High Expression of *LGR6* is A Poor Prognostic Factor In Esophageal Carcinoma

Takehito Ehara

First Department of Surgery

Takeshi Uehara (✉ tuehara@shinshu-u.ac.jp)

Department of Laboratory Medicine

Takahiro Yoshizawa

First Department of Surgery

Tomoyuki Nakajima

Department of Laboratory Medicine

Yasuhiro Kinugawa

Department of Laboratory Medicine

Shota Kobayashi

Department of Laboratory Medicine

Shiho Asaka

Department of Laboratory Medicine

Mai Iwaya

Department of Laboratory Medicine

Tadanobu Nagaya

Department of Gastroenterology

Yusuke Miyagawa

First Department of Surgery

Hiroyoshi Ota

Department of Laboratory Medicine

Yuji Soejima

First Department of Surgery

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Abstract

Background: Leucine-rich repeat-containing G-protein-coupled receptor 6 (LGR6) promotes carcinogenesis and progression in some cancer types. However, there are few reports of *LGR6* expression in esophageal squamous cell carcinoma (ESCC). *LGR6* expression and clinicopathological features in ESCC were investigated by RNAscope, a high-sensitivity RNA *in situ* hybridization method.

Methods: Appropriate tumors were selected from 41 cases of ESCC from which tissue microarrays were generated, and *LGR6* expression was identified by RNAscope.

Results: Thirty-seven patients had *LGR6* expression. High *LGR6* expression was observed in 17 cases and low *LGR6* expression in 24 cases. *LGR6* expression was significantly higher in high histological grade ESCC than in low histological grade ESCC ($P=0.0023$). ESCC patients who received neoadjuvant chemotherapy had significantly higher *LGR6* expression than those without neoadjuvant chemotherapy ($P=0.0109$). Furthermore, high *LGR6* expression showed a poorer prognosis than low *LGR6* expression (log-rank test, $P=0.0365$).

Conclusions: *LGR6* may be a prognostic factor and a potential new therapeutic target in ESCC.

Background

Leucine-rich repeat-containing G-protein-coupled receptor 6 (LGR6) belongs to the leucine-rich repeat-containing subgroup of the G-protein-coupled 7-transmembrane protein superfamily [1]. LGR6 has homology to LGR4 and LGR5 [1], and is a stem cell marker for hair follicles [2], lung [3], and mammary gland [4]. *LGR6* is involved in tissue development, regeneration, and injury repair.

LGR6 is also involved in cancer initiation and progression in lung [5], breast [4], and ovarian cancer [6].

Esophageal squamous cell carcinoma (ESCC) has a poor prognosis [7], and improvements are required in treatment outcome. To improve the survival rate of patients, the establishment of new biomarkers and targets is required. In this study, we analyzed the clinicopathological features of *LGR6* and examined the possibility of their application to the treatment of ESCC.

Methods

Patients and materials

We identified 56 ESCC cases who underwent surgical resection between 2010 and 2019 at Shinshu University Hospital, Matsumoto, Japan, and evaluated their clinicopathological features. Among these patients, cases with invasion deeper than the submucosa were selected. Stage IV cases were excluded as well as those that were negative for the positive control (housekeeping gene). A re-evaluation of the cases was undertaken by two pathologists (T.U. and M.I.), and 41 ESCC cases remained as candidates for

analysis. This study was approved by the ethics committee of Shinshu University, Japan (approval no. 4088).

Histopathology

Paraffin blocks fixed with 8% formaldehyde containing sufficient tumor for analysis were prepared for hematoxylin and eosin (HE) staining and tissue microarray analysis by extracting a core with a diameter of 3 mm from each case.

LGR6 RNA in situ hybridization

LGR6 mRNA detection was performed using the RNAscope kit (Advanced Cell Diagnostics, Hayward, CA, USA), as described previously [8]. Additionally, a four-step evaluation method was used, as we previously reported [8]. *LGR6* mRNA expression was categorized as low expression (grades 0, 1+, and 2+) and high expression (3+ and 4+). We analyzed the relationship between *LGR6* expression and the clinicopathological data and prognosis of patients with ESCC, particularly regarding overall survival (OS). We also evaluated *LGR6* expression in non-cancerous tissues of the background mucosa of ESCC.

Statistical analysis

Fisher's exact test was applied to assess statistical significance. A *P*-value of < 0.05 was considered statistically significant. The OS rates of ESCC patients were calculated using the Kaplan–Meier method, and differences were compared using the log-rank test. A *P*-value of < 0.05 was considered to be statistically significant. Statistical analysis was performed using JMP version 13 (SAS Institute Japan, Tokyo, Japan).

Results

LGR6 expression in normal esophageal mucosa and ESCC

LGR6 expression was identified in the basal cell layer of non-tumorous regions. In ESCC patients, 37 of 39 cases showed *LGR6* expression. Among them, 17 cases were identified as having high *LGR6* expression (Fig. 1A and 1B), while *LGR6* expression was completely absent in two cases (Fig. 1D and 1E). *LGR6* expression varied from diffuse to scattered patterns.

Relationship between LGR6 expression and clinicopathological characteristics

LGR6 expression and clinicopathological data are shown in Table 1. *LGR6* expression was significantly higher in high histological grade ESCC than in low histological grade ESCC ($P=0.0023$). ESCC patients with neoadjuvant chemotherapy had significantly higher *LGR6* expression than ESCC patients without neoadjuvant chemotherapy ($P=0.0109$). No significant differences were identified between high *LGR6* expression and low *LGR6* expression regarding age, sex, vascular invasion, or TNM stage.

Table 1
LGR6 expression and clinicopathological characteristics

<i>LGR6</i> expression				
Factors	n	High (n=17)	Low (n=24)	<i>P</i> -value
Age				0.0624
>71years	19	11	8	
≤70years	22	6	16	
Sex				0.6786
Male	34	15	19	
Female	7	2	5	
Neoadjuvant chemotherapy				0.0109
Yes	21	13	8	
No	20	4	16	
Vascular invasion				1.000
Present	21	9	12	
Absent	20	8	12	
TIL				1.000
High	20	8	12	
Low	21	9	12	
Histological grade				0.0023
High	31	17	14	
Low	10	0	10	
TNM stage				1.0000
I–II	20	8	12	
III	21	9	12	
TIL: tumor infiltrating lymphocyte; TNM: tumor, node, metastasis staging system				

Prognostic value of *LGR6* in ESCC

The prognostic value of *LGR6* expression in ESCC was analyzed by the Kaplan–Meier method and log-rank test (Fig. 2). The median OS for the study patients was 537 (range; 269.5–2299.2) days. High *LGR6*

expression [median OS: 322 (range; 175–555.5) days] was correlated with significantly worse OS than low *LGR6* expression [median OS: 992 (range; 435.5–1825.5) days] (log-rank test, $P=0.0365$).

Discussion

This is the first report of RNAscope analysis of *LGR6* expression in the esophagus. The correlation of poor prognosis with high *LGR6* expression is consistent with previous immunohistochemical reports [9]. *LGR6* expression is associated with ESCC differentiation in immunostaining studies [9]. However, maintenance of epithelial tissue is promoted by resident stem cells [10]. Given that *LGR6* is used as a stem cell marker in the epidermis [2], identification of the position of stem cells exhibiting *LGR6* expression in the esophageal epithelium, which is the same stratified squamous epithelium in the skin, may lead to applications such as regenerative medicine.

In a murine study, it was reported that *LGR6* has the characteristics of cancer stem cells (CSCs) in squamous cell carcinoma of the skin [11]. *LGR6* interacts with R-spondin 1-4 and is involved in the Wnt/ β -catenin signaling pathway via phosphorylation, exerting various biological actions [9] [12, 13] [14] [15]. The Wnt/ β -catenin signaling pathway plays an important role in the pathogenesis of various human diseases and tumors [13]. *LGR6* has been reported to promote the initiation and progression of cancers such as lung adenocarcinoma [5] and ovarian cancer including serous carcinoma [6]. *LGR6* may contribute to the initiation and progression of ESCC by activating the Wnt/ β -catenin signaling pathway, but further research is needed to confirm this hypothesis.

High *LGR6* expression in neoadjuvant chemotherapy cases indicated that *LGR6*-expressing cells may have CSC-like characteristics with chemoresistance. In fact, *LGR6* expression has also been reported to contribute to stem cell and chemoresistance in ovarian cancers, including serous carcinoma [6]. The function of chemoresistance is also considered to be influenced by the Wnt/ β -catenin pathway.

Nonetheless, it has been reported that *LGR6* acts as a tumor suppressor gene in colorectal cancer [16]. These findings may suggest that the functional control of *LGR6* is complex, and its role depends on the tumor type. The clinical significance and function of *LGR6* in ESCC has been rarely reported thus far, but on the basis of our current study, it seems to have a function as a CSC marker.

Therefore, the function of *LGR6* needs to be verified in cultured cells. It is particularly interesting as to whether *LGR6* is involved in tumor progression or tumor suppression. It is also necessary to search for differences between *LGR6* and *LGR5*. Further analyses in additional cases are desired.

Conclusions

We have demonstrated that *LGR6* may be an important prognostic factor in ESCC. *LGR6* may also be useful as a CSC marker and may be applicable in future cancer treatments.

List Of Abbreviations

ESCC, esophageal squamous cell carcinoma; LGR6, leucine-rich repeat-containing G-protein-coupled receptor 6; HE, hematoxylin and eosin; OS, overall survival.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shinshu University School of Medicine (approval no. 4088). The requirement of informed consent was waived by the ethics committee of Shinshu University School of Medicine, and an opt-out method was used because of the retrospective design of the study. The investigation was conducted in compliance with the Helsinki Declaration.

Consent for publication

Not applicable.

Availability of data and materials

All data generated and analyzed in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TE participated in the design of the study, performed the pathological analysis, and drafted the manuscript. TU, SK, SA, and MI helped with the pathological analysis. TU performed the statistical analysis. TN and YK conducted the immunohistochemistry. TN and US examined the clinical data of cases. HO and TU critically revised the draft for important intellectual content.

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Figures

Figure 1

Representative images of LGR6. Representative features in cases with high LGR6 expression (A and C) and low LGR6 expression (B and D). (A and B, HE; C and D, LGR6).

Figure 2

Prognostic value of LGR6 by Kaplan–Meier analysis. High LGR6 expression [median OS: 322 (range; 175–555.5) days] was associated with significantly worse OS than low LGR6 expression [median OS: 992 (range; 435.5–1825.5) days] (log-rank test, $P=0.0365$).