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ORIGINAL ARTICLE

Correlation of Lgr5 expression with clinicopathological features of colorectal cancer and its diagnostic and prognostic values

Ning Xu[1], Kai Gao[2], Hui Luo[3], Yuan Wu[4], Bo Shen[4]*, Ke Liu[5]*

[1]Hepatobiliary & Pancreatic Surgery, the second affiliated Hospital of Kunming Medical University, Kunming 650000, P.R. China. [2]Department of Gastrointestinal Surgery, the third affiliated Hospital of Central South University, Central South University, Changsha 410013, PR China. [3]Department of Oncology, the first affiliated Hospital of Nanchang University, Nanchang 330000, P.R. China. [4]Department of Oncology, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, Nanjing Medical University Affiliated Cancer Hospital, Nanjing 210009, P.R. China. [5]Department of Medical Oncology, Changzheng Hospital, Naval Medical University, Shanghai 200072, P.R. China.

*Drs Bo Chen and Ke Liu contributed equally to this study.

Summary

Purpose: *To investigate the relationship between Leucine-rich repeat G-protein coupled receptor 5 (Lgr5) expression and clinicopathological features of colorectal cancer and its diagnostic and prognostic values.*

Methods: *The cancer and adjacent tissues of 98 patients with colorectal cancer undergoing resection in Jiangsu Cancer Hospital from 2011 to 2013 were enrolled. The intestinal mucosal tissue of 50 healthy subjects was enrolled as a control group. Western blot was used to detect the expression level of Lgr5 in colorectal cancer, Kaplan-Meier method to analyze the correlation of Lgr5 expression with the 5-year survival rate, Cox regression to analyze prognostic risk factors for the patients, and receiver operating characteristics (ROC) curve to analyze the diagnostic value of Lgr5 of the disease.*

Results: *The expression level of Lgr5 in the intestinal mucosal and adjacent tissues was significantly lower than that in the cancer tissue ($p<0.05$). The 5-year survival in the Lgr5 low expression group was higher than that in the Lgr5 high expression group ($p=0.002$). Lgr5 was an independent risk factor for the prognosis of colorectal cancer. The sensitivity, specificity and the area under the curve (AUC) of Lgr5 were 90.00%, 79.59% and 0.880, respectively ($p<0.001$).*

Conclusion: *Patients with a low 5-year survival rate have a high expression of Lgr5, which is closely related to the clinical staging, differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis and distant metastasis. Lgr5 is an independent prognostic risk factor for the patients and a better indicator for the diagnosis of colorectal cancer.*

Key words: *clinicopathological features, colorectal cancer, diagnostic value, Lgr5, prognosis*

Introduction

Colorectal cancer is the third malignant tumor diagnosed worldwide, the mortality rate of which ranks fourth [1,2]. The incidence and mortality rates of this disease are rising rapidly [3]. The pathogenesis and mechanism of colorectal cancer remain unclear [4]. Early screening, timely detection and treatment can effectively improve the survival rate of the patients, although the disease has complex biological behaviors [5]. Therefore, it is of great significance to discover the pathogenesis and find new biomarkers and prognostic indicators.

Leucine-rich repeat–containing G protein-coupled receptor 5 (Lgr5) belongs to the G protein-coupled receptor family. According to recent studies,

Corresponding author: Bo Shen, MD. Department of Oncology, Jiangsu Institute of Cancer Research, Nanjing Medical University Affiliated Cancer Hospital, No.42 Baiziting, Nanjing 210009, P.R.China.
E-mail: 45352126@qq.com
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Lgr5 is a marker for colorectal cancer stem cells [6] and intestinal stem cells, the expression of which is limited to the crypt base of small and large intestine [7,8]. Studies have shown that the deletion of Lgr5 inhibits the proliferation and survival of colorectal cancer cells and increases apoptosis through inhibiting Wnt/β-catenin signaling pathway in a targeted way, as well as induces apoptosis through loss of mitochondrial membrane potential (MMP) [9]. The expression, clinicopathological significance and diagnostic value of Lgr5 in colorectal cancer still need further exploration. Therefore, Lgr5 expression was analyzed in this study in terms of its diagnostic value and its correlation with clinicopathological features and prognosis of patients with colorectal cancer.

Methods

Research object

The cancer and adjacent tissues of 98 patients with colorectal cancer undergoing resection in Jiangsu Cancer Hospital from 2011 to 2013 were evaluated in this study. The patients consisted of 56 cases with colon cancer and 42 cases with rectal cancer, aged 42-76 years old. The intestinal mucosal tissue of 50 healthy volunteers in the same period was enrolled as the control group. Inclusion criteria: Patients pathologically diagnosed with colon cancer or rectal cancer; patients who had not received radiotherapy and chemotherapy before operation; patients without abnormal bleeding or coagulation disorders; patients with complete medical records and follow-up data. Exclusion criteria: Patients with lumps over 10cm in diameter; patients with other lung diseases or diseases of the chest wall; patients with other benign or malignant tumors; patients with history of tumors; patients with malignant pleural effusion; patients with cancer-related inflammatory bowel diseases and familial adenomatous polyposis; patients with heart, brain, liver, kidney, lung and other organ diseases; patients with sepsis and other severe infections; pregnant or lactating women. This study was approved by the Medical Ethics Committee of Jiangsu Cancer Hospital. Patients and their families were informed, and an informed consent form was signed.

Western blot analysis

The tissue protein was extracted with protein extracting solution (Bestbio, Shanghai, BB-3531), separated by polyacrylamide gel electrophoresis with an initial voltage of 90 V, transferred to the membrane at 100 V for 100 min, and sealed at 37°C for 60 min. Then, hybridization with antibodies was carried out. The membrane, together with primary antibody (mouse anti-human Lgr5 monoclonal antibody, Shanghai Qunji Biotech Co., Ltd., MAB17138), was incubated overnight at 4°C, washed with phosphate buffered saline (PBS) over 10 min for three times the next day, and then incubated at 37°C for 2 h together with secondary antibody (HRP-labeled goat anti-mouse IgG, Otwo Biotech Co., Ltd., Shenzhen, PL03-0375R). After that, the membrane was fixed with electrochemiluminescence. Quantity One was used to statistically analyze the protein bands scanned in the film. The relative expression level of protein was: the gray value of protein bands/the gray value of internal reference, with β-actin as the internal reference. Polyacrylamide gel electrophoresis buffer was purchased from Xiamen Huijia Biotechnology Co., Ltd., Item No.: orb154330; Western blot detection kit was purchased from Shanghai Junrui Biotechnology Co., Ltd., Item No.: UFC04948.

Observational indexes

Chi-square test was used to analyze the correlation of Lgr5 expression with clinicopathological features of patients with colorectal cancer, Kaplan-Meier method to analyze the correlation of Lgr5 expression with the 5-year survival rate, univariate and multivariate Cox regression analyses to analyze prognostic risk factors for the patients, and ROC curve to analyze the diagnostic value of Lgr5 in the disease.

Statistics

SPSS 19.0 (Asia Analytics Formerly SPSS China) was used to analyze the data. Measurement data were expressed as %, and x^2 test was used for comparison of rates. Count data were expressed as mean±standard deviation (mean±SD), and t-test was used for comparison between the two groups, and analysis of variance (ANOVA) was used for comparison between groups. Univariate and multivariate Cox regression analyses were used to analyze the prognostic risk factors of the patients, Kaplan-Meier survival to analyze the correlation of Lgr5 expression with the survival time, ROC curve to analyze the diagnostic value of Lgr5 in colorectal cancer. P<0.05 indicated a statistically significant difference.

Results

Analysis of Lgr5 expression level

According to western blot analysis, the expression level of Lgr5 was 1.120±0.144 in the intestinal

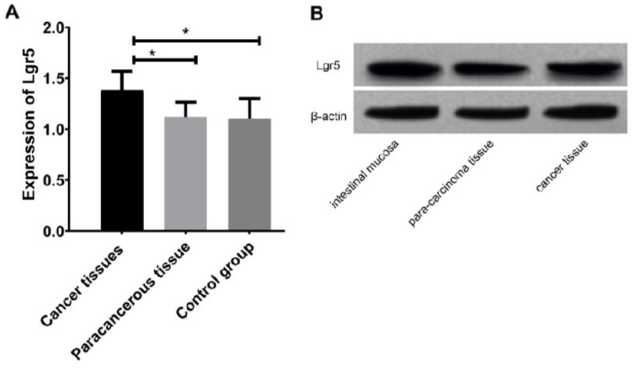

Figure 1. Analysis of Lgr5 expression levels. **A:** Expression level of Lgr5 in colorectal cancer tissues. **B:** The representative results of western blot analysis. *indicates p<0.05.

mucosal tissue, 1.382±0.184 in the cancer tissue and 1.104±0.197 in the adjacent tissue. The expression level of Lgr5 in the intestinal mucosal and adjacent tissues was significantly lower than that in the cancer tissue (p<0.05), whereas there was no statistically significant difference between the intestinal mucosal and adjacent tissues (p>0.05) (Figure 1).

Correlation of Lgr5 expression with clinicopathological features

Based on the median expression level of Lgr5 in the cancer tissue, the patients were divided into high (>1.379) and low (≤1.379) expression groups. High expression of Lgr5 was closely related to the clinical staging, differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis and distant metastasis of patients with colorectal cancer (p<0.05), but not significantly correlated with gender, age, tumor size and tumor location (p>0.05). More details are shown in Table 1.

Correlation of Lgr5 expression with prognosis

Based on the median expression level of Lgr5 in the cancer tissue, the patients were divided into high (>1.379) and low (≤1.379) expression groups. According to Kaplan-Meier survival analysis, the 5-year survival rate was 36.74% (18 cases) in the Lgr5 high expression group, lower

Table 1. Clinicopathological features of patients in both groups

Features	*High expression group (n=49) n (%)*	*Low expression group (n=49) n (%)*	x^2	*p*
Gender			0.373	0.541
Male	26 (53.06)	29 (59.18)		
Female	23 (46.94)	20 (40.82)		
Age, years			0.373	0.541
≥60	29 (59.18)	26 (53.06)		
<60	20 (40.82)	23 (46.94)		
Clinical staging			9.583	0.002
I-II	22 (44.90)	37 (75.51)		
III-IV	27 (55.10)	12 (24.49)		
Tumor size, cm			1.224	0.289
≥5	17 (34.69)	12 (24.49)		
<5	32 (65.31)	37 (75.51)		
Tumor location			2.622	0.105
Colon cancer	30 (61.22)	22 (44.90)		
Rectal cancer	19 (38.78)	27 (55.10)		
Differentiation			13.364	<0.001
Poorly differentiated	31 (63.27)	13 (26.53)		
Highly differentiated	18 (36.73)	36 (73.47)		
Depth of infiltration			11.204	0.001
Into serosa	26 (53.06)	10 (20.41)		
Not into serosa	23 (46.94)	39 (79.59)		
Lymph node metastasis			13.500	<0.001
Yes	37 (75.51)	19 (38.78)		
No	12 (24.49)	30 (61.22)		
Vascular invasion			27.671	<0.001
Yes	27 (55.10)	3 (6.12)		
No	22 (44.90)	46 (93.88)		
Liver metastasis			11.240	0.001
Yes	26 (53.06)	10 (20.41)		
No	23 (46.94)	39 (79.59)		
Distant metastasis			4.780	0.029
Yes	12 (24.49)	4 (8.16)		
No	37 (75.51)	45 (91.84)		

than 71.43% (35 cases) in the Lgr5 low expression group (p=0.002). More details are shown in Figure 2.

Analysis of prognostic risk factors

Cox univariate analysis showed that clinical stage, grade of differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis, distant metastasis and Lgr5 levels were related to prognosis of colorectal cancer patients. Cox multivariate showed that all these factors were also independent factors for the prognosis of patients with colorectal cancer (Tables 2, 3 and 4).

Diagnostic value of Lgr5

The sensitivity of Lgr5 in the diagnosis of colorectal cancer was 90.00%, the specificity was 79.59% and the AUC was 0.880, with a diagnostic level of 1.241 and 95%CI of 0.8229-0.9379 (p<0.001) (Figure 3).

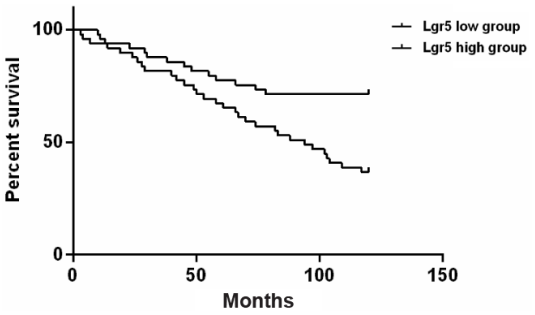

Figure 2. Relationship between Lgr5 levels in cancer tissues and prognosis in patients with colorectal cancer. The 5-year survival rate of patients with low expression of Lgr5 was higher than that of patients with high expression of Lgr5 (p=0.002).

Discussion

Colorectal cancer, more common in middle-aged men, has a high incidence rate among malignant tumors, which is the main cause of cancer death. Early detection and surgical resection of precancerous lesions can reduce its mortality rate. According to reports, the risk of cancer in patients with colorectal cancer screening is reduced by 44% compared with patients without screening, while the patients that can be detected in the early stage account for approximately only 2% [10-12], one reason for which is the unclear pathogenesis of the disease. Therefore, it is of great significance for elucidating the pathogenesis to find molecular biomarkers related to clinical features and prognosis of patients with colorectal cancer.

Table 2. Assignment table

Gender	*Male= 1, Female= 0*
Age	A continuous variable
Clinical staging	I-II= 1, III-IV= 0
Tumor size	≥5 cm= 1, <5 cm= 0
Tumor location	Colon cancer= 1, rectal cancer= 0
Differentiation	Lowly differentiated= 1, highly differentiated= 0
Depth of infiltration	Into serosa= 1, not into serosa= 0
Lymph node metastasis	Yes= 1, no= 0
Vascular invasion	Yes= 1, no= 0
Liver metastasis	Yes= 1, no= 0
Distant metastasis	Yes= 1, no= 0
Prognosis	Survived= 1, dead= 0
Lgr5	A continuous variable

Table 3. Univariate analysis of prognostic risk factors

Variables	*B*	*SE*	*Wald*	*df*	*Sig.*	*Exp(B)*	*95.0% CI*	
							Lower part	*Upper part*
Gender	-0.237	0.298	0.630	1	0.427	0.789	0.440	1.416
Age	0.020	0.015	1.794	1	0.180	1.020	0.991	1.051
Clinical staging	1.399	0.525	7.116	1	0.008	4.053	1.449	11.333
Tumor size	1.604	0.313	26.335	1	<0.001	4.973	2.695	9.176
Tumor location	-0.057	0.298	0.037	1	0.847	0.944	0.526	1.694
Differentiation	2.082	0.379	30.355	1	<0.001	8.017	3.823	16.811
Depth of infiltration	2.255	0.342	43.474	1	<0.001	9.537	4.878	18.644
Lymph node metastasis	1.791	0.440	16.587	1	<0.001	5.998	2.533	14.204
Vascular invasion	1.528	0.309	24.506	1	<0.001	4.610	2.517	8.442
Liver metastasis	1.784	0.323	30.484	1	<0.001	5.954	3.160	11.216
Distant metastasis	1.583	0.348	20.693	1	<0.001	4.870	2.462	9.632
Lgr5	1.787	0.780	5.256	1	0.022	5.972	1.296	27.522

Table 4. Multivariate analysis of prognostic risk factors

Variables	*B*	*SE*	*Wald*	*df*	*Sig.*	*Exp(B)*	*95.0% CI*	
							Lower part	*Upper part*
Clinical staging	-0.881	0.661	1.779	1	0.182	.414	0.113	1.512
Tumor size	-1.273	1.619	0.619	1	0.431	0.280	0.012	6.679
Differentiation	2.083	0.538	14.977	1	<0.001	8.030	2.796	23.064
Depth of infiltration	1.754	0.594	8.728	1	0.003	5.775	1.804	18.483
Lymph node metastasis	1.813	0.515	12.386	1	<0.001	6.127	2.233	16.814
Vascular invasion	-1.869	0.546	11.737	1	0.001	0.154	0.053	0.449
Liver metastasis	0.638	0.404	2.492	1	0.114	1.893	0.857	4.178
Distant metastasis	2.682	0.451	35.372	1	<0.001	14.609	6.037	35.352
Lgr5	0.957	0.483	3.925	1	0.048	2.603	1.010	6.706

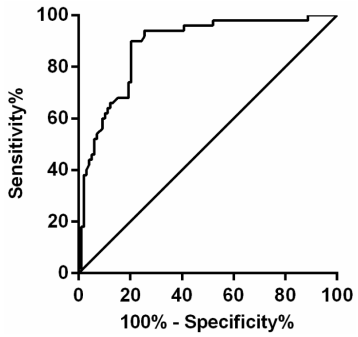

Figure 3. nostic value of Lgr5 in colorectal cancer. The sensitivity of Lgr5 in the diagnosis of colorectal cancer was 90.00%, and the specificity was 79.59%; the AUC was 0.880, and the diagnostic level was 1.241.

The diagnostic value of Lgr5 in colorectal cancer and its correlation with prognosis and clinicopathologic features were explored in this study. The expression level of Lgr5 in the colorectal cancer tissue was significantly higher than that in the adjacent tissue, and the high expression of Lgr5 was closely related to the clinical staging, differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis and distant metastasis of patients with colorectal cancer. According to Kaplan-Meier survival analysis, the 5-year survival rate was 71.43% (35 cases) in the Lgr5 low expression group, higher than 36.74% (18 cases) in the Lgr5 high expression group. According to Cox multivariate analysis, the clinical staging, differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis, distant metastasis and Lgr5 were independent risk factors for the prognosis of patients with colorectal cancer. In a recent study, the expression of Lgr5 in colorectal cancer was related to the tissue differentiation, depth of tumor infiltration, lymph node metastasis, soft tissue nodules, liver metastasis and TNM staging, among which the positive expression of Lgr5, lymph node metastasis, liver metastasis and clinical staging were independent prognostic factors of colorectal cancer [13]. According to Rosiq et al [14], Lgr5 expression in colorectal cancer is enhanced, which is positively correlated with histological grade, depth of infiltration, lymph node metastasis, distant metastasis and TNM staging. These findings are similar to the results of this study.

There are recent studies on the specific role of Lgr5 in tumors. According to a study by Barker et al [15] in mouse models, intestinal tumors originate from Lgr5-positive cells, indicating that Lgr5 marks intestinal cancer stem cells. As the most mature marker for intestinal stem cells and a negative regulator of tumors, Lgr5 activates Wnt signal transduction of colorectal cancer cells, enhances adhesion and promotes tumor development [16]. Lgr5 gene knockout inhibits the proliferation and colony formation of colorectal cancer cells and promotes apoptosis, causing cells more sensitive to chemotherapeutic drugs. On the contrary, the overexpression of Lgr5 promotes the proliferation and enhances the drug resistance of cells [17]. According to Hirsch et al [18], silencing Lgr5 inhibits the proliferation and migration of colon cancer SW480 cells possibly through down-regulating Notch signaling pathway, which also reduces the tumorigenicity of the cells after xenotransplantation. Importantly, the overexpression of Lgr5 can be detected in the early stage of colorectal tumors [19], which makes it possible for Lgr5 to play a role in the early diagnosis of colorectal cancer. Colonoscopy is greatly limited in colorectal cancer screening due to its low sensitivity and specificity, great trauma and high cost [20], although it remains the gold standard for the early diagnosis of the disease [21]. In this study, the sensitivity of Lgr5 in the diagnosis of colorectal cancer was 90.00%, the specificity was 79.59% and the AUC was 0.880, in-

dicating that Lgr5 has a better application value in the diagnosis of the disease. There are currently few studies on Lgr5 in the diagnosis of colorectal cancer. Therefore, the diagnostic value of Lgr5 in the disease still needs to be confirmed, but its potential value is undeniable.

In conclusion, Lgr5 is highly expressed in the cancer tissue of colorectal cancer. Patients with a low 5-year survival rate have a high expression of Lgr5, which is closely related to the clinical staging, differentiation, depth of infiltration, lymph node metastasis, vascular invasion, liver metastasis and distant metastasis. Lgr5 is an independent prognostic risk factor for patients and a better indicator for the diagnosis of colorectal cancer.

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Conflict of interests

The authors declare no conflict of interests.

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