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RESEARCH ARTICLE

G protein-coupled receptor LGR6 is an independent risk factor for colon adenocarcinoma

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Abstract **LGR6 is a member of the G protein-coupled receptor family that plays a tumor-suppressive role in colon cancer. However, the relationship between LGR6 expression in patients and clinicopathological factors remains unclear. This study aimed to clarify whether the expression level of LGR6 is correlated with colon adenocarcinoma progression. Immunohistochemistry was used to detect LGR6 expression in colon adenoma tissues (n = 21), colon adenocarcinoma tissues (n = 156), and adjacent normal tissues (n = 124). The expression levels of LGR6 in colon adenoma and adenocarcinoma were significantly higher than those in normal colon epithelial tissues (P < 0.001). Low LGR6 expression predicted a short overall survival in patients with colon adenocarcinoma (log-rank test, P = 0.016). Univariate and multivariate survival analyses showed that, in addition to N and M classification, LGR6 expression served as an independent prognostic factor. Thus, low expression of LGR6 can be used as an independent prognostic parameter in patients with colon adenocarcinoma.**

Keywords LGR6; colon adenocarcinoma; immunohistochemistry; prognosis

Introduction

In China, colorectal cancer is the third most common cancer in women and the fifth most common in men [1]. Many colorectal cancers follow the adenoma–carcinoma sequence model [2] in which adenomas (i.e., precancerous lesions of colorectal cancer) may develop into cancer over more than 10 years [3]. Colonoscopy is an effective screening method for diagnosing and resecting these lesions; however, at late stages, surgery and neoadjuvant and adjuvant therapies are needed [2]. In spite of early endoscopic screening and aggressive treatments, colorectal cancer diagnostic methods remain insufficient and molecular studies have been given increasing attention. Colorectal cancer can be classified into three molecular subtypes, namely, chromosomal instability [4], microsatellite instability [4,5], and CpG island methylation subtypes [6,7]. The identification of novel, reliable molecular biomarkers associated with clinical factors for targeted therapy is crucial [8].

G protein-coupled receptors (GPCRs) are membrane proteins with seven transmembrane domains that regulate various physiologic processes associated with multiple diseases [9]. Leucine-rich repeat-containing GPCR 6 (LGR6) exhibits high homology to LGR4 and LGR5 [10], which play roles in activating the Wnt pathway [11]. Among the three receptors, LGR5 and LGR6 play pivotal roles in adult stem cells: LGR5 is a marker for proliferative stem cells in the intestine, stomach, colon, and hair follicle; and LGR6 is a marker for multiple types of adult stem cells in the skin and nails [12,13].

In addition, the three LGRs are relevant to several types of cancer. LGR4 contributes to lymphatic invasiveness and metastasis in human colon carcinoma [14]. LGR4 is also upregulated in gastric cancer and associated with lymph node metastasis [15]. LGR5 is related to several clinical variables and predicts poor survival in lung adenocarcinoma [16]. The expression and localization of LGR5 are closely related to the occurrence and development of gastric cancer, and patients with $LGR5^+$ gastric cancer exhibit poorer prognoses than those with $LGR5^-$ gastric cancer [17]. $LGR5^+$ stem cells drive intestinal regeneration

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and facilitate cancer initiation through Yap-dependent reprogramming [18]. Some researchers have speculated that elevated LGR5 expression may be associated with poor survival in colorectal cancer patients [19]. Paradoxically, other research has indicated that loss of LGR5 enhances invasion, growth, and carcinogenesis in colorectal cancer cell lines, indicating that LGR5 functions as a tumor suppressor [20,21]. Therefore, the role of LGR5 in colorectal cancer remains controversial.

$LGR6^+$ non-small cell lung cancer cells are likely to undergo self-renewal and progression, indicating their high carcinogenetic potential [22]. A new study has shown that LGR6 is a marker for a group of basal and luminal progenitor cells that induce the occurrence of luminal mammary tumors [23]. LGR6 is elevated in gastric cancer and is associated with local tumor growth; notably, LGR6 expression predicts better survival in poorly cohesive gastric cancer [15]. *LGR6* is commonly mutated in colorectal cancer as confirmed by whole-exon sequencing [24]. LGR6 is also hypermethylated in 20%–50% of colon cancer cases, and the discovery of loss-of-function mutations in cancer cells indicates that *LGR6* may serve as a tumor suppressor gene [25,26]. However, these findings were observed at the cellular level and have not been validated in patients. To date, no in-depth study on the prognostic signature of LGR6 expression at the protein level in patients with colon adenocarcinoma has been conducted.

In this study, we examined the expression levels of LGR6 in colon normal mucosa, adenoma, and adenocarcinoma tissues. We also evaluated potential correlations between LGR6 expression and the clinicopathological and prognostic characteristics of patients with colon adenocarcinoma.

Materials and methods

Participants and tissue specimens

Adenoma tissue specimens (n = 21) were collected from patients who underwent endoscopic treatment in the Department of Gastroenterology of Peking University Third Hospital between January 2015 and December 2016. Tissue specimens were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and confirmed by our pathologist by light microscopy. Human colon adenocarcinoma and adjacent normal colon tissue samples were derived from two tissue microarrays (TMAs) that were purchased from Shanghai Core Technology (Shanghai, China; lot numbers: HCol-Ade180Sur-01, HCol-Ade180-Sur-03). The TMAs consisted of 90 colon adenocarcinoma tissues and 90 adjacent normal colon tissues for a total of 180 cores with a diameter of 1.5 mm. Adjacent normal colon tissues were obtained at least 2 cm from the tumor tissues of the same patients. In the course of the experiment, certain cases were omitted because of tissue slicing issues or incomplete clinicopathological data. As a result, 156 colon adenocarcinoma and 124 normal colon tissues were used for analyses. Pathological diagnoses were based on WHO classification, and clinicopathological stage was based on American Joint Committee on Cancer (AJCC) classification. Survival period was calculated from the day of surgery to death from colon adenocarcinoma or to the date of the end of the follow-up period. The median follow-up time for overall survival was 48 months (range, 1–73 months). None of the patients had received chemotherapy prior to surgery. The study and the use of patient tissue specimens and clinicopathological data were approved by the local Ethics Committee. Characteristics of the 156 patients with colon adenocarcinoma are summarized in Table 1, and raw clinical data of the 156 patients are shown in Supplemental Table 1.

Immunohistochemistry

The 21 colon adenoma tissue sections and two TMAs were formalin fixed and paraffin embedded. Prior to immuno-

Table 1 Clinicopathological factors of patients with colon adenocarcinoma (n = 156)

Characteristic	Value/number of patients	Ratio
Age median (range), year	67 (24–91)	
Gender		
Male	85	54.50%
Female	71	45.50%
Age, year		
<60	30	19.20%
60–91	126	80.80%
Tumor Nodes Metastases category		
T1	4	2.60%
T2	9	5.80%
T3	113	72.40%
T4	30	19.20%
N0	85	54.50%
N1	54	34.60%
N2	17	10.90%
M0	149	95.50%
M1	7	4.50%
American Joint Committee on Cancer category		
I	11	7.10%
II	73	46.80%
III	65	41.70%
IV	7	4.50%
Survival status		
Alive	88	56.40%
Dead	68	43.60%

histochemistry, tissue sections were deparaffinized in xylol twice for 20 min each and hydrated in a series of descending alcohol concentrations. Endogenous peroxidase activity was blocked by incubating the samples in 0.3% hydrogen peroxide for 10 min at 25 °C. Antigen retrieval was carried out at 99 °C in a solution of 10 mmol/L sodium citrate buffer (pH 6.0) for 20 min. After cooling to 25 °C, the sections were incubated in a moist chamber at 4 °C overnight with LGR6 or LGR5 primary antibody (Cell Signaling Technology, CST, USA). PV9000 universal two-step detection kit (Zhong Shan Jin Qiao, Beijing, China) was applied as the secondary antibody at 25 °C. Subsequently, staining was visualized by incubation with diaminobenzidine substrate. Sections were counterstained with hematoxylin. For negative controls, the primary antibody was replaced with PBS.

Assessment of immunohistochemical staining intensity

Two professional pathologists blind to clinicopathological characteristics evaluated all sections independently. Inconsistent scores were discussed by the two pathologists until agreement was reached. Staining scores were categorized into four grades: 0 for no staining (Fig. 1A), 1 + for weak staining (Fig. 1B), 2 + for moderate staining (Fig. 1C), and 3 + for strong staining (Fig. 1D). We recorded the scores as 0, 0–1, 1–2, or 2–3. A score of 0–1 was defined as low expression, and scores higher than 1 were defined as high expression.

Statistical analysis

All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Chi-square, Fisher's exact, and Kruskal–Wallis tests were used to analyze the association between LGR6 expression and clinicopathological factors of patients with colon adenocarcinoma. The Kaplan–Meier (log-rank) test was used for univariate survival analysis to screen for prognostic factors, and the Cox proportional hazard regression model was used for multivariate survival analysis to examine variables significantly associated with survival in univariate analysis. Statistical significance was determined as $P < 0.05$.

Results

LGR6 expression is significantly higher in adenoma and adenocarcinoma than in normal tissues

We explored LGR6 expression in normal colon ($n = 124$), adenoma ($n = 21$), and adenocarcinoma ($n = 156$) tissues,

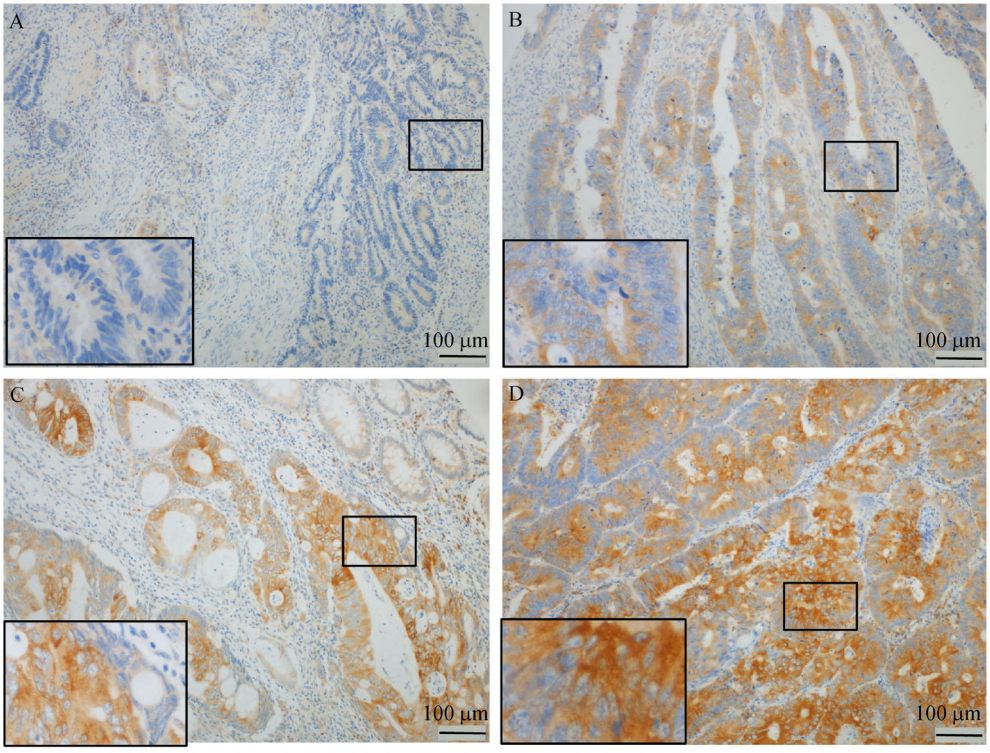

Fig. 1 LGR6 is expressed in colon adenocarcinoma. Immunohistochemical staining was performed to examine LGR6 expression in colon adenocarcinomas. Representative photographs are shown, and corresponding regions are enlarged. Staining scores were categorized into four grades: (A) no staining (0); (B) weak staining (1+); (C) moderate staining (2+); (D) strong staining (3+).

and representative staining images of LGR6 expression are presented in Fig. 2. Positive immunoreactions were primarily detected in the membrane and cytoplasm. In normal colon tissue (Fig. 2A and 2C), the membrane and cytoplasm of mucosal epithelial cells were seldom stained, whereas stromal cells were strongly stained. Normal (Fig. 2C) and adenoma (Fig. 2D) tissues were extracted from a large tissue section that enclosed the adenoma and normal colon mucosa (Fig. 2B). In the same tissue sample, the staining of the adenoma was significantly stronger than that of the adjacent normal tissue. Similarly, the expression of LGR6 in colon adenocarcinoma epithelial cells was much higher than that in normal tissues (Fig. 2E). One-way ANOVA indicated significant differences in staining intensity among the three groups. Specifically, when comparing the staining intensity scores of normal and adenoma tissues with a *t*-test, LGR6 was significantly overexpressed in adenoma tissues ($P < 0.001$). Similarly, by comparing normal and adenocarcinoma tissues using the same method, LGR6 was found to be significantly overexpressed in adenocarcinoma tissues ($P < 0.001$). Nevertheless, no significant difference in staining was observed between adenoma and adenocarcinoma tissues ($P > 0.05$; Fig. 2F). These findings demonstrate that, in the progression of colon adenocarcinoma, LGR6 expression is elevated at the stage of precancerous lesion formation, which may serve as an early diagnostic indicator. Furthermore, we examined LGR6 expression in Sabates–Bellver colon (Fig. 3A) and Skrzypczak colorectal (Fig. 3B) data sets from Oncomine. We found that LGR6 expression levels were higher in adenoma and adenocarcinoma than in normal tissues, and these findings are consistent with our experimental results. We also queried CEA, KRAS, and MSI expression statuses in the UCSC and Oncomine databases to assess correlations among KRAS, MSI expression statuses, and LGR6 expression. Unfortunately, no data on CEA and LGR6 expression were available. As for KRAS, UCSC data showed no significant correlations between the expression of KRAS and LGR6 expression in normal and tumor tissues ($P = 0.1810$, $P = 0.2177$, respectively; Fig. 3C and 3D). However, Oncomine data indicated that LGR6 expression was significantly higher in KRAS mutant than in KRAS wild-type specimens ($P = 0.0006$; Fig. 3E). As for microsatellite statuses, the Oncomine database showed that LGR6 expression was lower in specimens with microsatellite instability than in those with microsatellite stability ($P = 0.0220$; Fig. 3F). Previous

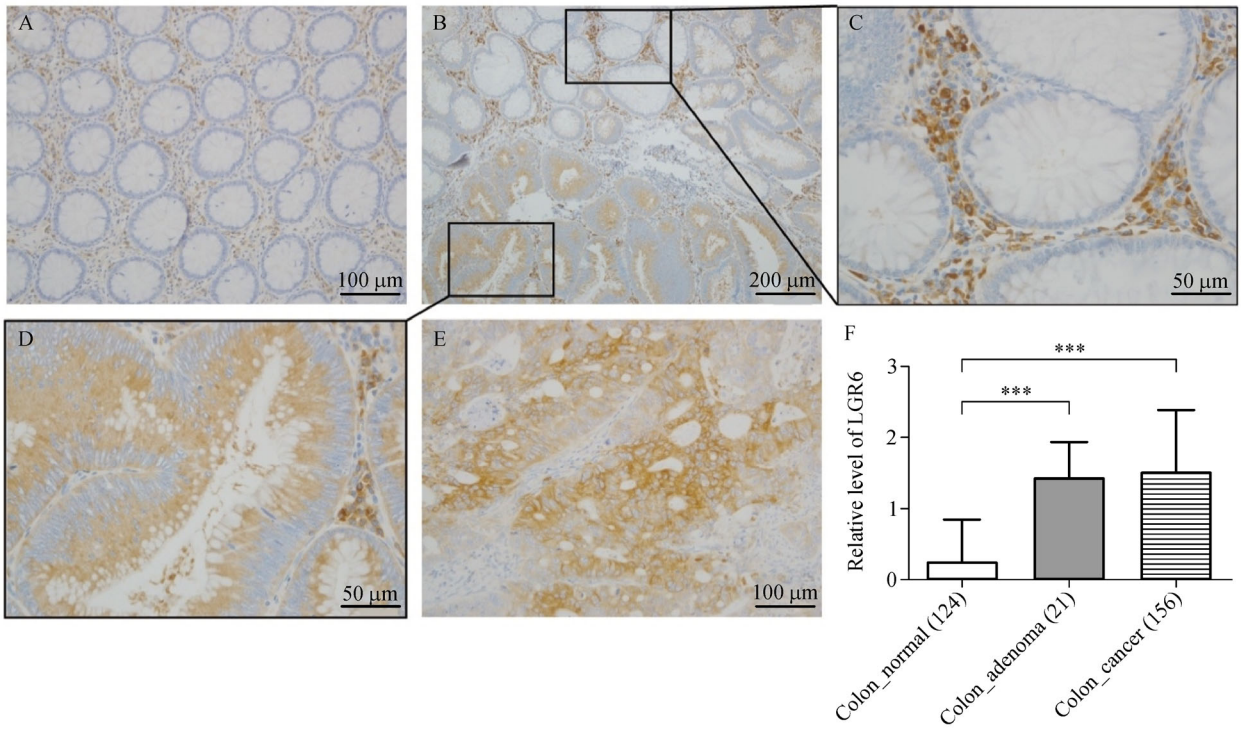

Fig. 2 Expression of LGR6 in normal, adenoma, and adenocarcinoma colon tissues. (A) Normal colon mucosa; (B) large tissue section enclosing adenoma and normal colon mucosa; (C) normal tissue extracted from Fig. 2B; (D) adenoma tissue extracted from Fig. 2B; (E) colon adenocarcinoma tissue; (F) LGR6 expression is higher in colon adenoma and adenocarcinoma tissues than in normal colon mucosa (*t*-test, $P < 0.001$), whereas differences between adenoma and adenocarcinoma are insignificant (*t*-test, $P > 0.05$).

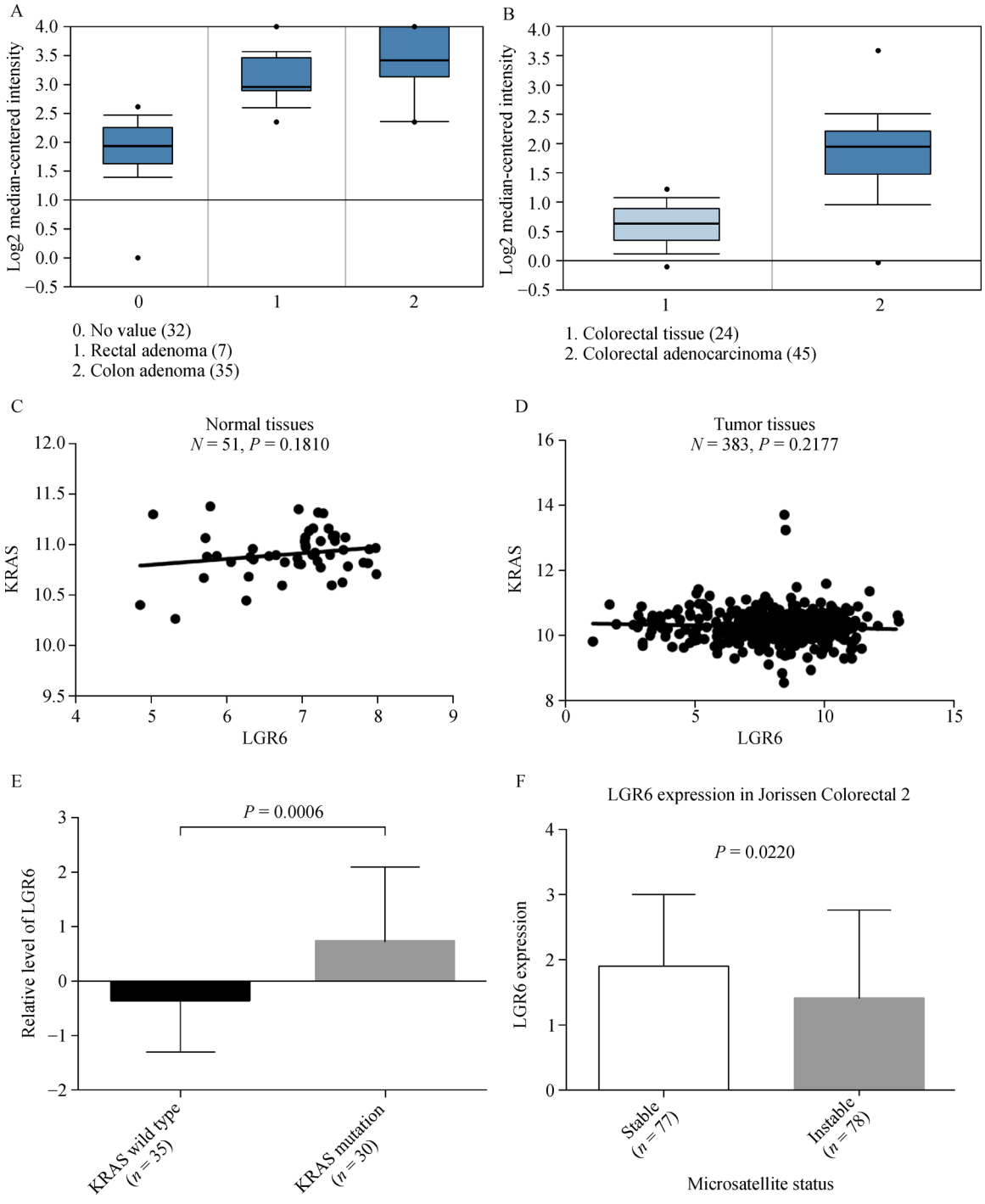

Fig. 3 Expression of LGR6 in colorectal adenoma and adenocarcinoma. (A) LGR6 expression in Sabates–Bellver colon data set in Oncomine: rectal and colonic adenomas highly express LGR6; (B) LGR6 expression in Skrzypczak colorectal data set in Oncomine: LGR6 expression in colorectal adenoma is significantly higher than that in normal tissue; (C and D) relationships between KRAS and LGR6 expression in normal and tumor tissues, respectively, in the UCSC database (P = 0.1810; P = 0.2177). (E) LGR6 expression is significantly higher in KRAS mutant than in KRAS wild-type specimens in the Oncomine database (P = 0.0006). (F) LGR6 expression is lower in specimens with microsatellite than in those with microsatellite stability in Oncomine (P = 0.0220).

studies have shown that the prognosis of patients with microsatellite is poorer than that of patients with microsatellite stability, which is consistent with our findings on LGR6 expression in relation to survival as shown below.

The relationship between LGR5 and colorectal cancer is controversial. Thus, we also compared LGR5 expression

in normal colon, adenoma, and adenocarcinoma tissues; however, we found no significant differences. Therefore, the role of LGR5 in colorectal cancer requires further study.

LGR6 expression is associated with adenocarcinoma patient survival

We analyzed associations between LGR6 expression and clinical features of the 156 patients with colon adenocarcinoma (Table 2). Among the adenocarcinoma patients, 59 (37.82%) patients exhibited low expression and 97 (62.18%) exhibited high expression of LGR6. Correlations between LGR6 expression and gender (Chi-square test, $P = 0.271$), age (Chi-square test, $P = 0.420$), T classification (Kruskal–Wallis test, $P = 0.068$; Fisher's exact test, $P = 0.123$), M classification (Chi-square test, $P = 0.063$), N classification (Fisher's exact test, $P = 0.367$), and AJCC classification (Kruskal–Wallis test, $P = 0.285$; Fisher's exact test, $P = 0.146$) were insignificant. However, LGR6 expression was significantly associated with survival (Chi-square test, $P = 0.036$).

Low LGR6 expression predicts poor prognosis in patients with colon adenocarcinoma

To determine the clinical significance of LGR6 expression in colon adenocarcinoma, we performed an in-depth survival analysis. Among the 156 patients, the median survival time was 48 months. At the end of the follow-up period, 88 (56.41%) patients had survived and 68 (43.59%) patients had died. A total of 61 (39.10%) survivors had high LGR6 expression and 27 (17.30%) had low expression. Among the patients who had died, 36 (23.10%) had high expression and 32 (20.50%) had low expression.

The survival analysis results are presented in Table 3. Univariate analysis showed that factors significantly correlated with the length of survival of patients with colon adenocarcinoma included N classification (N0 vs. N1–N2, $P = 0.002$), M classification (M0 vs. M1, $P = 0.000$), AJCC classification (I –II vs. III–IV, $P = 0.001$), and LGR6 expression (high vs. low, $P = 0.018$). On the contrary, gender ($P = 0.653$) and age ($P = 0.612$) were insignificantly related to survival.

Table 2 Association between LGR6 expression levels and clinical variables in colon adenocarcinoma

Characteristic	Low-expression group				High-expression group				*P*
	0		(0–1)		(1–2)		(2–3)		
	No. of patients	Ratio	No. of patients	Ratio	No. of patients	Ratio	No. of patients	Ratio	
Gender									0.271
Male	5	3.20%	31	19.90%	31	19.90%	18	11.50%	
Female	7	4.50%	16	10.30%	30	19.20%	18	11.50%	
Age, year									0.420
<60	2	1.30%	11	7.10%	8	5.10%	9	5.80%	
60–91	10	6.40%	36	23.10%	53	34.00%	27	17.30%	
Tumor Nodes Metastases category									
T1	1	0.60%	3	1.90%	0	0.00%	0	0.00%	0.068[a]
T2	1	0.60%	1	0.60%	6	3.80%	1	0.60%	0.123[b]
T3	8	5.10%	32	20.50%	42	26.90%	31	19.90%	
T4	2	1.30%	11	7.10%	13	8.30%	4	2.60%	
N0	6	3.80%	28	17.90%	26	16.70%	25	16.00%	0.063
N1–N2	6	3.80%	19	12.20%	35	22.40%	11	7.10%	
M0	11	7.10%	44	28.20%	58	37.20%	36	23.10%	0.367
M1	1	0.60%	3	1.90%	3	1.90%	0	0.00%	
American Joint Committee on Cancer									
I	1	0.60%	4	2.60%	5	3.20%	1	0.60%	0.285[a]
II	5	3.20%	23	14.70%	21	13.50%	24	15.40%	0.146[b]
III	5	3.20%	17	10.90%	32	20.50%	11	7.10%	
IV	1	0.60%	3	1.90%	3	1.90%	0	0.00%	
Survival status									
Alive	4	2.60%	23	14.70%	36	23.10%	25	16.00%	0.036*
Dead	8	5.10%	24	15.40%	25	16.00%	11	7.10%	

[a] Kruskal–Wallis test. [b] Fisher's exact test. Other *P*-values are based on Chi-square tests. *$P < 0.05$.

Table 3 Univariate and multivariate analysis of overall survival in 156 patients with colon adenocarcinoma

Characteristic	Univariate survival analysis			Multivariate survival analysis		
	P	HR	95% CI	*P*	HR	95% CI
Gender						
Male	0.653	0.896	0.554–1.499			
Female						
Age, year						
<60	0.612	1.174	0.629–2.191			
60–90						
Tumor Nodes Metastases category						
T1–T2	0.063	3.794	0.929–15.505			
T3–T4						
N0	0.002*	2.122	1.307–3.444	0.003*	2.109	1.283–3.467
N1–N2						
M0	0.000*	5.437	2.305–12.825	0.002*	4.029	1.681–9.657
M1						
American Joint Committee on Cancer						
I–II	0.001*	2.229	1.370–3.626			
III–IV						
Relative level of LGR6						
High expression	0.018*	1.780	1.103–2.872	0.008*	1.919	1.182–3.114
Low expression						

Patients with $P<0.15$ in univariate analysis were selected for multivariate analysis of survival. The AJCC (American Joint Committee on Cancer) classification is directly derived from the TNM classification. Thus, it is excluded in the multivariate survival analysis. * $P<0.05$.

Subsequently, Cox proportional hazard model analysis was used to determine the independent prognostic factors for survival. Multivariate survival analysis results indicated that N classification (HR 2.109, $P = 0.003$), M classification (HR 4.029, $P = 0.002$), and LGR6 expression (HR 1.919, $P = 0.008$) served as independent prognostic factors. The Kaplan–Meier survival curve (Fig. 4) demonstrates that elevated expression of LGR6 predicts long overall survival time (log-rank test, $P = 0.016$).

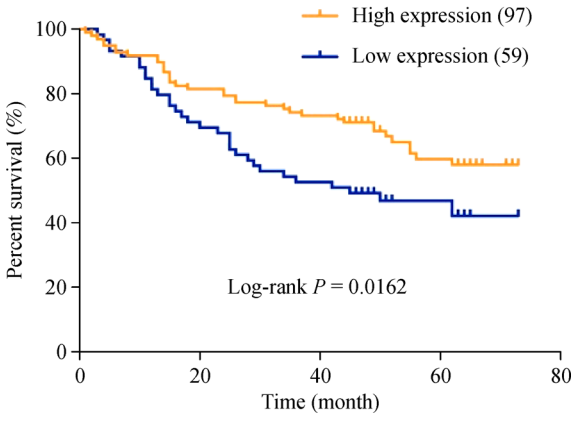

Fig. 4 Elevated expression of LGR6 predicts long overall survival for patients with colon adenocarcinoma. Kaplan–Meier analysis of two groups of patients with colon adenocarcinoma with high or low LGR6 expression was determined by log-rank test ($P < 0.05$), which shows that elevated expression of LGR6 correlates with better overall survival for patients with colon adenocarcinoma.

Discussion

The development of colonoscopy has improved the diagnostic rate of colorectal cancer, but this cancer remains a common cause of death worldwide and accounts for more than 600 000 deaths each year [2]. Multiple studies have investigated molecular targets in colorectal cancer, but few independent prognostic factors have been reported. Thus, to determine appropriate treatments and improve colorectal cancer prognoses, predictive and effective targeted molecular approaches are needed. However, previous studies on LGR6 were limited to the understanding of cellular and molecular mechanisms, and no studies have analyzed the clinical correlation of LGR6 in terms of disease progression, especially the prognostic value of LGR6 expression in colorectal cancer patients. Intriguingly, our investigation identified that LGR6 is an independent prognostic factor in colon adenocarcinoma. For the first time, LGR6 is clearly linked with colon adenocarcinoma patient progression, indicating that LGR6 is a potential prognostic marker and may also be a therapeutic target for colorectal cancer.

In this study, we used 21 adenoma tissues to analyze precancerous expression of LGR6. We also used 156 cancer tissues to analyze the relationship between LGR6 expression and clinicopathological features. We found that LGR6 is upregulated in colon adenocarcinoma and even in the early stages of adenoma, indicating that it is expressed during colorectal carcinogenesis. Our findings suggested that LGR6 acts as a protective factor against cancer progression. Indeed, protective mechanisms are usually activated during cancer progression. For example, p53, HOX family proteins, and various other molecules are reported to be cancer inhibitors that are upregulated in cancer [27,28]. Previous findings of loss-of-function mutations in cancer cells and promoter hypermethylation strongly argue that LGR6 functions as a tumor suppressor in colon cancer. Another previous study indicated that LGR6 is not involved in cancer cell proliferation [26]. We have also previously examined the potential role of LGR6 in cancer cell proliferation and migration, but no such role was established. Thus, LGR6 may act as an oncogene in other ways, which require further research.

In addition to its role in colorectal cancer, LGR6 is involved in other cancers, such as gastric, lung, and breast cancers. These results indicate that a single molecule may have pleiotropic effects in different diseases or different subtypes of the same disease. Molecules playing different roles in different organs or tissues are commonly observed in cancer pathogenesis. Guinot *et al*. [22] reported that LGR6 promotes lung cancer progression. However, aberrant Wnt/β-catenin signaling is known to play a critical role in the pathogenesis of multiple human diseases and various types of cancer, including colon cancer [29]. LGR4–6 are modulators of the Wnt/β-catenin signaling pathway. Interactions with their R-spondin receptors (RSPO1, RSPO2, RSPO3, or RSPO4) can modulate Wnt/β-catenin signaling and play pleiotropic roles in various aspects [11,30]. This mechanism explains how LGR6 promotes lung cancer progression. LGR6 can bind to and interact with RSPO1–3, thereby positively impacting Wnt/β-catenin signaling through phosphorylation in colon cancer [26]. However, this condition does not explain why high LGR6 expression predicts good outcome for colorectal patients. Mutations and other molecular features of LGR6 in the digestive system require further study.

Colorectal cancer is a heterogeneous disease. Different subtypes of cells may exist in the same tumor, and patients suffering from the same type of cancer may exhibit different therapeutic outcomes and prognoses. This heterogeneity brings difficulty in selecting appropriate target therapies for each patient. Therefore, a deep understanding of tumor heterogeneity in colorectal cancer, especially in relation to clinical features, is necessary. The cell membrane signaling GPCR proteins, including LGR6, are excellent candidates for targeted molecular treatment of cancer [31]. Notably, our data demonstrate that LGR6 may be a potential therapeutic target in colon adenocarcinoma. Increased expression or inhibition of degradation to interfere with tumor progression can provide potential therapeutic strategies. Expression of LGR6 can be combined with other independent prognostic factors for the diagnosis and treatment of colorectal cancer.

The limitations of the current study include the relatively small number of patients and the use of immunohistochemical methods only for the detection of LGR6 expression. Despite these limitations, our results demonstrate a significantly positive correlation between LGR6 expression and colon adenocarcinoma patient prognosis. In the future, additional precancerous lesions should be examined, and further functional investigations should be performed to elucidate the molecular mechanisms by which LGR6 expression is involved in the progression of colon cancer. This need is especially true in terms of the pathogenesis of precancerous lesions such that interventions can be performed early on during disease progression.

In summary, this study is the first to demonstrate that LGR6 expression occurs early and remains high in colon adenoma and adenocarcinoma tissues. Moreover, elevated expression of LGR6 is associated with improved prognosis in patients with colon adenocarcinoma. These findings provide a basis for the potential utility of LGR6 as an early prognostic biomarker and a target gene for early therapeutic intervention.

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Compliance with ethics guidelines

Wenjing Wang, Shigang Ding, Hejun Zhang, Jun Li, Jun Zhan, and Hongquan Zhang declare no conflicts of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the ethical committee of Peking University Health Science Center, China) and with the *Helsinki Declaration* of 1975, as revised in 2000. Informed consent was obtained from all patients enrolled in the study.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-018-0633-0 and is accessible for authorized users.

References

1. Chen W, Zheng R, Zeng H, Zhang S. The incidence and mortality of major cancers in China, 2012. Chin J Cancer 2016; 35(1): 73
2. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet 2014; 383 (9927): 1490–1502
3. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007; 50(1): 113–130
4. Markowitz SD, Bertagnolli MM. Molecular origins of cancer: molecular basis of colorectal cancer. N Engl J Med 2009; 361(25): 2449–2460
5. Sinicrope FA, Sargent DJ. Molecular pathways: microsatellite instability in colorectal cancer: prognostic, predictive, and therapeutic implications. Clin Cancer Res 2012; 18(6): 1506–1512
6. De Sousa E Melo F, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, de Jong JH, de Boer OJ, van Leersum R, Bijlsma MF, Rodermond H, van der Heijden M, van Noesel CJ, Tuynman JB, Dekker E, Markowetz F, Medema JP, Vermeulen L. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 2013; 19(5): 614–618
7. Carmona FJ, Azuara D, Berenguer-Llergo A, Fernández AF, Biondo S, de Oca J, Rodriguez-Moranta F, Salazar R, Villanueva A, Fraga MF, Guardiola J, Capellá G, Esteller M, Moreno V. DNA methylation biomarkers for noninvasive diagnosis of colorectal cancer. Cancer Prev Res (Phila) 2013; 6(7): 656–665
8. Vatandoost N, Ghanbari J, Mojaver M, Avan A, Ghayour-Mobarhan M, Nedaeinia R, Salehi R. Early detection of colorectal cancer: from conventional methods to novel biomarkers. J Cancer Res Clin Oncol 2016; 142(2): 341–351
9. Böhme I, Beck-Sickinger AG. Illuminating the life of GPCRs. Cell Commun Signal 2009; 7(1): 16
10. Hsu SY, Kudo M, Chen T, Nakabayashi K, Bhalla A, van der Spek PJ, van Duin M, Hsueh AJ. The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. Mol Endocrinol 2000; 14(8): 1257–1271
11. de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ, Clevers H. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature 2011; 476(7360): 293–297
12. Leushacke M, Barker N. Lgr5 and Lgr6 as markers to study adult stem cell roles in self-renewal and cancer. Oncogene 2012; 31(25): 3009–3022
13. Lehoczky JA, Tabin CJ. Lgr6 marks nail stem cells and is required for digit tip regeneration. Proc Natl Acad Sci USA 2015; 112(43): 13249–13254
14. Gao Y, Kitagawa K, Hiramatsu Y, Kikuchi H, Isobe T, Shimada M, Uchida C, Hattori T, Oda T, Nakayama K, Nakayama KI, Tanaka T, Konno H, Kitagawa M. Up-regulation of GPR48 induced by down-regulation of p27Kip1 enhances carcinoma cell invasiveness and metastasis. Cancer Res 2006; 66(24): 11623–11631
15. Steffen JS, Simon E, Warneke V, Balschun K, Ebert M, Röcken C. LGR4 and LGR6 are differentially expressed and of putative tumor biological significance in gastric carcinoma. Virchows Arch 2012; 461(4): 355–365
16. Ryuge S, Sato Y, Jiang SX, Wang G, Kobayashi M, Nagashio R, Katono K, Iyoda A, Satoh Y, Masuda N. The clinicopathological significance of Lgr5 expression in lung adenocarcinoma. Lung Cancer 2013; 82(1): 143–148
17. Simon E, Petke D, Böger C, Behrens HM, Warneke V, Ebert M, Röcken C. The spatial distribution of $LGR5^{+}$ cells correlates with gastric cancer progression. PLoS One 2012; 7(4): e35486
18. Gregorieff A, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL. Yap-dependent reprogramming of $Lgr5^{+}$ stem cells drives intestinal regeneration and cancer. Nature 2015; 526(7575): 715–718
19. Takahashi H, Ishii H, Nishida N, Takemasa I, Mizushima T, Ikeda M, Yokobori T, Mimori K, Yamamoto H, Sekimoto M, Doki Y, Mori M. Significance of $Lgr5^{+ve}$ cancer stem cells in the colon and rectum. Ann Surg Oncol 2011; 18(4): 1166–1174
20. Walker FZhang HH, Odorizzi A, Burgess AW. LGR5 is a negative regulator of tumourigenicity, antagonizes Wnt signalling and regulates cell adhesion in colorectal cancer cell lines. PLoS One 2011; 6(7): e22733
21. de Sousa E Melo F, Colak S, Buikhuisen J, Koster J, Cameron K, de Jong JH, Tuynman JB, Prasetyanti PR, Fessler E, van den Bergh SP, Rodermond H, Dekker E, van der Loos CM, Pals ST, van de Vijver MJ, Versteeg R, Richel DJ, Vermeulen L, Medema JP. Methylation of cancer-stem-cell-associated Wnt target genes predicts poor prognosis in colorectal cancer patients. Cell Stem Cell 2011; 9(5): 476–485
22. Guinot A, Oeztuerk-Winder F, Ventura JJ. miR-17–92/p38α dysregulation enhances Wnt signaling and selects $Lgr6^{+}$ cancer stem-like cells during lung adenocarcinoma progression. Cancer Res 2016; 76(13): 4012–4022
23. Blaas L, Pucci F, Messal HA, Andersson AB, Josue Ruiz E, Gerling M, Douagi I, Spencer-Dene B, Musch A, Mitter R, Bhaw L, Stone R, Bornhorst D, Sesay AK, Jonkers J, Stamp G, Malanchi I, Toftgård R, Behrens A. Lgr6 labels a rare population of mammary gland progenitor cells that are able to originate luminal mammary tumours. Nat Cell Biol 2016; 18(12): 1346–1356
24. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Willis J, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Bachman KE, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. Science 2006; 314(5797): 268–274
25. Chan TA, Glockner S, Yi JM, Chen W, Van Neste L, Cope L, Herman JG, Velculescu V, Schuebel KE, Ahuja N, Baylin SB. Convergence of mutation and epigenetic alterations identifies common genes in cancer that predict for poor prognosis. PLoS Med 2008; 5(5): e114
26. Gong X, Carmon KS, Lin Q, Thomas A, Yi J, Liu Q. LGR6 is a high affinity receptor of R-spondins and potentially functions as a tumor suppressor. PLoS One 2012; 7(5): e37137
27. Wang X, Sun Q. TP53 mutations, expression and interaction networks in human cancers. Oncotarget 2017; 8(1): 624–643
28. Zhan J, Niu M, Wang P, Zhu X, Li S, Song J, He H, Wang Y, Xue L, Fang W, Zhang H. Elevated HOXB9 expression promotes differentiation and predicts a favourable outcome in colon

adenocarcinoma patients. Br J Cancer 2014; 111(5): 883–893
29. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell 2012; 149(6): 1192–1205
30. Carmon KS, Gong X, Lin Q, Thomas A, Liu Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/β-catenin signaling. Proc Natl Acad Sci USA 2011; 108(28): 11452–11457
31. Lappano R, Maggiolini M. G protein-coupled receptors: novel targets for drug discovery in cancer. Nat Rev Drug Discov 2011; 10 (1): 47–60