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Clinicopathological and prognostic significance of aberrant G protein-couple receptor 110 (GPR110) expression in gastric cancer

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ABSTRACT

Background: GPR110 is a member of the adhesion G protein-coupled receptor family, which has been identified as an oncogene in various cancers, including hepatocellular carcinoma, lung cancer, prostatic cancer and glioma. Whereas the expression and the clinical relevance of GPR110 in gastric cancer has not been investigated. The research purpose of this study was to explore the expression pattern of GPR110 and evaluate its clinical-pathological and prognostic value in gastric cancer.

Methods: In this study, the expression of GPR110 was detected in 117 paired gastric cancer tissues and adjacent non-tumorous tissues by using qRT-PCR and immunohistochemical assays. Univariate Kaplan-Meier and multivariate Cox analysis were used to determine the prognostic value of GPR110 in GC.

Results: We demonstrated that the mRNA and protein levels of GPR110 in GC tissues were overexpressed than the adjacent non-tumorous tissues. Furthermore, elevated GPR110 protein expression was correlated with decreased overall and recurrence-free survival ($P = 0.001$ and $P = 0.000$, respectively). Univariate and multivariate analysis indicated that GPR110 protein level may serve as an independent prognostic indicator for determining prognosis of GC patients.

Conclusions: Our study revealed that high expression of GPR110 predicts the poor prognosis of GC patients, and GPTR110 may function as a potential biomarker for the diagnosis of GC.

1. Introduction

Gastric cancer (GC) is an etiologically multifactorial disease which represents one of the most common malignant tumors worldwide [1]. Although, the therapeutic tools of GC are varied including surgery, chemotherapy, radiotherapy, gene therapy and other options, the morbidity and mortality rates still remain high globally, especially in China [2,3]. Therefore, there is an urgent need to explore new diagnostic and prognostic targets for GC patients.

Adhesion GPCRs are characterized by extremely long N-terminal regions and a GPCR-like-seven-pass transmembrane domain [4]. Recent years, several adhesion GPCR molecules have been identified associated with cancer development in various cancers [5–9]. GPR110, a number of the adhesion G protein-coupled receptor family, has also been identified as an oncogene during the development of several cancers, including lung and prostate adenocarcinomas, breast cancer and hepatocellular carcinoma [10–13]. In 2010, Amy et al. identified that orphan receptor GPR110 was overexpressed in lung and prostate cancer [10]. In addition, ma et al. revealed that GPR110 deficiency decelerates carcinogen-induced hepatocarcinogenesis via activation of the IL-6/STAT3 pathway [11]. Furthermore, a study published recently revealed that GPR110 could function as a potential new target in HER2+ breast cancer through GPCR profiling [12]. However, to the best of our knowledge, the expression of GPR110, as well as its prognostic significance, has not been investigated in GC.

In the present study, the mRNA and protein levels of GPR110 were examined in 117 paired GC specimens and adjacent non-tumorous tissues. The association between GPR110 expression and the clinicopathological parameters as well as the 5 year overall and recurrence-free survival of GC patients was analyzed. The results indicate that GPR110 may be a predictive biomarker for the poor prognosis in patients with GC.

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Table 1
Relationship between GPR110 expression in tumorous tissues and clinicopathological characteristics in patients with gastric cancer.

Clinicopathological characteristic	N	GPR110		Pearson χ^2	P-value
		Low (%)	High (%)		
Total	117	32 (27.4)	85 (72.6)		
Gender				0.511	0.475
Male	86	22 (25.6)	64 (74.4)		
Female	31	10 (32.3)	21 (67.7)		
Age (years)				0.365	0.546
≤60	57	14 (24.6)	43 (75.4)		
> 60	60	18 (30.0)	42(70.0)		
Tumor size				1.317	0.251
< 5 cm	45	15 (33.3)	30 (66.7)		
≥5 cm	72	17 (23.6)	55 (76.4)		
Differentiation				6.19	**0.013**
Low grade	51	8 (15.7)	43 (84.3)		
Middle and high grade	66	24 (36.4)	42 (63.6)		
T classification				8.878	**0.003**
T1/T2	23	12 (52.2)	11 (47.8)		
T3/T4	94	20 (21.3)	74 (78.7)		
Nodal involvement				6.781	**0.009**
Negative	34	15 (44.1)	19 (55.9)		
Positive	83	17 (20.5)	66 (79.5)		
TNM stage				7.031	**0.008**
I + II	50	20 (40.0)	30 (60.0)		
III + IV	67	12 (17.9)	55 (82.1)		

Note: Bold values have statistical significance.

2. Material and methods

2.1. Patients and samples

We obtained 117 paired GC tissues and adjacent non-tumorous tissues from patients who underwent a radical resection from the same surgical team and were histologically and clinically diagnosed with GC. None of the patients had received preoperative treatments, for example chemotherapy, radiotherapy or other related anti-tumor treatments. Surgeries were performed at the Department of Gastrointestinal Surgery, the Affiliated Wenling Hospital of Wenzhou Medical University between June 2010 and November 2012. The clinicopathological parameters, including age, gender, tumor differentiation, tumor size, lymph node metastasis, tumor stage and TNM stage were retrospectively collected. All tissue samples were immediately collected and cryopreserved in liquid nitrogen for further study. A portion of each specimen was fixed with 10% paraformaldehyde and embedded in paraffin blocks. Written informed consent was obtained from the patients prior to participation in this clinical trial and research, and the research protocols were approved by the ethics committee of the Affiliated Wenling Hospital of Wenzhou Medical University. The clinicopathological characteristics of the patients are presented in Table 1.

2.2. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissue sample using TRIzol reagent (Invitrogen, Grand Island, NY, USA) as described by the manufacturer and RNAs (500 ng) were reverse transcribed using the PrimeScript RT Master Mix (Takara, Dalian, China). Quantitative real-time PCR was performed to detect the expression levels of GPR110 using the SYBR Premix Ex Taq (Takara, Dalian, China) on the ABI Prism 7900 H T (Applied Biosystems, Foster City, CA, USA). The relative expression levels of GPR110 were normalized to GAPDH. The reactions were incubated in a 384-well optical plate at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The primer sequences were as follows: GPR110 Forward: GCCCAGTCGAAGAATATCAGC, Reverse: GCCCATGTGACCATAATAATGGA; GAPDH Forward: CGCTGAGTACGT CGTGGAGTC Reverse: GCTGATGATCTTGAGGCTGTTGTC. The $2^{-\Delta\Delta Ct}$ method was used to quantify the relative CHOP expression levels.

2.3. Immunohistochemistry

Tissue sections (4 lm thick) were deparaffinized with xylene, rehydrated, and subjected to microwave antigen retrieval in citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase was quenched with 3% hydrogen peroxide for 10 min. The sections were then separately incubated with rabbit anti-human antibody against GPR110 (Atlas Antibodies, Stockholm, Sweden) at 4 C overnight. After washing, sections were incubated with horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology) for 20 min. Sections were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were included by omitting the primary antibody. Photographs were taken with the microscope (Nikon, ECLIPSE 50i) and software NIS-Elements v4.0. Using Image-Pro Plus software (v. 5.0), average values of integrated optical density (IOD) was obtained by analyzing five random fields per slide. Every index was detected a minimum of three times. According to the higher 95% confidence interval (CI) of the IOD value of IHC staining of GPR110 expression in adjacent samples, we divided the GC patients into two groups: GPR110 high group and GPR110 low group.

2.4. Statistical analysis

All statistical analyses were performed using SPSS 19.0 (SPSS Inc, Chicago, IL, USA) software and presented with the GraphPad prism software (GraphPad Software, San Diego, CA, USA). Results of quantitative real-time PCR were expressed as mean ± S.E.M. The Student's *t* test and the Chi-square (χ^2) test were used to evaluate statistical differences of GPR110 expression in different samples and examine the relationship between GPR110 expression and clinicopathological features. Patient survival and their differences were determined using the Kaplan-Meier method and the log-rank test. Univariate and multivariate prognosis analyses were performed using the Cox proportional hazards regression model. $P < 0.05$ indicated that the differences were statistically significant.

3. Results

3.1. Expression levels of GPR110 mRNA and protein in GC tissues and adjacent non-tumorous tissues

Firstly, the mRNA level of GPR110 in 117 pairs of fresh GC tissues and paired adjacent non-tumorous tissues was determined by qRT-QCR. It was identified that GPR110 mRNA expression was significantly higher in GC tissues than in the adjacent non-tumorous tissues ($P < 0.001$, Fig. 1A). Next, to further confirm GPR110 expression level and localization, we performed immunohistochemical (IHC) staining of GPR110 in 117 pairs of paraffin-embedded GC and paired adjacent non-cancerous tissues. The results showed that GPR110 was mainly localized in the cytoplasm and cell membrane (Fig. 1B). In addition, we analysed the mRNA and protein expression of GPR110 in the tumorous tissues from the same 117 GC patients, and found a positive correlation between their expressions ($r^2 = 0.7003$, $P < 0.0001$) (Fig. 1D). According to the integrated optical density (IOD) value of IHC staining, we found that the protein level of GPR110 in GC tumors was higher than in the matched adjacent non-cancerous tissues ($P < 0.001$, Fig. 1C), which was consistent with the results of qRT-PCR. To distinguish the expression level of GPR110 in GC cancerous samples, we divided the GC patients into two groups: GPR110 high (n = 85) and GPR110 low

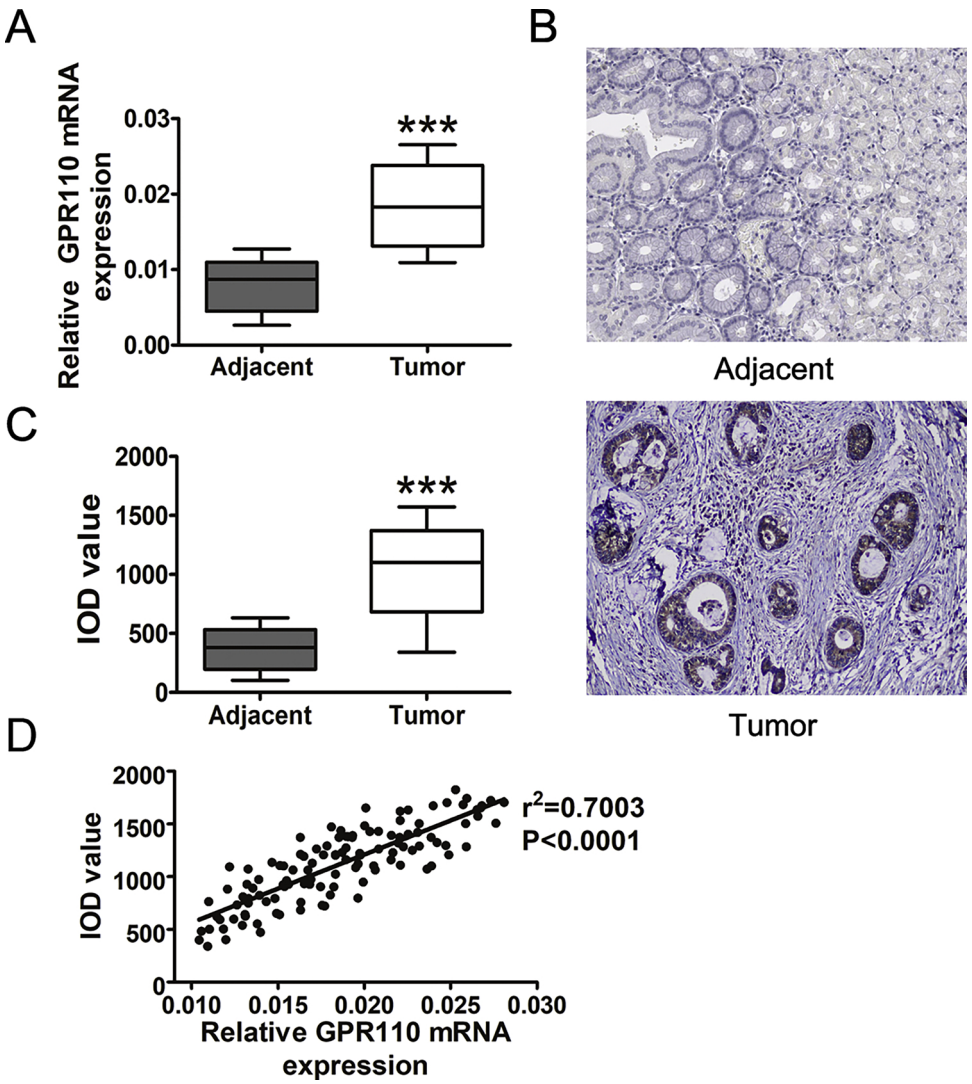

Fig. 1. Analysis of GPR110 expression in human gastric cancer. (A) The levels of GPR110 mRNA in 117 paired GC and corresponding adjacent normal tissues were detected by qRT-PCR. (B, C) Paraffin sections from 117 paired GC tissues and adjacent non-tumorous tissues were used to examine GPR110 protein expression via IHC with IOD value examined based on IHC (original magnification ×200). (D) A positive correlation between the mRNA and protein expression of GPR110 in the tumorous tissues from the same 117 GC patients. *** P < 0.001. P < 0.05 was regarded as statistically significant.

(n = 32) according to the higher 95% confidence interval (CI) of the IOD value of IHC staining of GPR110 expression in adjacent samples. Images of representative immunostaining are presented in Fig. 2.

3.2. Association between GPR110 protein expression and clinicopathological parameters of GC patients

Next, the associations between GPR110 protein expression and the clinicopathological parameters in GC patients were analyzed. As shown in Table 1, it was identified that high protein expression of GPR110 was significantly associated with tumor differentiation ($P = 0.013$), T classification ($P = 0.003$), nodal involvement ($P = 0.009$) and TNM stage ($P = 0.008$). However, no association was identified between GPR110 immunoreactivity and other clinical features, including age, gender and tumor size.

3.3. Prognosis value of GPR110 expression in GC patients

To further explore the association between GPR110 protein expression and the clinical outcome of GC patients, the survival curve (including OS, overall survival time and RFS, recurrence-free survival time) of our cohort was plotted. Kaplan-Meier curve analysis showed that patients with high expression of GPR110 protein (33.292 months; 95% CI 29.434–37.150) had shorter OS compared to GPR110 low patients (47.574 months; 95% CI 41.807–53.342; $P = 0.001$; Fig. 3A). Similarly, the RFS was notably worse in patients with GPR110-high expression (30.824 months; 95% CI 26.986–34.662) than in those with GPR110-low expression (46.092 months; 95% CI 39.930–52.253; $P = 0.000$; Fig. 3B). Univariate analysis indicated that tumor expression of GPR110, tumor differentiation, T classification, nodal involvement and TNM stage had significant prognostic influences on OS (Table 2) and RFS (Table 4) of GC patients. Furthermore, multivariate analysis revealed that intra-tumoral GPR110 staining as an independent poor prognostic marker for OS (HR = 2.250, 95% CI 1.194–4.238, $P = 0.012$) and RFS (HR = 2.489, 95% CI 1.348–4.594, $P = 0.004$). Additionally, T classification and nodal involvement were also independent prognostic factors for OS and RFS (Tables 3 and 5).

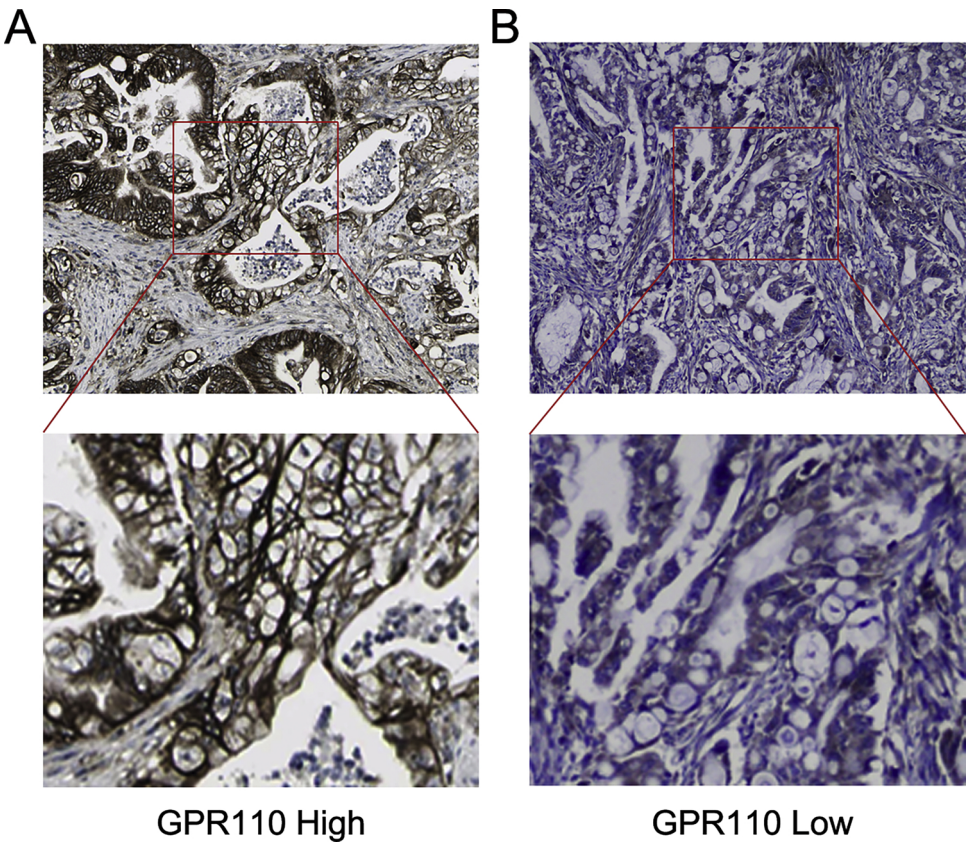

Fig. 2. The protein expression of GPR110 in GC tissues examined by IHC. (A) Representative high expression of GPR110 protein in GC tissues. (B) Representative low expression of GPR110 protein in GC tissues. Original magnification ×200.

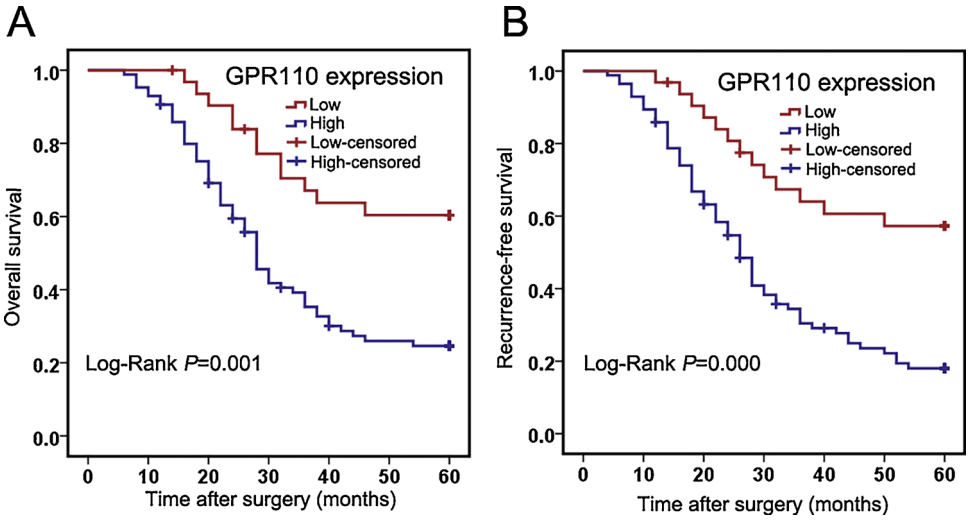

Fig. 3. The Kaplan-Meier curves of the overall survival (A) and recurrence-free survival (B) of GC patients.

4. Discussion

It is well known that gastric cancer is one of the leading causes of cancer-related deaths worldwide [14–17]. Although great advancements in the treatments for gastric cancer have been acquired in recent years, the 5-year survival rate of patients diagnosed with advanced gastric cancer is still poor [18,19]. Hence, it is urgent to explore more effective biomarkers for monitoring prognosis and early diagnosis of GC patients. GPCRs represent a diverse and biologically ubiquitous group of protein receptors, which have been found to be involved in a variety of diseases, including cancers [20–23]. In consideration of that approximately 40% of the modern medicinal drugs are targeting GPCRs [24], we can achieve great clinical benefits through investigations on the associations between GPCRs and cancers.

Table 2
Univariate analysis of the correlation between clinicopathological parameters and overall survival time of patients with gastric cancer.

Variable	Mean survival time (m)	95% CI	Log-Rank Test	*P* value
Gender				
Male	36.937	32.996-40.877	0.156	0.692
Female	37.890	31.032-44.749		
Age (years)				
≤60	36.649	31.786-41.513	0.078	0.779
> 60	37.760	32.948-42.573		
Tumor size				
< 5 cm	35.687	30.421-40.953	0.463	0.496
≥5 cm	38.224	33.757-42.691		
Differentiation				
Low grade	30.950	26.119-35.781	9.817	**0.002**
Middle and high grade	42.102	37.663-46.540		
T classification				
T1/T2	49.439	43.331-55.547	8.482	**0.004**
T3/T4	34.433	30.684-38.181		
Nodal involvement				
Negative	48.615	42.673-54.558	16.240	**0.000**
Positive	32.606	28.891-36.321		
TNM stage				
I + II	43.521	38.415-48.627	10.361	**0.001**
III + IV	32.375	28.133-36.617		
GPR110 expression				
High	33.292	29.434-37.150	12.091	**0.001**
Low	47.574	41.807-53.342		

Note: Bold values have statistical significance.

Table 3
Multivariate analysis of OS for GC patients.

Variables	HR	95% CI	*P* value
Gender			
Male vs female	1.133	0.658-1.951	0.653
Age			
≤60 vs > 60 years	1.151	0.682-1.945	0.598
Tumor size			
< 5 cm vs ≥5 cm	1.577	0.923-2.695	0.096
Differentiation			
Low grade vs middle and high grade	1.542	0.950-2.504	0.080
T classification			
T1/T2 vs T3/T4	0.413	0.195-0.876	**0.021**
Nodal involvement			
Negative vs positive	0.409	0.203-0.823	**0.012**
GPR110 expression			
High vs low	2.250	1.194-4.238	**0.012**

Note: Bold values have statistical significance.

The class of adhesion G protein–coupled receptors (aGPCRs), which contain adhesion domains in their extracellular region, is the second largest family of GPCRs [25]. Recent years, studies have found that many adhesion GPCRs are involved in the development of various cancers through regulating cell proliferation, cell migration and invasion capacities, tumor angiogenesis and so on [23,26,27]. As one of the adhesion GPCRs, GPR110 has been identified as an oncogene in human lung and prostate cancer, indicating that GPR110 may exert important influence in the process of tumor formation [10]. In a murine model of liver cancer induced by DEN plus CCl_4, researchers found that GPR110 deficiency decelerates carcinogen-induced hepatocarcinogenesis via activation of the IL-6/STAT3 pathway [11]. It was also reported that GPR110 was highly expressed in some glioma patients and the overexpression of GPR110 can inhibit the phosphorylation and activation of STAT3 [28]. In a study published recently, researchers revealed that GPR110 was overexpressed in advanced stage of osteosarcoma and could function as a potential novel prognostic biomarker in osteosarcoma [13].

In the present study, we revealed that GPR110 was significantly overexpressed in GC tissues compared with that in adjacent noncancerous tissues. We also found that the high expression level of GPR110 was associated with poorer differentiation, deeper infiltration, more nodal involvement and worse TNM stage. Univariate analysis showed that patient with poor differentiation, deep infiltration, more nodal involvement, worse TNM stage and high GPR110 expression had a poor 5-year OS and RFS. Further stratified analysis demonstrated that T classification, nodal involvement and GPR110 expression were validated as independent prognostic factors of survival in multivariate Cox regression. Our findings suggest that the high expression of the orphan receptor GPR110 was correlated with unfavorable prognosis of GC patients.

Although studies screening for the receptor agonists of GPR110 have been conducted, we still have no idea about the function of GPR110 endogenous ligand. STAT3 pathway has been reported to be involved in GPR110 pathway, whether this interaction exists in GC pathology is not yet known. Further study should be carried out to explore the underlying molecular mechanism of action of this molecule in GC development. A limitation of the present study is the smaller size of tissue samples used in the tissue analysis. In addition, the gastric cancer samples were collected from a single institution, which could introduce selection bias. Therefore, more gastric cancer specimens collected from different hospitals are needed to confirm the present analysis, and additional studies are required to elucidate the precise role of GPR110 in the development of GC and to examine its potential role as a therapeutic target.

5. Conclusions

In summary, our study explored the mRNA and protein levels of GPR110 in patients with GC for the first time, and revealed its correlations with the clinical stages of GC patients. Furthermore, univariate and multivariate analyses identified it as an independent prognostic biomarker for the overall survival and recurrence-free survival of patients with GC.

Identification of GPR110 as a novel biomarker for GC would be helpful for both disease mechanism elucidation and clinical prognosis improvement.

Table 4
Univariate analysis of the correlation between clinicopathological parameters and recurrence-free survival time of patients with gastric cancer.

Variable	Mean survival time (m)	95% CI	Log-Rank Test	*P* value
Gender				
Male	34.473	30.466-38.480	0.260	0.610
Female	36.467	29.405-43.528		
Age (years)				
≤60	34.196	29.376-39.016	0.647	0.421
> 60	35.922	30.877-40.968		
Tumor size				
< 5 cm	33.764	28.270-39.258	0.146	0.703
≥5 cm	35.854	31.348-40.361		
Differentiation				
Low grade	28.834	23.875-33.794	9.458	**0.002**
Middle and high grade	39.848	35.312-44.384		
T classification				
T1/T2	47.725	41.251-54.200	7.894	**0.005**
T3/T4	32.114	28.315-35.913		
Nodal involvement				
Negative	46.283	40.107-52.458	14.924	**0.000**
Positive	30.457	26.661-34.252		
TNM stage				
I + II	42.274	37.133-47.414	12.555	**0.000**
III + IV	29.502	25.222-33.783		
GPR110 expression				
High	30.824	26.986-34.662	14.316	**0.000**
Low	46.092	39.930-52.253		

Note: Bold values have statistical significance.

Table 5
Multivariate analysis of RFS for GC patients.

Variables	HR	95% CI	*P* value
Gender			
Male vs female	1.241	0.735-2.095	0.420
Age			
≤60 vs > 60 years	1.184	0.724-1.936	0.501
Tumor size			
< 5 cm vs ≥5 cm	1.533	0.926-2.539	0.097
Differentiation			
Low grade vs middle and high grade	0.661	0.416-1.051	0.080
T classification			
T1/T2 vs T3/T4	0.448	0.227-0.886	**0.021**
Nodal involvement			
Negative vs positive	0.480	0.254-0.906	**0.024**
GPR110 expression			
High vs low	2.489	1.348-4.594	**0.004**

Note: Bold values have statistical significance.

Conflict of interest

All the authors declared that there is no conflict of interest.

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