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ORIGINAL ARTICLE

Basic Study

Overexpression of G protein-coupled receptor 31 as a poor prognosticator in human colorectal cancer

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Abstract

AIM

To investigate the expression of G protein-coupled receptor 31 (GPR31) and its clinical significance in human colorectal cancer (CRC).

METHODS

To determine the association between the GPR31 expression and the prognosis of patients, we obtained paraffin-embedded pathological specimens from 466 CRC patients who underwent initial resection. A total of 321 patients from the First Affiliated Hospital of Sun Yat-sen University from January 1996 to December 2008 were included as a training cohort, whereas 145 patients from the Sixth Affiliated Hospital of Sun Yat-sen University from January 2007 to November 2008 were included as a validation cohort. We examined GPR31 expression levels in CRC tissues from two independent cohorts via immunohistochemical staining. All patients were categorized into either a GPR31 low expression group or a GPR31 high expression group. The clinicopathological factors and the prognosis of patients in the GPR31 low expression group and GPR31 high expression group were compared.

RESULTS

We compared the clinicopathological factors and the prognosis of patients in the GPR31 low expression group and GPR31 high expression group. Significant differences were observed in the number of patients in pM classification between patients in the GPR31 low expression group and GPR31 high expression group ($P = 0.007$). The five-year survival and tumor-free survival rates of patients were 84.3% and 82.2% in the GPR31 low expression group, respectively, and both rates were 59.7% in the GPR31 high expression group ($P < 0.05$). Results of the Cox proportional hazard regression model revealed that GPR31 upregulation was associated with shorter overall survival and tumor-free survival of patients with CRC ($P < 0.05$). Multivariate analysis identified GPR31 expression in colorectal cancer as an independent predictive factor of CRC patient survival ($P < 0.05$).

CONCLUSION

High GPR31 expression levels were found to be correlated with pM classification of CRC and to serve as an independent predictive factor of poor survival of CRC patients.

Key words: G protein-coupled receptor 31; Colorectal cancer; Predictive factor; Metastasis; Clinical significance

Core tip: G protein-coupled receptor 31 (GPR31) is a member of the G protein-coupled receptor superfamily whose biological function remains unclear in colorectal cancer (CRC). Expression of GPR31 and its prognostic significance in human CRC have not been studied. The present study aimed to investigate the expression of GPR31 and its clinical significance in human CRC. In our study, high GPR31 expression levels were found to be correlated with pM classification of CRC and to serve as an independent predictor of poor survival in patients with CRC.

Rong YM, Huang XM, Fan DJ, Lin XT, Zhang F, Hu JC, Tan YX, Chen X, Zou YF, Lan P. Overexpression of G protein-coupled receptor 31 as a poor prognosticator in human colorectal cancer. *World J Gastroenterol* 2018; 24(41): 4679-4690 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i41/4679.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i41.4679

INTRODUCTION

Based on the statistics of the American Cancer Society (ACS), colorectal cancer (CRC) has become the second common cause of cancer deaths[1]. CRC causes over 600 thousand deaths each year all over the world and is the fifth common cause of cancer deaths in China[2,3]. The incidence rate of CRC is predicted to increase both in urban and rural areas in the next few years[4]. The prognosis of CRC has improved recent years because of the continued advancements in CRC diagnosis and treatment[5,6]. However, many CRC patients are incurable because of various reasons[7,8].

Nowadays, the American Joint Committee on Cancer (AJCC) staging system is still the gold standard to predict the prognosis of CRC patients. However, the AJCC staging system has certain limitations due to the heterogeneity of CRC. Other reliable biomarkers, which can provide guidance to the treatment of CRC and help predict treatment prognosis, have gradually received interest of clinicians[9,10].

G protein-coupled receptor 31 (GPR31) is a member of the G protein-coupled receptor (GPCR) superfamily and has been identified as a target receptor for 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE][11]. 12(S)-HETE is an eicosanoid product of arachidonate metabolism by the enzyme 12-lipoxygenase (12-LOX), which was first demonstrated by Hamberg and Samuelsson[12]. 12(S)-HETE plays an important role in many physiological and pathological processes, such as cell growth, adhesion, differentiation, angiogenesis, inflammation, atherosclerosis and cancer promotion[13-17]. Previous studies have demonstrated that 12(S)-HETE promotes cell migration during tumor progression by eliciting a wide variety of physiological and pathological responses[18-22]. It is showed that 12(S)-HETE could

induce changes of cancer cell cytoskeleton and result in enhanced tumor invasion and motility[23,24], which in turn enhances tumor cell motility[16]. Exogenous addition of 12(S)-HETE induces overexpression of proteinases[25-27], vascular endothelial growth factor[28], integrins[23,29] and fibronectin[30] in cancer cells, thereby prolonging cell survival[21,31]. 12(S)-HETE promotes adhesion of tumor cell by inducing the nondestructive retraction of monolayers in endothelial cells[32,33]. 12(S)-HETE also promotes tube formation by enhancing the motility of isolated endothelial cells[34]. The diverse biological effects of this important proinflammatory metabolite suggest that 12(S)-HETE potentially acts through a cognate receptor, which was identified as GPR31[11].

In this present study, we aimed to elucidate the association between the expression level of GPR31 and CRC progression.

MATERIALS AND METHODS

Patients

To determine the association between the expression of GPR31 and the prognosis of CRC patients, we obtained tissue specimens from 466 CRC patients who underwent surgery. A total of 321 patients treated at the First Affiliated Hospital of Sun Yat-sen University (SYSU) from January 1996 to December 2008 were included as a training cohort, whereas 145 patients treated at the Sixth Affiliated Hospital of SYSU from January 2007 to November 2008 were included as a validation cohort. The patients who underwent initial colorectal resection were included in this study. Patients who received neoadjuvant therapy were excluded. Abdominal ultrasonography, chest X-ray, magnetic resonance imaging (MRI), computed tomography (CT), bone scans or positron emission tomography-computed tomography (PET-CT) were performed to identify tumor recurrence and distant metastasis. Clinical data including demographics, surgical method, tumor location, differentiation, TNM status and follow-up data were collected from the CRC database of each hospital. MRI and/or CT were used to evaluate the patients at 3, 6, 9, 12, 18 and 24 mo after surgery in the first two years, and annually after that. The primary endpoint of this study was described as the overall survival (OS) and was defined as the time interval between the first surgery to clinical death.

TMA construction and IHC analysis

Tissue microarrays (TMA) were considered as an array fashion to facilitate multiplex analysis of histology[35]. In our study, the TMAs were constructed respectively by two skilled researchers. During the process of TMA experiment, the central portion of neoplasm tissue was selected by two skilled pathologists, after which two pieces of each sample were picked out and deposited into the tissue array instrument (Beecher Instruments, Alphelys, France). TMA blocks were subsequently sliced into 5-μm thick sections before immunohistochemistry (IHC) staining. TMA slides were deparaffinized, rehydrated, exposed to the antigen retrieval system, endogenous peroxidase blocked, primary antibody incubated, stained with diaminobenzidine and counterstained with hematoxylin according to the methods of our previous study[2]. The primary antibody of GPR31 (Santa Cruz Biotechnology, Inc., Dallas, TX, United States) was used in a dilution of 1:200.

Evaluation of IHC analysis

Immunoreactivity of GPR31 protein was examined according to previous studies[1,36]. Spots of TMA were scored ranging from 0 to 3 according to the intensity by two separated researchers. The percentage of positive cancer cells was described as 0%, 5%, …, 95%, 100%. The H score (0 to 300) was determined by calculating the sum of the product of the intensity score and the proportion of the corresponding stained area for each intensity score. The average H score was calculated by two professional researchers finally.

Cut-off point determination

Receiver operating characteristic (ROC) curve was used to determine the cutoff score according to previous studies[1,37]. The H score which met both highest sensitivity and highest specificity was determined as the final cut-off point. Neoplasm tissues were described as "low expression" if they had scores lower than or equal to the cutoff point, whereas neoplasm tissues with scores above the cutoff point were designated as "high expression". Clinicopathological features including differentiation, pT status, pN status, pM status, TNM stage, and survival were dichotomized for ROC curve analysis, the same as our previous study[1].

Statistical analysis

Relationship between GPR31 protein levels and the clinicopathological characteristics were analyzed using methods according to our previous study[1]: Chi-square test for categorical variables, Student's *t*-test for quantitative data which meet homogeneity and normality, Kaplan-Meier curves with a log-rank test for the correlation of the GPR31 and patient survival, and forward stepwise method for construction of a multivariate Cox proportional hazard regression model. SPSS (v19.0, Chicago, IL, United States) was used for our statistical analyses. $^{a}P < 0.05$ was considered statistically significant in this study.

RESULTS

Patient clinical features

The baseline clinical features of the two cohorts were listed in Table 1. Four hundred and sixty-six patients with CRC were included for analysis. Three hundred and twenty-one patients were included in the training cohort and 145 in the validation cohort. Two hundred and sixty-five males and 201 females were recruited. There were

Table 1 Clinicopathological characteristics of patients with different G protein-coupled receptor 31 expression levels in colorectal cancer *n* (%)

Variable	GPR31 expression							
	Training cohort				Validation cohort			
	Cases	Low	High	*P* value	Cases	Low	High	*P* value
Age (yr)	321	57.7 ± 14.3	59.6 ± 13.7	0.223	145	61.6 ± 13.4	64.0 ± 13.1	0.324
Gender				0.674				0.921
Female	148	89 (60.1)	59 (39.9)		53	39 (73.6)	14 (26.4)	
Male	173	108 (62.4)	65 (37.6)		92	67 (72.8)	25 (27.2)	
BMI (kg/m^2)	315	21.1 ± 4.0	21.5 ± 3.1	0.350	71	21.8 ± 3.1	22.8 ± 2.2	0.192
Preoperative ileus				0.051				0.402
Yes	25	20 (80.0)	5 (20.0)		33	26 (78.8)	7 (21.2)	
No	294	177 (60.2)	117 (39.8)		112	80 (71.4)	32 (28.6)	
CEA (ng/mL)				0.949				0.954
< 5	200	125 (62.5)	75 (37.5)		90	66 (73.3)	24 (26.7)	
⩾ 5	97	61 (62.9)	36 (37.1)		42	31 (73.8)	11 (26.2)	
CA199 (ng/mL)				0.399				0.534
< 37	218	142 (65.1)	76 (34.9)		105	77 (73.3)	28 (26.7)	
⩾ 37	64	38 (59.4)	26 (40.6)		21	14 (66.7)	7 (33.3)	
Tumor location				0.764				0.404
Colon	156	93 (59.6)	63 (40.4)		64	49 (76.6)	15 (23.4)	
Rectal	163	103 (63.2)	60 (36.8)		81	57 (70.4)	24 (29.6)	
Size (cm)	320	5.1 ± 2.2	4.9 ± 2.0	0.380	143	4.8 ± 2.0	4.5 ± 1.7	0.446
Histopathology				0.551				0.406
Adenocarcinoma	283	172 (60.8)	111 (39.2)		128	95 (74.2)	33 (25.8)	
Others	38	25 (65.8)	13 (34.2)		17	11 (64.7)	6 (35.3)	
Differentiation				0.464				0.305
Well/moderate	271	164 (60.5)	107 (39.5)		112	84 (75.0)	28 (25.0)	
Poor	50	33 (66.0)	17 (34.0)		29	19 (65.5)	10 (34.5)	
pT classification				0.592				0.006[a]
T1/T2	60	35 (58.3)	25 (41.7)		39	35 (89.7)	4 (10.3)	
T3/T4	261	162 (62.1)	99 (37.9)		106	71 (67.0)	35 (33.0)	
pN classification				0.643				0.767
N0	194	117 (60.3)	77 (39.7)		81	60 (74.1)	21 (25.9)	
N1	124	78 (62.9)	46 (37.1)		64	46 (71.9)	18 (28.1)	
pM classification				0.007[a]				0.018[a]
M0	298	189 (63.4)	109 (36.6)		127	97 (76.4)	30 (23.6)	
M1	23	8 (34.8)	15 (65.2)		18	9 (50.0)	9 (50.0)	
TNM stage				0.885				0.360
Ⅰ/Ⅱ	188	116 (61.7)	72 (38.3)		76	58 (76.3)	18 (23.7)	
Ⅲ/Ⅳ	133	81 (60.9)	52 (39.1)		69	48 (69.6)	21 (30.4)	

[a]*P* < 0.05; GPR31: G protein-coupled receptor 31; BMI: Body mass index; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 19-9.

99 early stage patients (stage Ⅰ or Ⅱ) and 367 advanced stage patients (stage Ⅲ or Ⅳ). For all cases, the mean follow-up period was 58.4 mo (range, 0.5 to 123.5 mo). One hundred and seventy-nine patients died during the follow-up period. In the training cohort, 173 patients were male (53.9%) and 148 (46.1%) were female, with an average age of 58.7 years. Tumors were located in the colon in 156 (48.6%) patients and the rectum in 163 (50.8%) patients (two patients had no record). The mean follow-up period was 60.1 mo. In the validation cohort, 92 (63.4%) patients were male, and 53 (36.6%) were female, with an average age of 57.3 years. These patients included 64 (44.1%) colon cancer patients and 81 (55.9%) rectal cancer patients and had a mean follow-up period of 55.42 mo.

Cut-off point of GPR31 expression levels

The H score, ranging from 0 to 300, was determined by calculating the sum of the product of the intensity score and the proportion of the corresponding stained area for each intensity score. ROC curve analysis was used to figure out the cut-off point of the different patterns of GPR31 expression. According to the generated ROC curve, the cut-off point of GPR31 expression levels was 185. H score more than 185 was categorized as "high expression", otherwise it was categorized as "low expression". In the training cohort, 124 samples were categorized as "high expression" and 197 were categorized as "low expression" based on the H scores. Thirty-nine were categorized as "high expression" and 106 were categorized as "low expression" in the validation cohort. Figure 1 shows the representative IHC staining for GPR31 in CRC tissue and adjacent normal colorectal mucosa. Figure 2 shows the corresponding area under curve (AUC).

Correlation between GPR31 level and clinicopathological characteristics

Advanced correlation analyses revealed that GPR31 level was notably associated with pM classification in the

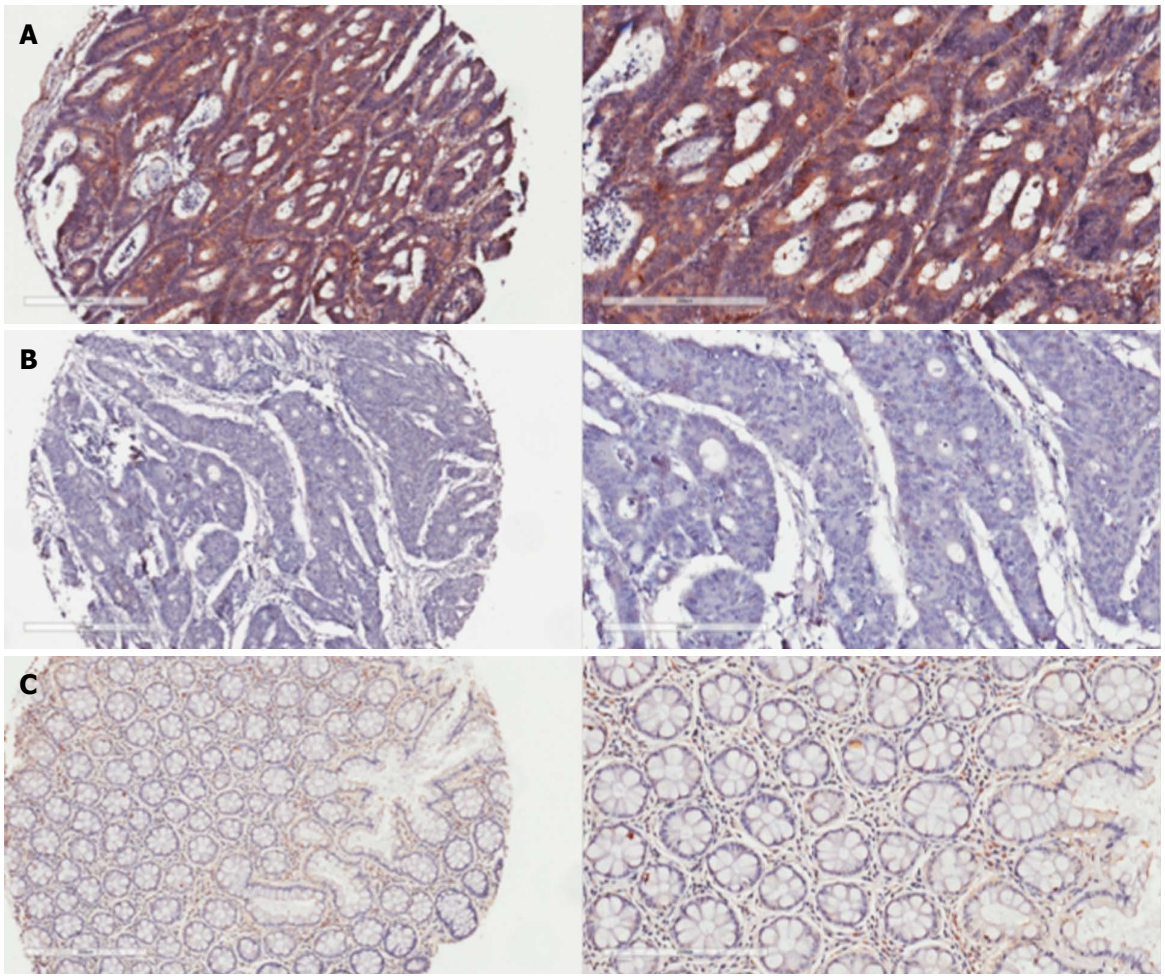

Figure 1 Immunohistochemistry staining of representative high- and low-G protein-coupled receptor 31-expressing samples of colorectal cancer and adjacent normal colorectal mucosa. A: High G protein-coupled receptor 31 (GPR31) expression in colorectal cancer (CRC) tissue. The intensity was assigned a score of 3; B: Low GPR31 expression in CRC tissue. The intensity was assigned a score of 2; C: The corresponding adjacent mucosal tissue stains negative for GPR31 expression. GPR31: G protein-coupled receptor 31; CRC: colorectal cancer.

training cohort (P = 0.007) (Table 1). pT classification (P = 0.006) and pM classification (P = 0.018) were significantly different between high- and low-GPR31 expressing patients in the validation cohort, and results showed that strong GPR31 expression was highly correlative with neoplasm metastasis (Table 1).

GPR31 level as a novel prognostic biomarker

In the training cohort, the survival analysis showed that a high GPR31 level was correlated with decreased OS (^bP < 0.001, Figure 3 and Table 2). In addition, the following clinical characteristics were also identified as prognostic factors: age (P = 0.010), carcinoembryonic antigen (CEA) (^bP < 0.001), carbohydrate antigen 19-9 (CA199) (P = 0.010), tumor differentiation (P = 0.001), pT (P = 0.039), pN (^bP < 0.001) and pM classification (^bP < 0.001) (Table 2). Roughly the same results were obtained showing the prognostic meaning of high GPR31 expression (log-rank, ^bP < 0.001 and ^bP < 0.001, Figure 3 and Table 2) in the validation cohort. Univariate analysis demonstrated that the undermentioned clinicopathological characteristics notably influenced overall patient survival: CEA (P = 0.034), pT (^bP < 0.001), pN (^bP < 0.001) and pM classification (^bP < 0.001) (Table 2).

In the training cohort, we found that high GPR31 expression levels were correlated with lower disease-free survival (DFS) (log-rank, ^bP < 0.001 and ^bP < 0.001, Figure 3 and Table 3). In addition, DFS was correlated with age (P = 0.021), CEA (P = 0.001), CA199 (P = 0.014), tumor differentiation (P = 0.002), pT (P = 0.020), pN (^bP < 0.001) and pM (^bP < 0.001) (Table 3). Similar outcomes were obtained in the validation cohort, in which high GPR31 expression was found to be correlated with decreased DFS (log-rank, ^bP < 0.001 and ^bP < 0.001, Figure 3 and Table 3). Results of the univariate analysis demonstrated that pT (^bP < 0.001), pN (^bP < 0.001) and pM (^bP < 0.001) significantly influenced disease-free patient survival (Table 3).

GPR31 expression levels and the clinicopathological characteristics that were found to be significantly associated above were then examined *via* multivariate analysis. Multivariate analysis revealed that high GPR31 level was a significant independent prognostic factor for poor OS [hazard ratio (HR): 1.896; 95% confidence

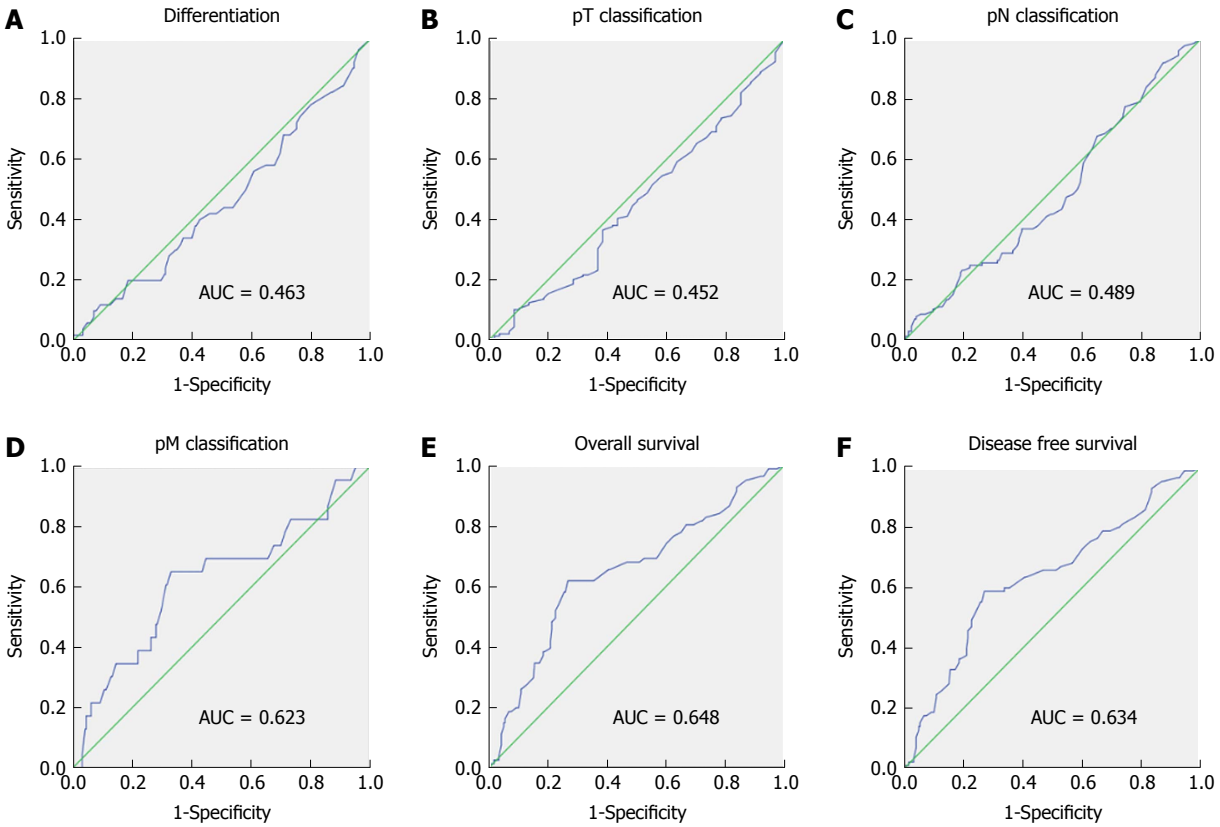

Figure 2 Receiver operating characteristic curve analysis used to determine the cutoff value for G protein-coupled receptor 31 expression levels in colorectal carcinoma. The following sensitivity and specificity parameters for each outcome were plotted. A: Differentiation (AUC = 0.463); B: pT status (AUC = 0.452); C: pN status (AUC = 0.489); D: pM status (AUC = 0.623); E: Overall survival (AUC = 0.648); F: Disease-free survival (AUC = 0.634). AUC: Area under curve.

interval (CI): 1.123-3.202; P = 0.017; Table 4] and DFS (HR: 1.766; 95%CI: 1.069-2.917; P = 0.026; Table 4) after adjusting for tumor differentiation, pT, pN, pM and TNM stage in the training cohort. Similar outcomes were obtained in the validation cohort, in which GPR31 expression was found to correlate with OS (HR: 2.254; 95%CI: 1.168-4.349; P = 0.015; Table 4) and DFS (HR: 1.825; 95%CI: 1.001-3.325; P = 0.049; Table 4).

DISCUSSION

Prognostic value of specific biomarkers was found to be more accurate than that of the AJCC staging system[38-40]. However, cancer heterogeneity limited the use of biomarkers and had produced disagreeing results[41,42]. Thus, large-scale prospective studies are required to validate the specificity and prognostic value of biomarkers.

G protein-coupled receptors (GPCRs) belong to a superfamily of the cell surface signaling proteins and are found only in eukaryotes. GPCRs mediate biological effects by coupling with G proteins. Currently, GPCRs are the target of approximately 40% of all modern medicinal drugs[43,44]. As a result, the molecular mechanisms and functions of GPCRs have been a hotspot in biomedical research.

G-protein coupled receptor 31 (GPR31), also known as 12(S)-HETE receptor, is a protein encoded by the *GPR31* gene that is located on chromosome 6q27 and consists of 319 amino acids[45]. GPR31 plays an important role in a variety of physiological and pathological processes, including inflammation and tumor progression[45]. GPR31 was first discovered in 1997, but its critical role as a 12(S)-HETE-specific receptor was first identified in 2011[11].

12(S)-HETE promotes tumor cell proliferation and metastasis directly or indirectly. Studies have demonstrated that 12-LOX upregulation leads to increased synthesis of 12(S)-HETE in cancer cells[21]. Treatment with LOX inhibitors, such as baicalin, can increase the activity of apoptotic proteases and lead to downregulation of the Bcl protein. In turn, Bcl downregulation promotes cell survival by inhibiting the expression of 12-LOX, thereby leading to cell cycle arrest and apoptosis. This effect can be reversed by exogenous addition of 12(S)-HETE[46]. In addition to promoting tumor cell survival, 12(S)-HETE can directly promote tumor metastasis by acting on vascular endothelial cells or inducing PKC-dependent cytoskeleton rearrangement[16]. Furthermore, 12(S)-HETE can promote the release of cathepsin B, disrupt the vascular endothelial basement membrane and promote penetration of blood vessels by tumor cells, thereby leading to tumor metastasis[25,47]. 12(S)-HETE can also inhibit cadherin E expression, disrupt the lymphatic endothelial cell membrane and promote the migration of tumor cells from the lymphatic vessels. Inhibition of 12(S)-HETE can significantly suppress tumor cell lymph

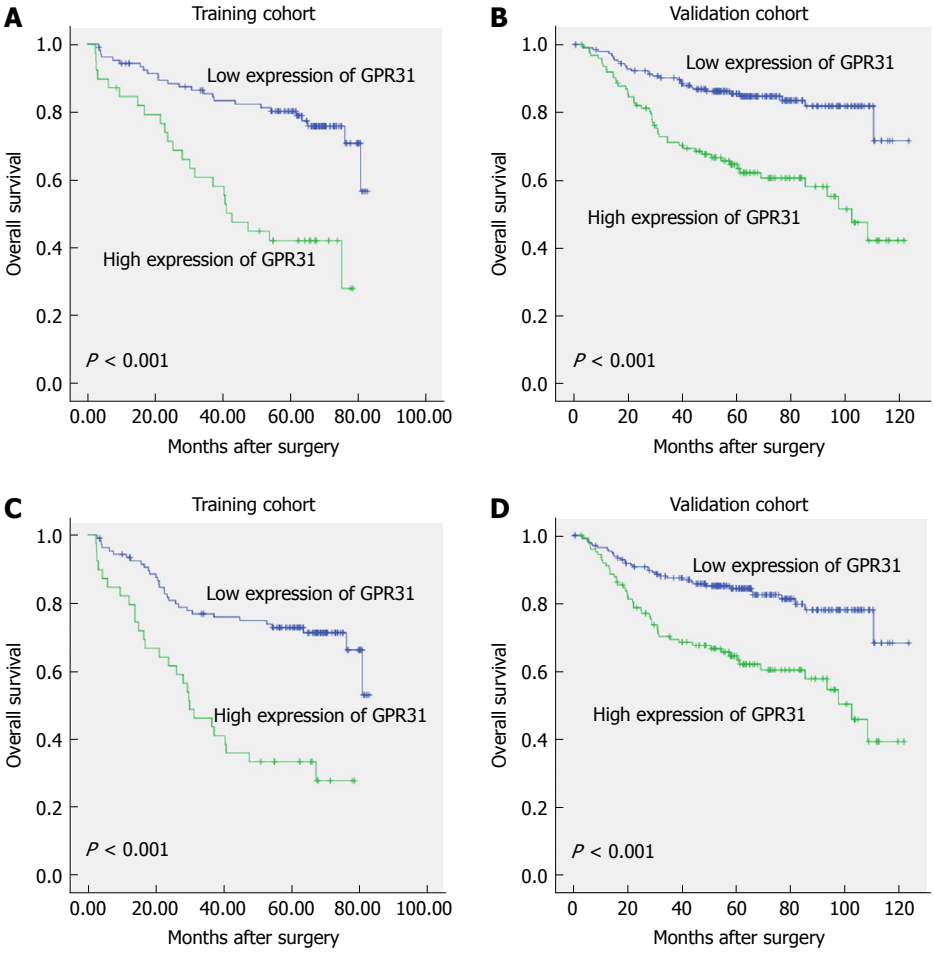

Figure 3 Survival curves for the training and validation cohorts according to G protein-coupled receptor 31 expression (log-rank test). A: Overall survival of the training cohorts: Low G protein-coupled receptor 31 (GPR31) expression, *n* = 197; high GPR31 expression, *n* = 124 (*P* < 0.001); B: Overall survival of the validation cohort: low GPR31 expression, *n* = 106; high GPR31 expression, *n* = 39 (*P* < 0.001); C: Disease-free survival of the training cohorts (*P* < 0.001); D: Disease-free survival of the validation cohorts (*P* < 0.001). GPR31: G protein-coupled receptor 31.

node metastasis[48,49].

GPR31 is a specific receptor for 12(S)-HETE. A study by Guo *et al*[11] of prostate cancer showed that 12(S)-HETE promotes invasion by tumor cells by specifically targeting GPR31. *In vitro* studies have revealed that activation of the 12(S)-HETE/GPR31 signaling pathway is a crucial factor that determines tumor invasion and metastasis[26,50].

Previous studies have shown that 12(S)-HETE can activate the extracellular signal regulated kinase (ERK)1/2, mitogen-activated protein kinase kinase (MEK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways[19,22,51] by specifically binding to GPR31[11]. GPR31 upregulation enhances the activation of ERK1/2, MEK and NF-κB *via* 12(S)-HETE, whereas GPR31 suppression can completely inhibit 12(S)-HETE-mediated activation of these signaling pathways. The ERK1/2, MEK and NF-κB pathways are involved in most human physiological and pathological processes and serve as important regulatory factors affecting immune and inflammatory processes. Moreover, NF-κB is an important tumor promoter[52]. 12(S)-HETE binds to GPR31 on the cell membrane and activates NF-κB by activating mitogen-activated protein kinases (MAPKs)/c-Jun N-terminal kinases (JNK)/ERK signaling[11]. NF-κB signaling pathways influence tumor cell invasion and angiogenesis by regulating a variety of tumor metastasis or invasion-related genes and cytokine expression, including matrix metalloproteinases, urokinase-type plasminogen activator (UPA), interleukin (IL)-8, inflammatory mediators of intercellular adhesion molecules, monocyte chemokines and cyclooxygenase-2 (COX-2)[53].

Results of the present study showed that GPR31 expression in colorectal cancer tissue was significantly higher than that in normal mucosa and that GPR31 expression levels are closely related to distant metastasis of tumors, which are consistent with findings reported in previous studies[54]. Further univariate and multivariate analyses showed that patients with high GPR31 expression had a worse prognosis and decreased OS and DFS than patients that exhibited low GPR31 expression. These results indicate that GPR31 is a critical prognostic factor of OS and DFS in CRC patients and suggest that GPR31 is closely related to the occurrence, development and prognosis of CRC. And GPR31 may become a novel

Table 2 Univariate analysis of G protein-coupled receptor 31 expression and clinicopathologic variables on overall survival

Variable	Training cohort			Validation cohort		
	All cases	Hazard ratio (95%CI)	*P* value	All cases	Hazard ratio (95%CI)	*P* value
Age (yr)			0.010[a]			0.054
< 58.4	153	1.0			1.0	
⩾ 58.4	168	1.832 (1.158-2.898)			1.937 (0.987-3.800)	
Gender			0.817			0.933
Female	148	1.0			1.0	
Male	173	1.053 (0.680-1.631)			1.026 (0.562-1.874)	
BMI (kg/m^2)			0.474			0.959
< 21.4	159	1.0			1.0	
⩾ 21.4	154	0.980 (0.927-1.036)			1.026 (0.394-2.673)	
Preoperative ileus			0.07			0.77
Yes	25	1.0			1.0	
No	294	1.438 (0.716-2.890)			0.901 (0.449-1.810)	
CEA (ng/mL)			< 0.001[b]			0.034[a]
< 5	200	1.0			1	
⩾ 5	97	2.435 (1.509-3.927)			1.919 (1.050-3.508)	
CA199 (ng/mL)			0.010[a]			0.279
< 37	218	1.0			1.0	
⩾ 37	64	1.988 (1.179-3.351)			1.504 (0.719-3.148)	
Tumor location			0.303			0.911
Colon	156	1.0			1	
Rectal	163	1.250 (0.818-1.910)			0.968 (0.545-1.720)	
Size (cm)			0.355			0.193
< 5.0	156	1.0			1.0	
⩾ 5.0	164	1.230 (0.793-1.907)			0.654 (0.345-1.239)	
Histopathology			0.091			0.537
Adenocarcinoma	283	1.0			1.0	
Others	38	1.671 (0.922-3.031)			1.311 (0.556-3.090)	
Differentiation			0.001[a]			0.07
Well/moderate	271	1.0			1.0	
Poor	50	2.363 (1.435-3.890)			1.811 (0.952-3.443)	
pT classification			0.039[a]			0.001[a]
T1/T2	60	1.0			1.0	
T3/T4	261	2.079 (1.038-4.163)			7.055 (2.191-22.722)	
pN classification			< 0.001[b]			< 0.001[b]
N0	194	1.0			1.0	
N1	124	2.293 (1.471-3.576)			3.130 (1.716-5.709)	
pM classification			< 0.001[b]			< 0.001[b]
M0	298	1.0			1.0	
M1	23	9.857 (5.825-16.680)			5.212 (2.764-9.828)	
GPR31 expression			< 0.001[b]			< 0.001[b]
Low	197	1.0			1.0	
High	124	2.888 (1.844-4.523)			3.413 (1.920-6.066)	

[a]$P < 0.05$; [b]$P < 0.001$; CI: Confidence interval; GPR31: G protein-coupled receptor 31; BMI: Body mass index; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 19-9.

biomarker and therapeutic target for CRC. Although few studies have discussed the role of GPR31 in tumors, it is reasonable to believe that GPR31 plays a key regulatory role in tumor development and progression by mediating a specific "switch" effect by 12(S)-HETE. Further studies are warranted to elucidate the detailed mechanisms underlying GPR31 function, specifically the molecular mechanisms by which GPR31 expression affects carcinogenesis process, such as tumor proliferation, differentiation, migration and invasion in CRC.

There are some limitations in this study. In order to study the clinical value and role of GPR31 in CRC more accurately, patients were divided into a training cohort and a validation cohort for analysis. However, due to the small sample size, relatively long sample age, poor storage conditions, single research center and other factors, the results still need further verification.

ARTICLE HIGHLIGHTS

Research background

G protein-coupled receptor 31 (GPR31) plays an important role in a variety of physiological and pathological processes, including inflammation and tumor progression. In this present study, we aimed to elucidate the association between the expression level of GPR31 and colorectal cancer (CRC) progression.

Research motivation

12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] plays an important role in cancer promotion. It potentially acts through GPR31. We aimed to elucidate the association between the expression level of GPR31 and CRC progression. We expect GPR 31 as one of reliable biomarkers can provide guidance to the treatment of CRC and help predict treatment prognosis.

Research objectives

GPR31 is a critical prognostic factor of overall survival and disease-free survival

Table 3 Univariate analysis of G protein-coupled receptor 31 expression and clinicopathologic variables on disease-free survival

Variable	Training cohort			Validation cohort		
	All cases	Hazard ratio (95%CI)	*P* value	All cases	Hazard ratio (95%CI)	*P* value
Age (yr)			0.021[a]			0.182
< 58.4	153	1.0			1.0	
⩾ 58.4	168	1.683 (1.082-2.619)			1.481 (0.832-2.636)	
Gender			0.832			0.959
Female	148	1.0			1.0	
Male	173	0.955 (0.624-1.462)			0.986 (0.573-1.697)	
BMI (kg/m2)			0.388			0.938
< 21.4	159	1.0			1.0	
⩾ 21.4	154	0.977 (0.926-1.030)			1.035 (0.431-2.488)	
Preoperative ileus			0.461			0.925
Yes	25	1.0			1.0	
No	294	1.299 (0.647-2.607)			0.971 (0.524-1.800)	
CEA (ng/mL)			0.001[a]			0.057
< 5	200	1.0			1.0	
⩾ 5	97	2.233 (1.400-3.563)			1.709 (0.985-2.966)	
CA199 (ng/mL)			0.014[a]			0.415
< 37	218	1.0			1.0	
⩾ 37	64	1.920 (1.143-3.225)			1.334 (0.668-2.666)	
Tumor location			0.101			0.199
Colon	156	1.0			1.0	
Rectal	163	1.416 (0.934-2.147)			0.713 (0.425-1.195)	
Size (cm)			0.210			0.686
< 5.0	156	1.0			1.0	
⩾ 5.0	164	1.316 (0.857-2.022)			0.893 (0.514-1.549)	
Histopathology			0.112			0.108
Adenocarcinoma	283	1.0			1.0	
Others	38	1.617 (0.894-2.924)			1.793 (0.879-3.658)	
Differentiation			0.002[a]			0.069
Well/moderate	271	1.0			1.0	
Poor	50	2.200 (1.342 -3.607)			1.732 (0.958-3.132)	
pT classification			0.020[a]			0.001[a]
T1/T2	60	1.0			1.0	
T3/T4	261	2.269 (1.135-4.537)			9.173 (2.867-29.350)	
pN classification			< 0.001[b]			< 0.001[b]
N0	194	1.0			1.0	
N1	124	2.228 (1.446-3.434)			2.667 (1.567-4.538)	
pM classification			< 0.001[b]			< 0.001[b]
M0	298	1.0			1.0	
M1	23	8.856 (5.259-14.913)			5.210 (2.895-9.375)	
GPR31 expression			< 0.001[b]			< 0.001[b]
Low	197	1.0			1.0	
High	124	2.576 (1.671-3.969)			3.277 (1.942-5.530)	

[a]$P < 0.05$; [b]$P < 0.001$; CI: Confidence interval; GPR31: G protein-coupled receptor 31; BMI: Body mass index; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 19-9.

in CRC patients and is closely related to the occurrence, development and prognosis of CRC. GPR31 may become a novel biomarker and therapeutic target for CRC.

Research methods

We obtained paraffin-embedded pathological specimens from 466 CRC patients. And we examined GPR31 expression levels in CRC tissues from two independent cohorts *via* immunohistochemical staining. All patients were categorized into either the GPR31 low expression group or GPR31 high expression group. The clinicopathological factors and the prognosis of patients in the GPR31 low expression group and GPR31 high expression group were compared.

Research results

Results of the present study showed that GPR31 expression in colorectal cancer tissue was significantly higher than that in normal mucosa and that GPR31 expression levels are closely related to distant metastasis of tumors, which are consistent with findings reported in previous studies. Further univariate and multivariate analyses showed that patients with high GPR31 expression had a worse prognosis and decreased overall survival and disease-free survival than patients that exhibited low GPR31 expression.

Research conclusions

We found that GPR31 was closely related to the occurrence, development, and prognosis of CRC. And GPR31 may become a novel biomarker and therapeutic target for CRC. Although few studies have discussed the role of GPR31 in tumors, it is reasonable to believe that GPR31 plays a key regulatory role in tumor development and progression by mediating a specific "switch" effect by 12(S)-HETE. Further studies are warranted to elucidate the detailed mechanisms underlying GPR31 function, specifically the molecular mechanisms by which GPR31 expression affects carcinogenesis process, such as tumor proliferation, differentiation, migration and invasion in CRC.

Research perspectives

High GPR31 expression levels were found to be correlated with pM classification of CRC and to serve as an independent predictive factor of poor survival of CRC patients. Further *in vivo* and *in vitro* experiments should be done to elucidate the molecular mechanisms by which GPR31 expression

Table 4 Cox multivariate analysis of prognostic factors on overall survival and disease-free survival

Variable	Training cohort			Validation cohort		
	Hazards ratio	95%CI	*P* value	Hazards ratio	95%CI	*P* value
Overall survival						
Age (≥ 58.4 *vs* < 58.4)	2.344	1.365-4.025	0.002[a]	1.722	0.788-3.760	0.173
CEA, ng/mL (≥ 5 *vs* < 5)	2.236	1.284-3.894	0.004[a]	1.437	0.745-2.773	0.279
CA199, ng/mL (≥ 37 *vs* < 37)	1.382	0.780-2.448	0.267	1.189	0.543-2.604	0.665
Differentiation (poor vs well/moderate)	1.913	1.045-3.503	0.036[a]	0.940	0.420-2.103	0.880
pT classification (T3/T4 *vs* T1/T2)	1.489	0.619-3.581	0.374	7.890	1.028-60.588	0.047[a]
pN classification (N1 *vs* N0)	1.855	1.116-3.084	0.017[a]	2.210	1.059-4.613	0.035[a]
pM classification (M1 *vs* M0)	11.836	5.801-24.148	< 0.001[b]	2.706	1.307-5.604	0.007[a]
GPR31 expression (high *vs* low)	1.896	1.123-3.202	0.017[a]	2.254	1.168-4.349	0.015[a]
Disease-free survival						
Age (≥ 58.4 *vs* < 58.4)	2.003	1.200-3.344	0.008[a]	1.159	0.598-2.247	0.661
CEA, ng/mL (≥ 5 *vs* < 5)	1.965	1.147-3.366	0.014[a]	1.247	0.686-2.267	0.469
CA199, ng/mL (≥ 37 *vs* < 37)	1.459	0.824-2.585	0.195	1.103	0.529-2.300	0.794
Differentiation (poor *vs* well/moderate)	1.609	0.884-2.929	0.119	0.881	0.424-1.830	0.734
pT classification (T3/T4 *vs* T1/T2)	1.749	0.719-4.254	0.218	13.092	1.738-98.636	0.013*
pN classification (N1 *vs* N0)	1.809	1.108-2.953	0.018[a]	1.787	0.936-3.412	0.079
pM classification (M1 *vs* M0)	10.233	5.128-20.420	< 0.001[b]	2.741	1.408-5.334	0.003[a]
GPR31 expression (high *vs* low)	1.766	1.069-2.917	0.026[a]	1.825	1.001-3.325	0.049[a]

[a]$P < 0.05$; [b]$P < 0.001$; CI: Confidence interval; GPR31: G protein-coupled receptor 31; BMI: Body mass index; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 19-9.

affects carcinogenesis process, such as tumor proliferation, differentiation, migration and invasion in CRC.

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