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Original Article

Increased expression of *LGR5* predicts poor prognosis in cervical cancer patients

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Abstract: Aims: The present study was designed to examine the expression of leucine-rich repeat-containing G protein-coupled receptor 5 (*LGR5*) in cervical cancer (CC) tissues, and evaluate the clinical significance of *LGR5* in prognosis of CC patients. Methods: The expression of *LGR5* in CC tissues and normal cervical tissues was detected by immunohistochemistry (IHC) method. Chi-square test was adopted to estimate the relationship of *LGR5* expression and clinical parameters of CC patients. Survival curves were plotted to describe the overall survival of CC patients using Kaplan-Meier method. Cox regression analysis was used to explore the correlation between *LGR5* expression and prognosis of CC patients. Results: The positive rate of *LGR5* expression was higher in CC tissues compared to the normal cervical tissues ($P<0.001$). *LGR5* expression was significantly associated with FIGO stage, lymph node metastasis and vascular invasion ($P<0.05$). The overall survival of patients with *LGR5* positive expression was significantly worse than those with *LGR5* negative expression ($P = 0.000$). Cox regression analysis confirmed that *LGR5* could be used as an independent prognostic biomarker for CC ($P = 0.000$, HR = 7.235, 95% CI = 3.462-15.119). Conclusion: Taken together, *LGR5* positively expressed in CC tissues and it was an independent prognostic biomarker for CC patients.

Keywords: Prognosis, cervical cancer, *LGR5*

Introduction

Cervical cancer (CC) is one of the most common female cancers and also one of the important causes of cancer-related deaths all over the world [1, 2]. Every year, about 273,000 patients died of CC globally, which mainly occurs in developing countries [3, 4]. As CC patients at early stage have no obvious signs and symptoms, they are often diagnosed at an advanced stage [5]. Currently, the potentially curative and optimal treatments for CC patients are mainly radiotherapy, surgical resection and chemotherapy [6, 7]. However, effective treatment options for CC patients with an advanced stage are limited and 30-35% of them are failed in the treatments [8]. Besides, the 5-year survival rate of patients with early stage could achieve 90%, while that of those with advanced stages are less than 40% [9, 10]. Therefore, identification of novel biomarkers for prognosis of CC patients is urgently needed.

Leucine-rich repeat-containing G protein-coupled receptor 5 (*LGR5*), which is also known as *GPR49*, is firstly identified as a member of G protein-coupled receptors, consisting of a seven transmembrane domain and a large extracellular domain with 17 leucine-rich repeats [11-13]. *LGR5* is found locating at chromosome 12q22-q23 with a length of about 144 kb [14]. Recently, *LGR5* has been regarded as a somatic stem cell biomarker which plays functionally important roles on the development of cells [15-17]. Besides, *LGR5* has been reported to be involved in various human cancers, such as basal cell carcinoma, colon cancer, hepatocellular carcinoma, ovarian cancer and endometrial cancer [18-21]. Qing Chen et al. [22] have demonstrated that *LGR5* could promote proliferation and tumor formation in CC cells by activating the Wnt/β-catenin pathways. Though previous reports have indicated the roles of *LGR5* in CC, there are few studies about the relationship between *LGR5* and the prognosis of CC patients.

Table 1. Different expression of *LGR5* in CC tissues and the normal cervical tissues

Tissues	Case NO.	*LGR5* expression		Positive rate	*P* value
		Positive	Negative		
CC	121	95	26	78.5%	<0.001
Normal	56	9	47	16.1%	

In this study, we tended to examine the expression of *LGR5* in CC tissues and explore the clinical value of *LGR5* as a potential prognostic factor in CC patients, then expected to provide new strategies for diagnosis and treatment of CC.

Materials and methods

Patients and specimens

CC tissues were collected from 121 patients who underwent surgical resection in Guizhou People's Hospital. Among the patients, 68 were squamous and 53 were adenocarcinoma with the age ranging from 21 to 74 years. The detailed clinical information of CC patients was enrolled from the hospital's records. All the patients were treated with the same strategy and received no radiotherapy or chemotherapy. The present study was approved by the Ethics Committee of Guizhou People's Hospital. Informed consents were provided by all the patients in advance. In addition, 56 healthy volunteers were randomly selected to serve as controls in the study.

Immunohistochemistry (IHC)

Immunohistochemical analysis was performed to assess the *LGR5* protein expression in human CC tissues and normal cervical tissues. Briefly, the paraffin-embedded sections were firstly deparaffinized in xylene and then washed in descending concentration of ethanol series. 3% H_2O_2 was used to block endogenous peroxidase for 10 min. After antigen revival, 10% goat serum was added to block nonspecific binding. The sections were incubated with *LGR5* purified rabbit polyclonal antibody (AP2745d, Abgent, San Diego, CA, USA; at 1:10 dilution) overnight at 4°C. Negative controls were carried out with the same procedure without primary antibody. Then the sections were washed three times with PBS followed by the addition of secondary antibody. At last, the sections were stained with DAB (GK500705, Dako, Glostrup, Denmark). Sections were counterstained with hematoxylin for 40 s, then rinsed in water. The result was recorded according to the staining percentage of cells. Tissues with the cell staining percentage more than 30% belonged to the positive group, and the others were in the negative group.

Statistical analysis

The data was analyzed by SPSS 18.0 (SPSS Inc, IL, USA). The difference of *LGR5* expression in tumor tissues and normal cervical tissues was compared by student's t test. The relationship between *LGR5* expression and clinical parameters of CC patients was evaluated by Chi-square test. The survival curves were plotted using the Kaplan-Meier method, and differences in the survival distributions were evaluated by log-rank test. Cox regression analysis was conducted to estimate the prognostic significance of *LGR5* in CC. It was considered statistically significant when *P* was less than 0.05.

Results

Positive expression of LGR5 in CC tissues

We firstly examined the expression of *LGR5* in 121 CC tissues and 56 normal cervical tissues using IHC method. Among the CC tissues, positive expression of *LGR5* was found in 95 cases, the positive rate was 78.5%. While positive expression of *LGR5* in the normal control cervical tissues was found in 9 cases, so the positive rate was only 16.1%. The result showed that *LGR5* expression between CC tissues and normal cervical tissues were significantly different (**Table 1**; **Figure 1**, $P<0.001$), indicating that *LGR5* expression might relate to the pathogenesis of CC.

Association of LGR5 expression and clinical characteristics of CC patients

Based on the expression of *LGR5* in CC tissues, we further analyzed its relationship with clinical characteristics of CC patients. As detailed in **Table 2**, *LGR5* expression shared no significant association with age, differentiation and tumor histology ($P>0.05$), but was tightly related with FIGO stage ($P = 0.029$), vascular invasion ($P = 0.021$) and lymph node metastasis ($P = 0.016$).

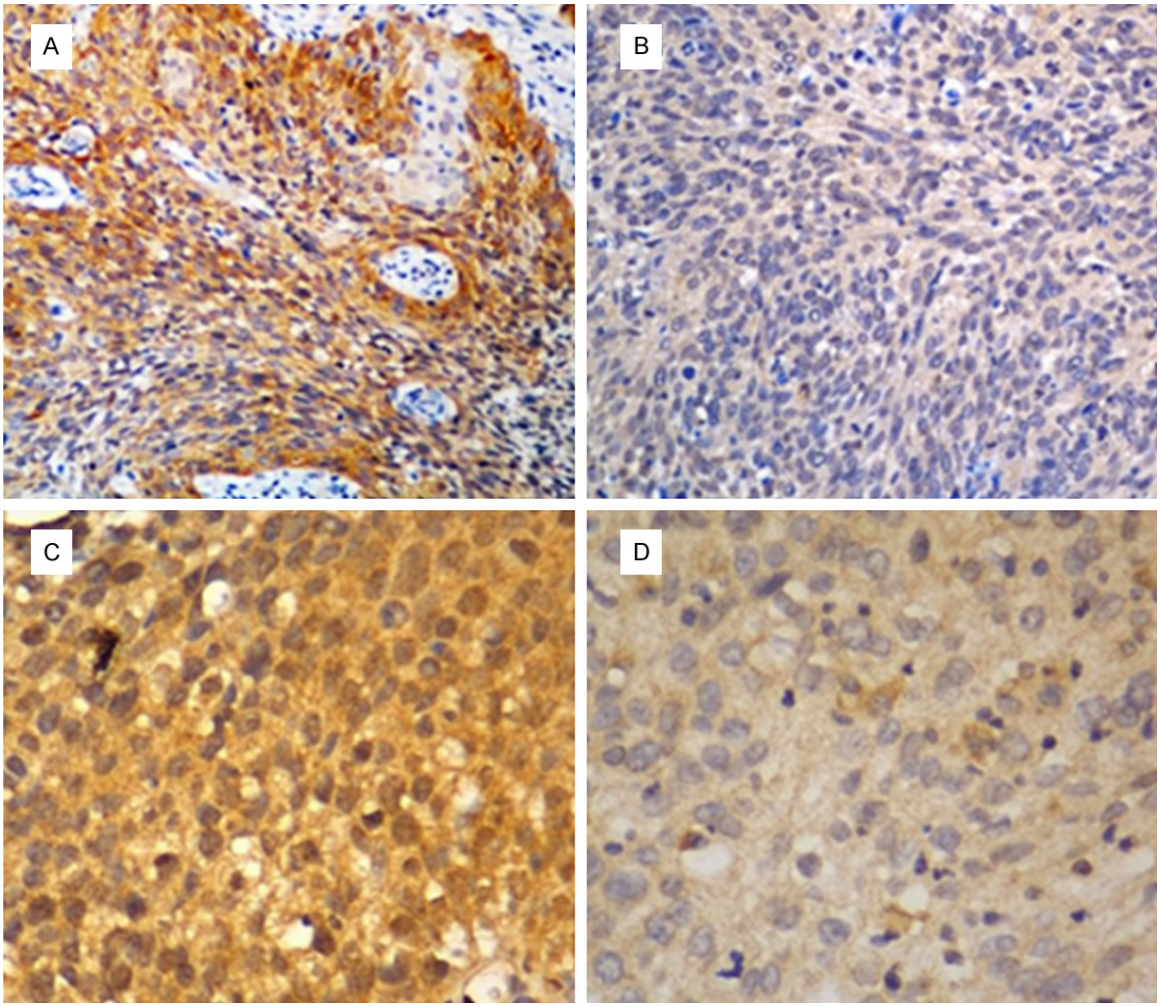

Figure 1. The result of *LGR5* immunohistochemical staining in CC tissues. A and C: CC tissues, high expression of *LGR5*; B and D: Normal controls, low expression of *LGR5* (A, B, 200×, C, D, 400× magnification).

Association of LGR5 expression with prognosis of CC patients

The survival curves showed that the patients with positive expression of *LGR5* lived shorter than those with negative expression of *LGR5* (**Figure 2**, log rank test, $P = 0.000$). Besides, multivariate analysis by the Cox regression method illustrated that *LGR5* expression was an independent factor for prognosis of CC patients (**Table 3**, $P = 0.000$, HR = 7.235, 95% CI = 3.462-15.119).

Discussion

Up to date, CC remains one of the most significant public health concerns all over the world [23]. Over the last few years, the incidence and recurrence rate of CC have increased, especially among the younger women [24]. Several biomarkers for CC have been identified in the past decades. For example Flotillin-2 was confirmed to predict poor clinical survival in CC patients according to the study of Liu et al. [25]. Yang et al. manifested that *microRNA-126* was related to prognosis of CC patients [6]. Besides, Guo et al. claimed that *MACC1* had also been found as a prognostic marker for CC patients [26]. In the present study, we attempted to find more biomarkers to further explain the pathogenesis and prognosis of CC.

In recent years, more and more studies have focused on exploring the relationship between *LGR5* expression and survival of patients due to the key roles of *LGR5* in tumorigenesis. Overexpression of *LGR5* has been reported in various cancers. High expression of *LGR5* was

Table 2. Relationship between *LGR5* expression and clinical features of CC patients

Clinical Features	Case NO.	*LGR5* expression		χ^2	*P* value
		Positive	Negative		
Age				0.343	0.558
≤50	62	50	12		
>50	59	45	14		
Differentiation				0.420	0.517
Poor	58	47	11		
Moderate + well	63	48	15		
Tumor histology				0.384	0.536
Squamous	68	52	16		
Adenocarcinoma	53	43	10		
FIGO stage				4.781	0.029
I+II	35	23	12		
III+IV	86	72	14		
Vascular invasion				5.315	0.021
Positive	83	70	13		
Negative	38	25	13		
Lymph node metastasis				5.774	0.016
Yes	67	58	9		
No	54	37	17		

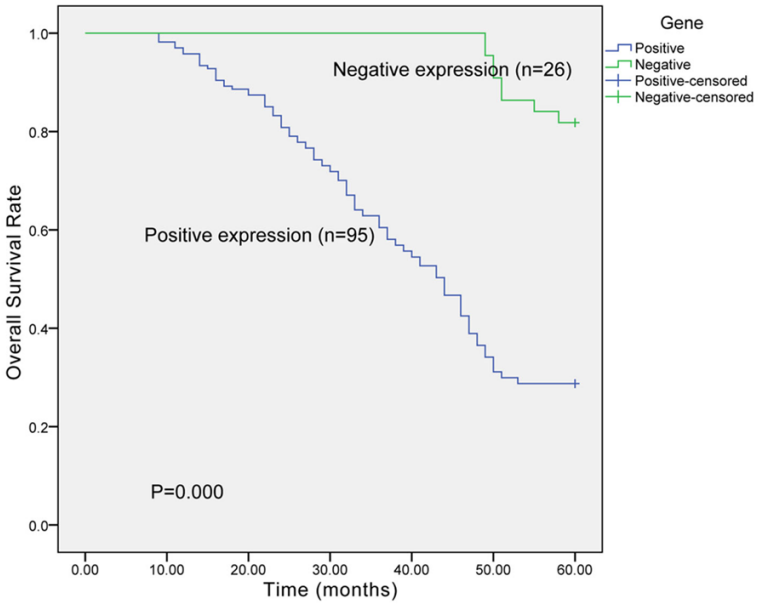

Figure 2. The overall survival of CC patients was described by Kaplan-Meier survival curves. The result showed that patients with positive expression of *LGR5* was more likely to die than those with negative expression of *LGR5* (*P* = 0.000). *P* value was analyzed by log-rank test.

found in colon cancer cells by Chen et al. [27]. Zheng et al. demonstrated *LGR5* expression was up-regulated in gastric cancer [28]. Bu et al. also illustrated that the expression of *LGR5* in gastric cancer tissues was higher than that in the adjacent normal tissues [29]. In this study, we firstly determined the expression of *LGR5* in CC tissues and the normal cervical tissues using IHC method. The result showed that the positive rate of *LGR5* expression was high in CC tissues compared to that in normal cervical tissues, which was in accordance with the above reports. Therefore, we speculated that *LGR5* was related to the carcinogenesis of CC.

Based on the conjecture, we further explored the correlation of *LGR5* expression and clinical features of CC patients. From the results we could conclude that *LGR5* expression in CC patients was significantly associated with FIGO stage, vascular invasion and lymph node metastasis, indicating that *LGR5* might relate to the process of CC. What's more, to better understand the prognostic value of *LGR5* in CC, survival analysis was conducted using Kaplan-Meier method, as well as Cox regression was employed for multivariate analysis. Our data exhibited that high positive rate of *LGR5* was tightly correlated with unfavorable overall survival and poor prognosis of CC patients. Therefore, *LGR5* might serve as a potential valuable prognostic biomarker for CC patients.

Although the expression of *LGR5* in CC has been identified and its relationship with clinical features of CC patients has been evaluated in this study, there are still several limitations in the present study. First, the number of tissue specimens was small.

Table 3. Multivariate analysis for the prognostic factors in the patients with CC

Clinical features	*P* value	HR	95% CI
Differentiation	0.336	0.838	0.584-1.202
Tumor histology	0.707	0.928	0.630-1.368
Vascular invasion	0.129	1.387	0.909-2.116
Lymph node metastasis	0.499	1.133	0.789-1.625
LGR5 expression	0.000	7.235	3.462-15.119

Second, only those CC patients that underwent surgery with no previous therapy were assessed. Third, the patients were selected from the same hospital, which might affect the prognostic evaluation because of the similar treatment methods. Thus, not only a larger number of CC tissues from different hospitals are needed but also individuals with neoadjuvant radiotherapy and chemotherapy are omitted. Therefore, it is essential to plan and conduct a further validated study with more accuracy and sensibility.

In conclusion, our present study demonstrated that the *LGR5* was positively expressed in CC tissues. The expression of *LGR5* was related to FIGO stage, lymph node metastasis and vascular invasion, but shared no relationship with age, differentiation and tumor histology. Besides, *LGR5* expression was associated with clinical survival of CC patients. *LGR5* can act as an independent prognostic biomarker for CC patients.

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Disclosure of conflict of interest

None.

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