125 also importantly affects cancer development. A previous study showed that up-regulation of GPR125 by cooperating of MLL/AF10 (OM-LZ) and KRASG12C promoted cell adhesion and contributed to myeloid sarcoma (MS) formation [16]. GPR125 was reported to be highly expressed in human brain cancer tissues, in which GPR125 was identified as an oncogene [17]. However, no information is available concerning the role of GPR125 in CRC formation.

In the present study, we investigated the clinical significance of GPR125 in CRC and its potential mechanism. Through bio-informatics analysis and experimental evidence, we found that GPR125 was expressed at higher levels in normal colon than in cancer tissue. In addition, we evaluated the prognostic value of GPR125 and demonstrated that patients with lower GPR125 expression had shorter survival and higher risk of tumor recurrence, KRAS mutation, and metastasis. Finally, we demonstrated the effects of GPR125 on inhibiting the Wnt/β-catenin signaling pathway.

Material and Methods

Clinical specimens

The human colorectal cancer tissues used in this study comprised 18 CRC specimens (average diameter: 5.32±1.02 mm; T stage: II–IV) with corresponding non-cancerous tissues, all acquired from our hospital. The human colon tissue microarray (BDCS118c) used in this study contained 50 CRC specimens and corresponding non-cancerous tissues and were purchased from Xi’an Alena Bio (Xi’an, China).

Cell culture and materials

Plasmids of GPR125, TOPFLASH/FOPFLASH vector, p-cDNA3.1, Renilla luciferase pRL-TK reporter vector were got from East China Normal University (ENCU). HCT116 cells were purchased from the Cell Bank of the Shanghai Academy of Science and were maintained in DMEM with 10% fetal bovine serum (Gibco) at 37°C and 5% CO₂ in an incubator.

Data mining

Three datasets (GSE20916, GSE21510, and GSE8671) were acquired from the GEO database https://www.ncbi.nlm.nih.gov/geo. The data was processed by bioconductor of R language for further analysis. We used the t test for statistical analysis of the differential expression of GPR125 between cancerous and non-cancerous colon tissues. The correlation of GPR125 expression with RFS was assessed based on the original data from the GEO database and analyzed using the survival package of R language. Another dataset containing 151 cases of colorectal cancer, download from The Cancer Genome Atlas (TCGA) CRC database https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm, was used to explore the association between GPR125 and clinicopathological features. Univariate and multivariate analyses were performed to analyze the association between GPR125 expression and overall survival (OS).

TOP/FOPFLASH reporter assay

Transfection of the GPR125 plasmid (0.375 ug per well) into HCT116 cells as an experimental group was performed in a 24-well plate with Lipofectamine 2000 (Invitrogen, Inc.) using the methods recommended by the manufacturer, while p-cDNA3.1 (0.375 ug per well) was transfected into HCT116 cells.
as a control group. For the measurement of TCF/β-catenin activity, TOPFLASH vector (0.375 ug per well) and Renilla luciferase pRL-TK reporter vector (37.5 ng per well) were co-transfected into HCT116 cells. The FOPFLASH vector used the same as the TOPFLASH vector as an internal reference. About 48 h later, Wnt3a-conditional media was used to activate the Wnt pathway for another 24 h. TCF/β-catenin activity was measured using the dual luciferase reporter system and normalized to Renilla luciferase activity.

**Immunofluorescence staining**

After transfection with GPR125 plasmid (3 ug per well) into HCT116 cells in 6-well plates for 48 h with DMEM as an experimental group, we incubated cells with 10% fetal bovine serum (Gibco) at 37°C and a 5% CO₂ incubator and stimulated them with Wnt3a-conditional media for another 2 h. HCT116 cells that adhered to the surface of elastic-bottomed plates were rinsed twice with PBS, fixed with 4% paraformaldehyde for 30 min, and rinsed twice with PBS again. Then, cells were incubated with the primary antibody (Abcam, mouse anti-active catenin, 1: 500) at 4°C for 12 h and the secondary antibody (Abcam, mouse-antibody, 1: 500) at room temperature for 2 h. After 3 washes in PBS, 4', 6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) (1 ug/ml, Sigma) was used to mark the nuclei for 5 min. Cell imaging was performed on a Leica TCS SP5 DMI6000 microscope (Leica, Wetzlar, Germany) using argon ion (488 nm), HeNe (543 nm), and violet diode (405 nm) lasers. Images were acquired sequentially by direct register using LAS AF Leica Confocal software.

**RNA extraction and real-time PCR analysis**

Total cellular or tissue RNA was extracted from the different groups using TRIzol reagent (Invitrogen, MA, USA). Subsequently, CDNA was synthesized with 900 ng of total RNA by reverse transcriptase (Takara, Shiga, Japan) according to the manufacturer's instructions. The CDNAs were amplified by PCR using the ABI 7500 Thermal Cycler Dice™ real-time system (Applied Biosystems, USA). The thermal cycler protocols included 3 min at 95°C and 40 cycles of 5 s at 95°C and 30 s at 60°C. The primer sequences are listed in Table 1. For each sample (GPR125, Axin2, c-Myc, cyclinD1, lef-1), relative mRNA expression levels were derived from the ratio of their expression to GAPDH expression as an internal standard using the 2−ΔΔCt method.

### Table 1. Human primers set for q-RTPCR analysis.

| Gene     | Direction | Primer sequence                  |
|----------|-----------|----------------------------------|
| GAPDH    | Forward   | TCAGAGGCGAACAACACAC             |
|          | Reverse   | GGCCTTTGCTAGTTTCAAA             |
| GPR125   | Forward   | TTTCAAGCTACAAATCTGG             |
|          | Reverse   | CCAAAAGCGTTAAGCGAACAGAAG       |
| C-MYC    | Forward   | TCAGAGGGCAACAACACAC             |
|          | Reverse   | GGCCTTTGCTATGTTCTCAAA           |
| Axin2    | Forward   | TACACTCCTTAATGCGGCTACTA        |
|          | Reverse   | GGCTGCTATCGTAAGGTGTTG           |
| Cycld1   | Forward   | GTGCCGTCTAGATGAAGGGAGA          |
|          | Reverse   | GGAGATTGCTAGAAGACCTG            |
| Lef-1    | Forward   | TGCCAAATATGAAATAGCGACCA         |
|          | Reverse   | GAGAAAAGTGCTCGTCACCTG           |

**IHC**

The colorectal cancer tissue microarray (TMA) was purchased from Xi’an Alena Bio (Xi’an, China), and then deparaffinized by xylene, followed by rehydration through a graded series of ethanol, and then incubated with blocking solution for 30 min at 25°C. Subsequently, TMA was stained with rabbit GPR125 antibody (1: 30, ab51705, Abcam, USA) at 4°C overnight. After washing 3 times, TMA was incubated for 1 h with biotinylated goat anti-rabbit antibody IgG and then for 30 min with Streptavidin-HRP peroxidase. Color reaction product was visualized by using diaminobenzidine (DAB)-H₂O₂ as the substrate for peroxidase. TMA was counterstained with hematoxylin.

**Statistical analysis**

Statistical analyses were performed by using SPSS 17.0 statistical package software (SPSS, Chicago, IL, USA) or GraphPad Prism (GraphPad Software Inc., San Diego, CA). The t test was performed to explore the expression pattern of GPR125 in CRC. Cumulative survival time was calculated by the Kaplan-Meier method and analyzed by the log-rank test. The correlation of GPR125 expression with clinicopathological parameters was evaluated by the X² test. Univariate and multivariate Cox regression analyses were performed to identify the factors that had a significant influence on survival by Cox proportional hazards model. P<0.05 was considered to indicate a significant difference.

**Results**

**GPR125 expression was down-regulated in colorectal cancer.**

To investigate the expression level of GPR125 mRNA between cancer and normal colon tissues in CRC patients, we first analyzed the information from 3 GEO datasets (GSE20916,
Figure 1. Differences in GPR125 expression in normal and tumor tissue analyzed by GEO database and human specimens. (A–C) Bioinformatics analysis for the expression of GPR125 in cancer tissues compared to normal tissues; (D) Expression of GPR125 mRNA in cancer (n=18) and adjacent normal tissue (n=18). The Q-PCR analysis verified that GPR125 expression was up-regulated in normal tissue. (Paired t test, P<0.001). (E) IHC analysis of GPR125 expression in human CRC tissues (upper; n=50) and normal colon tissues (lower; n=50). Typical fields of view are presented (Scale bar, 50 μm; original magnification, ×20 and ×40). GPR125 was highly expressed in normal tissues and found to stain mainly the cytoplasm and membrane of cells. (F) The semi-quantitative analysis of GPR125 immunohistochemical staining in human normal colon and cancer tissues.
As shown in Figure 1A–1C, GPR125 mRNA expression was markedly up-regulated in normal tissues in comparison to their cancerous counterparts.

In order to confirm the expression pattern of GPR125 in human colorectal cancer, we collected 18 pairs of CRC specimens with corresponding non-cancerous tissues, and then extracted their RNA. Our work also demonstrated that total expression levels of GPR125 mRNA were markedly up-regulated in normal tissues (P<0.01) (Figure 1D). Meanwhile, 83.3% of adjacent normal tissues expressed higher GPR125 level than their matched cancer tissues (Figure 1E).

Furthermore, we purchased a commercial colorectal cancer tissue microarray (TMA) with relatively complete pathological information. Besides cancer tissue, 50 matched normal tissue spots were found on this TMA. Expression of GPR125 in the TMA were detected by immunohistochemistry (IHC). We observed that in colorectal adenocarcinoma cells, GPR125 was found to stain mainly in the cytoplasm and membrane of cells (Figure 1F). In order to assess the difference in expression between carcinoma and normal tissue, we quantified the expression level of this protein. Two independent pathologists with no knowledge of the clinical characteristics of the patients were employed to score staining intensity (0–4) and extent (0–100) of GPR125. (t test, P<0.01). As expected, GPR125 expression was significantly reduced in the tumor tissues compared with the normal tissues; 78% of colorectal cancer tissues showed negative staining for GPR125, while 66% of normal tissues showed obvious positive staining (Figure 1G).

Correlations between GPR125 expression and clinicopathological features

To determine whether GPR125 expression is associated with the tumorigenesis and progression of CRC, we investigated the relationship of this gene with clinicopathological features. We divided CRC patients into low- and high-GPR125-expression groups on the basis of the median value. In the TMA cohort, results showed that early clinical stage (P=0.0008) and negative tumor metastasis (P=0.0467) were significantly related to high GPR125 expression (Table 2). We analyzed a TCGA dataset with data on 151 CRC patients to investigate the correlation between GPR125 and these specific clinicopathological features: KRAS mutation and microsatellite. The results showed high of microsatellite instability; MSI-L – low of microsatellite instability; MSM – microsatellite miss.

Table 2. Association between GPR125 and clinicopathological characteristics in TMA cohort.

| Expression | High | Low | p-Value |
|------------|------|-----|---------|
| n (%)      | 11 (22%) | 39 (78%) | 0.8805 |
| Gender     |      |      |         |
| Male       | 5 (10%) | 19 (38%) | 0.7435 |
| Female     | 6 (12%) | 20 (40%) |         |
| Age (year) |      |      |         |
| <50        | 3 (6%) | 15 (30%) | 0.2487 |
| >50        | 8 (16%) | 24 (48%) |         |
| Grade      |      |      |         |
| I          | 5 (10%) | 6 (12%) | 0.0008* |
| II         | 4 (8%) | 12 (24%) |         |
| III        | 2 (4%) | 11 (22%) |         |
| Tumor size |      |      |         |
| T1–2       | 8 (72.7%) | 6 (15.4%) |         |
| T3–4       | 3 (36.3%) | 33 (84.6%) |         |
| Node status|      |      |         |
| Negative   | 6 (12%) | 22 (44%) | 0.8152 |
| Positive   | 5 (10%) | 17 (34%) |         |
| Metastasis |      |      |         |
| Yes        | 4 (18.2%) | 29 (58%) | 0.0467* |
| No         | 7 (31.8%) | 10 (20%) |         |

Table 3. Association between GPR125 and clinicopathological characteristics in TCGA cohort.

| Expression | High | Low | p-Value |
|------------|------|-----|---------|
| n (%)      | 61 (40.1%) | 90 (59.6%) | 0.1198 |
| Gender     |      |      |         |
| Male       | 37 (60.7%) | 43 (47.8%) | 0.7162 |
| Female     | 24 (39.3%) | 47 (52.2%) |         |
| Age (year) |      |      |         |
| <50        | 32 (52.5%) | 44 (48.9%) | 0.006 |
| >50        | 29 (47.5%) | 46 (51.1%) |         |
| Stage      |      |      |         |
| T1–2       | 25 (41.0%) | 57 (63.3%) | 0.0368* |
| T3–4       | 36 (59.0%) | 33 (36.7%) |         |
| KRAS mutation |      |      |         |
| Positive   | 22 (36.1%) | 48 (53.3%) | 0.9248 |
| Negative   | 39 (63.9%) | 42 (46.7%) |         |
| Microsatellite |      |      |         |
| MSI-H      | 24 (39.3%) | 35 (38.9%) |         |
| MSI-L      | 20 (32.8%) | 32 (36.6%) |         |
| MSM        | 17 (27.9%) | 23 (25.5%) |         |

Correlations between GPR125 expression and clinicopathological features

To determine whether GPR125 expression is associated with the tumorigenesis and progression of CRC, we investigated the relationship of this gene with clinicopathological features. We divided CRC patients into low- and high-GPR125-expression groups on the basis of the median value. In the TMA cohort, results showed that early clinical stage (P=0.0008) and negative tumor metastasis (P=0.0467) were significantly related to high GPR125 expression (Table 2). We analyzed a TCGA dataset with data on 151 CRC patients to investigate the correlation between GPR125 and these specific clinicopathological features: KRAS mutation and microsatellite. The results showed...
that GPR125 expression was negatively associated with KRAS mutation (P=0.0368) and T3–T4 stage (Table 3). However, no significant associations were found between CPR125 expression and age or gender in either cohort (P>0.05).

High GPR125 expression is correlated with favorable recurrence-free survival (RFS)

To further explore the clinical significance of GPR125, RFS data were used to assess whether GPR125 had prognostic significance in CRC. Data mining in R2 was performed using the datasets GSE20916, GSE21510, and GSE8671. In GSE20916, Kaplan-Meier analysis revealed that patients with higher GPR125 expression levels had favorable RFS (log-rank P=0.0013). The Cox proportional hazard model revealed that low expression of GPR125 increased the hazard ratio (HR) for death in colorectal cancer patients (HR 1.51; 95% CI, 1.17-1.95, P=0.0014; Figure 2A). For GSE21510 and GSE8671, the results were (log-rank P=0.0233; HR 1.94; 95% CI, 1.10-3.48, P=0.0233) and (log-rank P=0.0151; HR 2.15; 95% CI, 1.16–3.99, P=0.0151) (Figure 2B, 2C). All 3 datasets demonstrated that higher GPR125 expression was significantly associated with favorable 10-year RFS.

Figure 2. The prognostic value of GPR125 in human colorectal cancer. (A–C) Kaplan-Meier analysis of the recurrence-free survival (RFS) in GPR125 high-expression (green) patients and low-expression (red) patients according to 3 datasets from the GEO cohort. The median value was used as a cut-off (log-rank test, P<0.0.5). Patients with low GPR125 expression had higher risk of recurrence.

Table 4. Univariate and multivariate analyses for over survival (OS) in TCGA CRC cohort.

| Variables          | Univariate analysis | Multivariate analysis |
|--------------------|---------------------|-----------------------|
|                    | HR | 95% CI   | P value | HR | 95% CI   | P value |
| Age                |    |          |         |    |          |         |
| <50                | 1  |          |         |    |          |         |
| >50                | 1.135 | 0.475–2.546 | 0.689 | 1  |          |         |
| Gender             |    |          |         |    |          |         |
| Male               | 1  |          |         |    |          |         |
| Female             | 1.221 | 0.734–2.345 | 0.566 | 1  |          |         |
| KRAS mutation      |    |          |         |    |          |         |
| Negative           | 1  |          |         |    |          |         |
| Positive           | 1.458 | 0.874–2.754 | 0.347 | 1  |          |         |
| T stage            |    |          |         |    |          |         |
| T1–2               | 1  |          |         |    |          |         |
| T3–4               | 2.356 | 1.367–4.545 | 0.008* | 2.124 | 1.281–4.314 | 0.011* |
| GPR125 expression  |    |          |         |    |          |         |
| High               | 1  |          |         |    |          |         |
| Low                | 2.364 | 1.293–4.455 | 0.007* | 2.011 | 1.127–3.971 | 0.018* |
High GPR125 expression improves overall survival (OS) of CRC patients.

The relationship between GPR125 expression and overall survival was then further analyzed in the TCGA CRC cohort. Univariate logistic regression analysis showed that the significant variables for favorable OS were early tumor stage (HR=2.356, 95% CI: 1.367–4.545; P=0.008) and high GPR125 expression (HR=2.364, 95% CI: 1.293–4.455; P=0.007). Multivariate logistic analysis using these 2 parameters also revealed that early tumor stage (HR=2.124, 95% CI: 1.281–4.314; P=0.011) and high GPR125 expression (HR=2.011, 95% CI: 1.127–3.971; P=0.018)

Figure 3. GPR125 overexpression inhibited Wnt/β-catenin signaling pathway in vitro. (A) Western blots analysis compared GPR125 expression in HCT116 cells transfected with or without GPR125 mimics. (B, C) TOPFLASH activity showed that GPR125 overexpression inhibited the β-catenin transcriptional activity in HCT116 cells. Luciferase activity in FOPFLASH remained unaffected, confirming a lack of nonspecific activation of the reporter system. (D) Immunofluorescence analysis was used to detect the amount of β-catenin transferred into the nucleus. GPR125 overexpression (Lower) inhibited the amount of β-catenin into the nucleus compared with the control group (Upper) (Scale bar, 100 um). (E) Overexpression of GPR125 down-regulated the mRNA expression levels of Axin2, CyclinD1, C-MYC, and Lef-1 in HCT116 cells.
were positively correlated with longer survival time (Table 4). In summary, GPR125 overexpression can improve the overall survival of CRC patients.

**GPR125 overexpression inhibited Wnt/β-catenin signaling pathway in vitro**

Next, we investigated the mechanism by which GPR125 suppresses CRC formation. Western blot analysis showed that GPR125 protein level was markedly increased in HCT116 cells after transfection of GPR125 mimics (Figure 3A). TOPFLASH assay showed that GPR125 overexpression reduced β-catenin transcriptional activation by 2-fold compared to the control group (Figure 3B). Luciferase activity in FOPFLASH remained unaffected, confirming the lack of nonspecific activation of the reporter system (Figure 3C). Consistent with the above results, immunofluorescence staining (IF) assay further confirmed the effects of GPR125 in significantly reducing the amount of β-catenin transferred into the nucleus (Figure 3D). In addition, the changing levels of the Wnt downstream proteins in response to GPR125 function are of importance. For quantitative determination of the mRNA expression levels of those proteins, qRT-PCR analysis was used to investigate the relative Axin2, c-Myc, cyclinD1, and lef1 mRNA levels. Our data show that GPR125 overexpression markedly down-regulated the expression levels of those proteins, especially for lef1 and c-Myc (Figure 3E). Thus, overexpression of GPR125 inactivated the classical Wnt/β-catenin signaling pathway.

**Discussion**

Colorectal cancer (CRC) develops via a series of genetic and epigenetic changes that result in the transformation of normal mucosa to a premalignant polyp, and ultimately to cancer [18,19]. Those regulatory genes are closely related with prognosis of patients, and their clinical significance provides strong evidence to guide disease diagnosis and treatment [20,21]. Thus, understanding the clinical significance of the regulatory gene and its mechanism is essential to optimizing current therapeutic strategies in CRC.

There are few recent studies on GPR125 in cancers, especially in CRC. No study has reported the potential role of GPR125 in CRC. Here, we examined the clinical implications of GPR125 in CRC. Our data first revealed that GPR125 was frequently down-regulated in clinical cancer tissue, particularly in the presence of advanced, metastatic, or KRAS mutation tumors. Also, GPR125 overexpression was significantly related with better prognosis of patients with CRC in recurrence-free survival (RFS) and overall survival (OS). These clinical data indicate that GPR125 is a suppressor gene in CRC. Additionally, to establish the mechanism of action of GPR125, we investigated the effects of GPR125 on the Wnt/β-catenin signaling pathway, showing that GPR125 can inhibit human CRC progression via inactivating this pathway.

The expression pattern of regulatory genes in cancers is of importance to measure their clinical significance. APC mutations occur in 80–90% of spontaneous colorectal cancers [22]. BRAF mutations occur in 5–22% of all colorectal cancers [23]. In our study, though bioinformatics analysis and tumor samples experiments, we found that the expression of GPR125 was more frequently down-regulated in cancer than in normal tissue. This clinical factor may alert clinicians to patients who have an increased probability of having a GPR125 mutation tumor.

In term of prognosis, an emerging body of literature shows that advanced tumors along with metastasis are associated with high risk of recurrence [24]. KRAS mutation status is a strong predictive marker of resistance to EGFR-targeted therapy in patients with metastatic colorectal cancer [25]. In our study, through analyzing the correlation of GPR125 expression with clinicopathological characteristics, we found that high GPR125 expression was positively correlated with early tumor stage, and benefited patients with less tumor metastasis and KRAS mutation, which suggests a role of GPR125 as a favorable prognostic indicator. Moreover, survival analysis showed that patients with low GPR125 expression were at significantly higher risk of tumor recurrence and had shorter survival. These findings therefore support that GPR125 is an independent protective factor for CRC and might function as a biomarker to provide evidence for therapeutic strategies.

Previous studies reported that GPR125 participates in the carcinogenesis of 2 cancers as a potential oncogene, and the reported effects of GPR125 on cancers did not agree with our results [16,17]. However, genetic alteration causing tumorigenesis is an extremely complex process that involves interaction among protein function areas, receptors, tumor-related pathways, and cancer types [26,27]. It is plausible that GPR125 influences CRC formation through other modes of action and works as a tumor suppressor in human CRC progression. Thus, studying the possible mechanism of GPR125 in CRC is important.

Wnt/β-catenin signaling pathway is the most relevant target-based approach for CRC treatment. However, it is still unclear if this signaling pathway is related with GPR125 in inhibiting colorectal cancer formation. A large-sample gene screening study reported that silencing GPR125 can up-regulate the Wnt pathway, but this needs to be confirmed by further experimental evidence [7]. Another report demonstrated that GPR125 influenced Wnt/PCP pathway activity, in part through modulating embryo development [28]. In the present study, we hypothesized that there is an association between the Wnt pathway...
and GPR125 in inhibiting CRC. Our data showed that GPR125 overexpression in HCT116 cell markedly inhibited β-catenin transcriptional activity though TOPFLASH activity and immunofluorescence staining. Moreover, qRT-PCR results demonstrated that GPR125 overexpression in HCT116 cells downregulated the expression levels of Axin2, cyclinD1, c-Myc, and lef-1 in the Wnt pathway. These findings indicate the Wnt/β-catenin signaling pathway may have important downstream effects on GPR125.

Conclusions

Using a combination of bioinformatics and biological experiments, we demonstrated that GPR125 is frequently down-regulated in clinical CRC samples and its overexpression is significantly related with favorable prognosis in terms of RFS and OS. We also explored the effects of GPR125 on the Wnt/β-catenin signaling pathway determine whether GPR125 inactivated this pathway by inhibiting the β-catenin transferred into the nucleus. Our results suggest that GPR125 is an essential factor in protecting against human CRC formation, and might be a potential target for clinical therapeutic strategies.

Conflict of interest

None.

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