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Clinical Implications and Prognostic Value of Leucine-Rich G Protein-Coupled Receptor 5 Expression as A Cancer Stem Cell Marker in Malignancies: A Systematic Review and Meta-Analysis

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Abstract

Leucine-rich G protein-coupled receptor 5 (*LGR5*) is a marker of cancer stem cells (CSCs) in various cancers. Based on different studies, conflicting reports exist on correlation between *LGR5* expression and poor prognosis/ clinicopathological parameters in cancer patients. Therefore, our purpose in conducting this study was to investigate correlation between *LGR5* expression and outcomes of cancer patients under study through a systematic review and meta-analysis. Relevant articles were searched and collected using EMBASE, PubMed, Science Direct, and Scopus databases until December 21, 2022. This study was conducted to examine correlation between *LGR5* expression and different clinical outcomes, such as recurrence-free survival (RFS), disease-free survival (DFS), overall survival (OS), and clinicopathological characteristics of the included cancer patients. To achieve this, hazard ratios (HRs) with 95% confidence intervals (CIs) and odds ratios (ORs) with 95% CIs were used as statistical measures. A meta-analysis was conducted using STATA 12.0 software. Finally, 53 studies including 9523 patients met the inclusion criteria. Significantly, high-level expression of *LGR5* was related to poor prognosis in terms of OS, higher tumor stage, presence of distant metastasis, and presence of lymph node metastasis. It was discovered through subgroup analysis that several factors, including the study area, evaluation method, and type of cancer, can influence the correlation between *LGR5* expression and negative prognosis in cancer patients. According to the results of our study, *LGR5* overexpression was related to poor OS in cancer patients. In addition, clinicopathological data indicated an unfavorable prognosis in cancer patients with high *LGR5* expression. In conclusion, *LGR5* may serve as a potential prognostic marker for predicting survival in certain cancer types.

Keywords: Cancer Stem Cells, Clinicopathological Features, *LGR5*, Prognostic Marker

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Introduction

Cancer remains a leading cause of death globally and a significant public health issue (1). Despite extensive studies in recent decades and progress in new systemic treatments, cancer treatment faces many challenges, including resistance to treatment and the existence of cancer stem cells (CSCs) (2). These challenges contribute to tumor recurrence, tumor progression, and mortality. Therefore, to effectively treat cancer and address the issues of invasion and metastasis and thus improve patient outcomes, it is essential to identify prognostic markers and new treatment options (3).

The CSCs theory is supported by data from various tumors and malignancies, indicating that CSCs have the potential to re-establish an entire tumor (4). These cells play a critical role in tumor development, spread, progression, metastasis, recurrence, and resistance to treatment due to their ability to self-renew, be flexible, and differente into heterogeneous cell lineages (5). Various factors regulate CSCs (6). Signaling pathways similar to those found in normal stem cells are also present in CSCs (7). Consequently, targeting the signaling pathways and genes involved in regulating CSCs is highly effective in eliminating these cells and preventing treatment failure, adverse outcomes, and side effects (8). Moreover, investigation of CSC markers could potentially provide prognostic information and new therapeutic targets (9).

LGR5, a G-protein coupled receptor, is encoded by the

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gene located on chromosome 12. This receptor contains a leucine-rich repeat and is also referred to as GPR49, which belongs to the G-protein-coupled receptor family (10). *LGR5* is a Wnt target gene and it has been identified as a CSC marker in intestinal cells (11). Its ability to maintain CSCs and promote cancer progression has been observed in various types of cancer, including breast, colorectal, hepatocellular, gastric, and ovarian cancers (12-15). Recent studies showed that *LGR5* expression levels could predict prognosis, recurrence, and survival rates in some cancer types (16, 17). High expression of *LGR5* has been linked to shorter survival rates and advanced clinicopathological features in several studies (14, 18-20).

This suggests that *LGR5* may serve as a potential prognostic biomarker as well as a therapeutic target for tumors. Moreover, *LGR5* expression has been shown to cause resistance to 5-FU-based chemotherapy and tumor recurrence. Therefore, checking *LGR5* expression may help identify cancer patients with poor clinical outcomes (21). Therapies targeting pathways related to *LGR5* signaling are important strategies to improve the efficacy of cancer treatment (22).

This study aimed to comprehensively evaluate prognostic significance of *LGR5* expression in various cancers, given the conflicting research on its association with poor prognosis (18, 23, 24). To accomplish this, a meta-analysis of 53 studies with 9523 patients was conducted, in order to investigate potential role of *LGR5* as a clinical and prognostic marker and clarify its relationship with clinical pathological parameters in different cancers.

Materials and Methods

Study strategy

This study was conducted according to PRISMA guidelines (PRISMA Checklist) (25). Two researchers independently searched databases including EMBASE, Science Direct, PubMed, and Scopus up to December 21, 2022. In this study, we used the following medical terms to search: ("Cancer" OR "Carcinoma" "Neoplasm" OR "Tumor") AND ("*LGR5*" OR "G-protein coupled receptor 67" OR "G-protein coupled receptor 49" AND "prognosis"). We first gathered the publication's summary and title, then carefully reviewed all selected articles to ensure they contained the necessary information. Any discrepancy was discussed with another researcher to reach a consensus.

Selection criteria

Eligibility criteria for inclusion in our study were as follows: i. Articles published in English, ii. Diagnosis of any type of cancer or malignancy in patients was confirmed by pathological identification, iii. Investigation of *LGR5* expression in human tissue samples were evaluated by any technique, iv. Studies in which the correlation of *LGR5* expression with overall survival (OS), recurrence-free survival (RFS), disease-free survival (DFS), and/or clinicopathological data of cancers were investigated and patients were separated into two groups (positive and negative, or high and low) according to *LGR5* expression, and v. Articles that calculated ORs for pathologic clinical features hazard ratios (HRs) f or prognostic outcomes. The study excluded book chapters, letters, reviews, or conference abstracts, as they lacked sufficient data, as well as articles on animals, cell lines, or blood samples, as well as studies that did not have sufficient useful information.

Data extraction

Two researchers (S.GH. and A.N.) independently assessed each eligible article and extracted data from qualifying publications. The study collected data from each publication, settling disagreements through conversation and using the Newcastle-Ottawa quality evaluation scale to appraise available studies. The most commonly collected data items included author, cancer type, sample size, detection method, publication year, nation, recruitment time, outcomes, HR acquire method, and Newcastle-Ottawa scale (NOS) score (26). S.J. and R.N. verified the all data.

Quality assessment

Two authors (S.GH. and S.J.) independently assessed quality of the articles using the NOS rating system, which rates articles on a scale of zero to nine stars, as shown in Table S1 (See Supplementary Online Information at www.celljournal.org). Articles scoring six or higher were deemed of good quality, and any disagreements were resolved through discussion.

Statistical analysis

The effect sizes of HR from each original article were extracted directly in Meta-analysis. Cochran's test evaluated heterogeneity and expressed it with the I^2 index. Pooled results used a random effects model. Subgroup analysis was conducted based on cancer type, ethnicity, and diagnosis method. To assess robustness of the results, sensitivity analysis was conducted by excluding one study or group of studies at a time. All statistical analysis was conducted using STATA software (version 12.0; STATA Corp, USA). Publication bias was assessed using Egger's test and funnel plots.

Results

Literature search

As shown in Figure 1, initially 958 articles were recognized using the primary search based on PRISMA guidelines. After removing overlapping studies, 695 studies were selected, and then the titles and abstracts of the selected studies were independently assessed by two authors to remove unrelated items. The authors examined

the remaining 322 articles carefully. 269 studies were excluded from our review for the following reasons: letters (n=6), reviews (n=21), blood samples (n=4), non-cancer studies (n=103), abstract of the meeting and congress (n=19), animal studies (n=61), cell line studies (n=23), and studies that do not have enough information (n=32). As a result, 53 articles met our inclusion criteria. Of these 53 selected studies, 27 articles were demonstradted from China, 10 papers were reported from Japan, five and four experiments were respectively obtained from Korea and USA, two actiles from Germany, two papers from Taiwan, and the remaining experiments were reported from Sweden, Iran, and Egypt. The included studies contained twelve types of cancer: colorectal cancer (n=19), gastric cancer (n=8), breast cancer (n=6), head and neck cancer (n=6), liver cancer (n=4), lung cancer (n=3), ovarian cancer (=2), cholangiocarcinoma (n=1), intrahepatic cholangiocarcinoma (n=1), cervical carcinoma (n=1), small intestinal adenocarcinoma (n=1), and pancreatic ductal carcinoma (n=1). *LGR5* expression level was investigated by immunohistochemistry (IHC) in 40 studies, by RNA in situ hybridization (ISH) in seven studies, by quantitative polymerase chain reaction (qRT-PCR) in five studies, and by western blot in one study. Due to different definitions, cut-off values for *LGR5* expression varied among the studies. Out of the 53 studies that were collected, in 48 studies, *LGR5* expression was analyzed in relation to clinicopathological features. Additionally, in 27 of 53 studies, *LGR5* expression was evaluated in relation to survival rates, including OS (n=24), DFS (n=3), and RFS (n=4), in cancer patients.

Study quality

In the selected studies, the NOS score ranged from six to eight. Results of the quality assessment of each study and further details about the papers are summarized in Table 1.

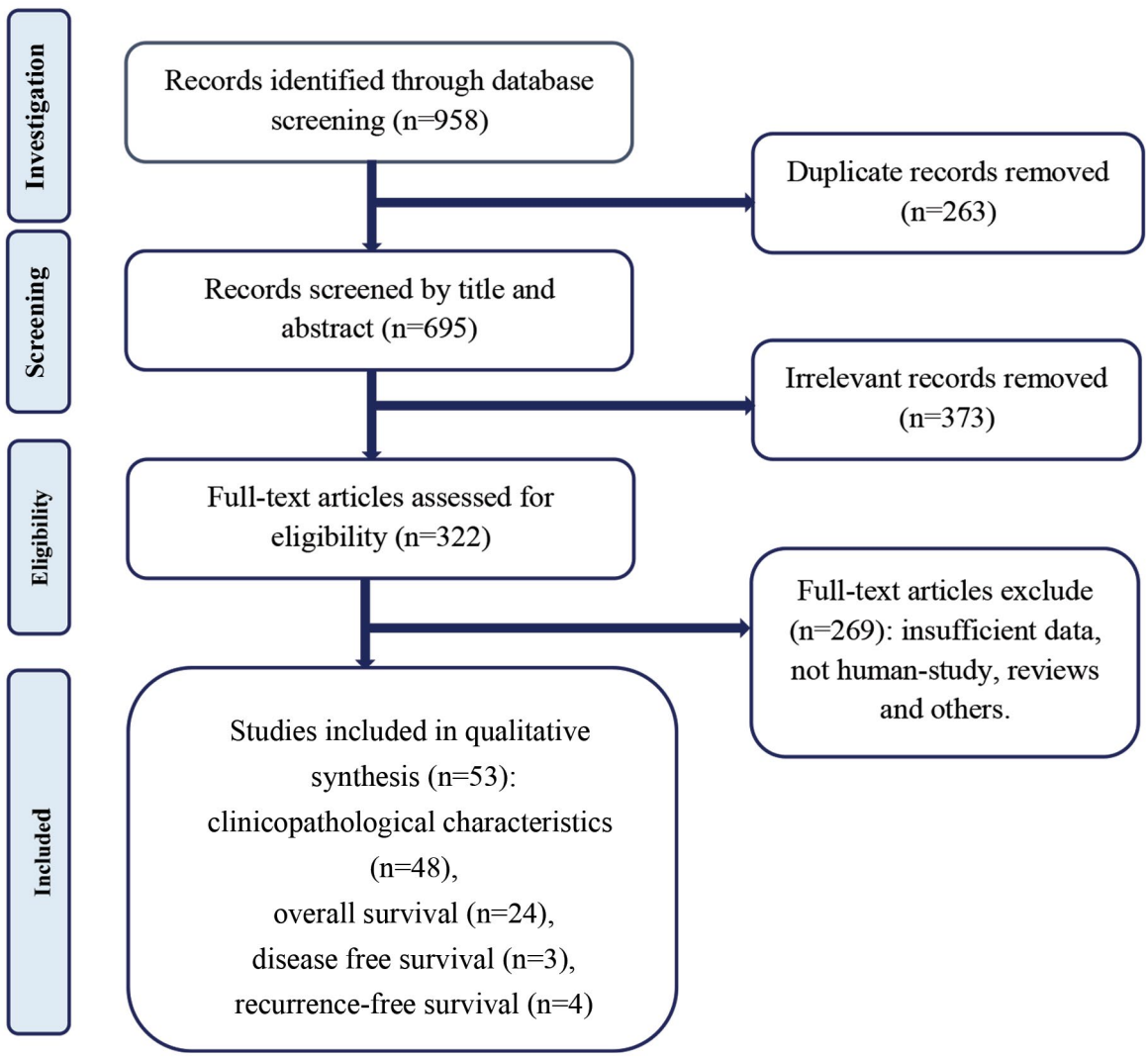

Fig.1: Flowchart for the study selection process.

Table 1: Characteristic of the included studies

Study	Year	Country	Cancer type	Sample size	Follow-up (month)	Detection method	Cut-off value	Evaluation of *LGR5* expression (H or L /+ or -)	NOS score	Expression associated with poor prognosis	Clinical feature	Outcome
Yoshizawa et al. (20)	2022	Japan	Cholangiocarcinoma	25	NA	IHC	NA	High/low	6	Low	Yes	NA
AbdelMageed et al. (27)	2021	Sweden	CRC	121	144	qRT-PCR	Median	Positive/Negative	6	High	No	DFS
Lee et al. (28)	2021	South Korea	TNBC	293	NA	IHC	NA	Positive/Negative	7	Positive	Yes	NA
Xu et al. (14)	2021	China	CRC	98	60	Western blot	Median	High/low	8	High	Yes	NA
Kawasaki et al. (29)	2021	Japan	ICC	59	NA	IHC	≥ 4	High/low	6	High	Yes	RFS
Abdelrahman et al. (30)	2021	Egypt	Colon cancer	60	40.8	IHC	NA	High/low	7	High	Yes	NA
Ehara et al. (16)	2021	Japan	GAS	41	NA	RNA-ISH	NA	High/low	6	High	Yes	OS
Hagerling et al. (31)	2020	USA	Breast cancer ER+	401	106	IHC	NA	High/low	6	High	Yes	OS
Ogasawara et al. (19)	2020	Japan	Breast carcinoma	43	NA	RNA-ISH	NA	High/low	6	High	Yes	OS
Kang et al. (32)	2020	China	CRC stage I, II	92	NA	IHC	≥ 4	High/low	7	High	Yes	OS, RFS
Zhang et al. (33)	2020	China	ESCC	45	48	IHC	Mean	Positive/Negative	7	NO relation	Yes	OS
Nagashima et al. (34)	2020	Japan	NSCLC	360	66	IHC	NA	High/low	7	High	Yes	OS, RFS
Ihemelandu et al. (35)	2019	USA	CRC	49	62.4	IHC	NA	High/low	8	Low	Yes	OS
Shen et al. (36)	2019	China	Breast carcinoma	112	3	IHC	Mean	Positive/Negative	7	High	Yes	NA
Shekarriz et al. (24)	2019	Iran	CRC	40	NA	IHC	Median	High/low	7	High	Yes	NA
Liu et al. (13)	2019	China	GC	100	56	IHC	NA	High/low	7	High	No	OS
Freiin Grote et al. (37)	2019	Germany	Gastric carcinoma	236	29.5	IHC	Median	High/Low	6	High	Yes	NA
Ko et al. (38)	2019	Taiwan	HCC	352	27	IHC	Median	High/Low	7	High	Yes	OS
Ma et al. (39)	2019	China	HCC	100	NA	IHC	Median	High/Low	7	High	Yes	OS
Rot et al. (40)	2019	Germany	OSCC	78	44.9	qRT-PCR	Median	High/Low	7	High	Yes	OS
Yu et al. (15)	2019	China	Epithelial ovarian cancer	210	NA	IHC	NA	Positive/Negative	7	High	Yes	NA
Kuraishi et al. (18)	2019	Japan	Pancreatic ductal	78	NA	RNA-ISH	NA	High/low	7	Low	Yes	NA
Hou et al. (41)	2018	Taiwan	Breast cancer	126	NA	IHC	Median	High/Low	6	High	Yes	NA
Jang et al. (42)	2018	Korea	CRC	788	NA	RNA-ISH	NA	Positive/Negative	7	High	Yes	OS
Kim et al. (43)	2018	Korea	CRC	337	NA	IHC	NA	High/Low	7	High	Yes	OS, DFS
Chen et al. (12)	2018	China	HCC	66	NA	IHC	NA	High/Low	7	High	Yes	OS
Harada et al. (44)	2017	Japan	Low rectal cancer	61	69.5	IHC	NA	Positive/Negative	6	High	Yes	NA
Lv et al. (45)	2017	China	ESCC	280	NA	IHC	NA	Positive/Negative	6	High	Yes	NA

Table 1: Continued

Study	Year	Country	Cancer type	Sample size	Follow-up (month)	Detection method	Cut-off value	Evaluation of *LGR5* expression (H or L /+ or -)	NOS score	Expression associated with poor prognosis	Clinical feature	Outcome
Liu et al. (46)	2017	China	HCC	139	31.15	IHC	NA	High/Low	8	High	Yes	NA
Wu et al. (47)	2017	China	OSCC	190	NA	IHC	NA	Positive/Negative	7	High	Yes	NA
Wu et al. (48)	2016	China	CRC	80	60	qRT-PCR	Median	High/Low	8	High	No	OS
Jang et al. (49)	2016	Korea	Gastric carcinomas	603	NA	RNA-ISH	NA	Positive/Negative	6	NA	Yes	NA
Sun et al. (50)	2015	China	Cervical carcinoma	94	46	qRT-PCR	NA	High/Low	7	High	Yes	OS, RFS
Yang et al. (51)	2015	China	Breast cancer	134	NA	IHC and TMA	NA	High/Low	7	High	Yes	NA
Gao et al. (52)	2015	China	Lung cancer	85	15.2	IHC	Median	Positive/Negative	7	High	Yes	OS
Sun et al. (53)	2015	China	Ovarian cancer	100	NA	IHC	NA	High/Low	7	High	Yes	NA
Wang et al. (54)	2015	China	Small intestinal adenocarcinomas	38	NA	IHC	NA	Positive/Negative	6	High	Yes	NA
Gao et al. (55)	2014	China	CRC stage IV	42	NA	IHC	Mean	Positive/Negative	6	High	Yes	NA
Liu et al. (56)	2014	China	CRC	366	NA	IHC	NA	Positive/Negative	7	High	Yes	NA
He et al. (57)	2014	China	CRC	53	48	IHC	Median	High/Low	7	High	Yes	OS
Chen et al. (58)	2014	China	SCCE	44	11.1	IHC	SI >4	High/Low	7	High	Yes	OS
Xi et al. (59)	2014	China	GC	318	NA	IHC	NA	High/Low	7	High	Yes	OS
Hsu et al. (60)	2013	China	CRC	218	28.3	IHC	NA	High/Low	7	High	Yes	DFS
Jang et al. (61)	2013	Korea	GC	159	NA	RNA-ISH	NA	Positive/Negative	6	High	Yes	NA
Zheng et al. (62)	2013	China	Gastric carcinoma	180	NA	IHC	NA	Positive/Negative	6	High	Yes	NA
Ryuge et al. (63)	2013	Japan	Lung adenocarcinoma	266	88	IHC	NA	Positive/Negative	7	High	Yes	OS
Bu et al. (64)	2013	China	GC stage I, II	257	NA	IHC	NA	Positive/Negative	7	High	Yes	NA
Wu et al. (65)	2012	China	Colorectal carcinoma	192	NA	IHC	NA	Positive/Negative	7	High	Yes	OS
Ziskin et al. (66)	2012	USA	Colorectal adenocarcinomas	891	NA	RNA-ISH	NA	High/Low	7	High	No	OS
Takahashi et al. (67)	2011	Japan	Colon and rectum	180	35.16	qRT-PCR	NA	High/Low	6	High	Yes	NA
Takeda et al. (68)	2011	Japan	CRC	60	NA	IHC	Median 5%	High/Low	6	High	Yes	NA
Becker et al. (69)	2010	USA	Barrett's esophagus and esophageal adenocarcinoma dote	81	32	IHC	SI >5	High/Low	6	High	No	OS
Fan et al. (70)	2010	China	CRC	102	NA	IHC	NA	Positive/Negative	7	Positive	Yes	NA

NOS; Newcastle-Ottawa scale, NA; Not available, CRC; Colorectal cancer, TNBC; Triple negative breast cancer, ICC; Intrahepatic cholangiocarcinoma, GAS; Gastric adenocarcinoma, ESCC; Esophageal squamous cell carcinoma, NSCLC; Non-small cell lung cancer, GC; Gastric cancer, HCC; Hepatocellular carcinoma, SCCE; Small cell carcinoma of the esophagus, IHC; Immunohistochemistry, qRT-PCR; Real-Time quantitative reverse transcription PCR, ISH; In situ Hybridization, TMA; tissue microarray, SI; Staining intensity, DFS; Disease-free survival, RFS; Relapse-free survival, and OS; Overall survival.

Relationship between the expression of the *LGR5* gene and verall survival

Among the 24 studies, including 4956 patients, correlation between *LGR5* expression and OS was significant. Therefore, meta-analysis of the total data of 24 studies using the random effect model revealed a positive and significant correlation between the expression of *LGR5* and OS [pooled HR (95% CI): 1.33 (1.02, 1.74, Fig.2A)]. There was a high and significant level of heterogeneity found among the studies (I^2=82.50%, P<0.001). Table 2 shows results of the subgroup meta-analysis according to cancer type, detection method, ethnicity, and model type. The association between OS and *LGR5* expression was significant for colorectal cancer groups [pooled HR (95% CI): 1.70 (1.06, 2.72); I^2=88.20%, P<0.001)], detection method of qRT-PCR [pooled HR (95% CI): 2.68 (1.27, 5.65); I^2=65.20%, P=0.056)], and multiple models [pooled HR (95% CI): 1.35 (1.01, 1.81); I^2=84.10%, P<0.001). The funnel plots showed symmetry (Fig.2B). Upon analysis, no evidence of publication bias was detected among the studies. (P for Egger's test=0.963). Meta-regression was used to determine how the effect sizes (HRs) were affected by the sample size and year of publication. The year of publication was a significant factor (beta=-0.11, SE=0.05, P=0.048) that may have contribution to heterogeneity between the studies. However, sensitivity analysis revealed that the exclusion of individual studies did not affect the overall effect size (HR).

Table 2: Subgroup analysis of the correlation between *LGR5* expression and OS

Cancer type	Number of studies	Pooled HR (95% CI)	P value	Heterogeneity	
				I^2	P value
Overall	24	1.33 (1.02, 1.74)	0.038	82.50%	<0.001
Cancer type					
Colorectal	8	1.70 (1.06, 2.72)	0.029	88.20%	<0.001
Gastric	3	1.17 (0.34, 4.04)	0.805	90.90%	<0.001
Breast	2	0.77 (0.53, 1.13)	0.189	0.00%	0.742
Head and neck	4	1.43 (0.69, 2.99)	0.337	56.50%	0.075
Lung	3	1.34 (0.71, 2.53)	0.368	63.60%	0.064
Liver	3	0.78 (0.19, 3.10)	0.719	93.00%	<0.001
Other	1	2.13 (0.81, 5.56)			
Detection method					
IHC	17	1.23 (0.85, 1.77)	0.273	82.10%	<0.001
qRT-PCR	3	2.68 (1.27, 5.65)	0.01	65.20%	0.056
RNA-ISH	4	1.03 (0.69, 1.54)	0.883	75.40%	0.007
Ethnicity					
Asian	19	1.29 (0.90, 1.83)	0.167	84.40%	<0.001
Non-Asian	5	1.35 (0.88, 2.08)	0.173	70.00%	0.01
Model type					
Multiple	21	1.35 (1.01, 1.81)	0.045	84.10%	<0.001
Univariate	3	1.15 (0.60, 2.20)	0.679	57.60%	0.094

HR; Hazard ratio, OS; Overall survival, CI; Confidence interval, IHC; Immunohistochemistry, qRT-PCR; Real-Time quantitative reverse transcription PCR, and ISH; In situ hybridization.

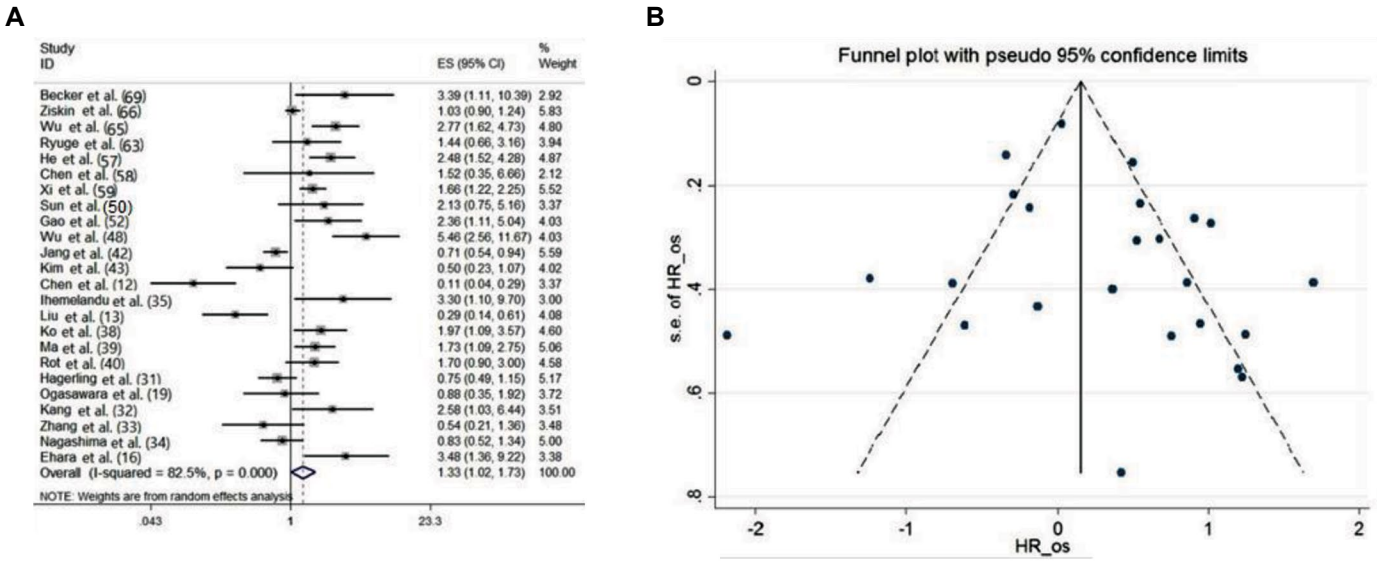

Fig.2: Relationship between the expression of the *LGR5* gene and OS. **A.** Forest plot and **B.** Funnel plots.

Relationship between the expression of *LGR5* gene and disease-free survival

Three studies, involving several colorectal cancer models, which included a total of 676 patients, reported a correlation between *LGR5* expression and DFS. Using the random effects model, meta-analysis of data from three studies did not reveal any significant correlation between *LGR5* expression level and DFS [pooled HR; (95% CI): 1.45 (0.54, 3.94); (I^2=88.5%, P<0.001, Fig.3A)]. None of the studies exhibited publication bias (P for Egger's test=0.105). Meta-regression analysis indicated that sample size and publication year were not the primary sources of heterogeneity (P>0.05). The sensitivity analysis revealed that upon excluding non-Asian studies that used qRT-PCR, the pooled hazard ratio for Asian studies detected by IHC was not statistically significant (pooled HR=1.12 (95% CI: 0.31, 4.01); I^2=89.9%, P=0.002).

Relationship between the expression of the *LGR5* gene and relapse-free survival

Four studies, all from Asia including 573 patients, reported a correlation between *LGR5* expression and RFS. Therefore, a meta-analysis of the total data of four studies using the random effect model did not find any significant correlation between high or positive *LGR5* expression and RFS [pooled HR; (95% CI): 2.20 (0.91, 5.33); (I^2=70.6%, P<0.017, Fig.3B)]. None of the studies showed any evidence of publication bias (P for Egger's test=0.963). Meta-regression analysis found that neither the publication year nor sample size were a significant source of heterogeneity (P>0.05). Sensitivity analysis indicated that excluding the study by Nagashima et al. (34) did not alter the results with the univariate model, while the pooled HR for multiple models was significant [pooled HR=3.62 (95% CI: 1.86, 7.06); I^2=1.1%, P=0.364]. However, by excluding the study with the detection method of qRT-PCR (Sun et al. 50), the pooled HR on studies with the detection method of IHC was not significant [pooled HR=1.79 (95% CI: 0.60, 5.36); I^2=68.1%, P=0.043].

Correlation between *LGR5* expression and clinicopathological features

A total of 48 studies, including 8250 patients, investigated relationship between *LGR5* expression level and clinical pathological features. Table 3 shows correlation between *LGR5* expression and clinicopathological characteristics in cancer patients. Results of the studies revealed that there was no correlation between the expression level of *LGR5* with gender [pooled OR (95% CI): 1.10 (0.97, 1.25), Fig.S1, See Supplementary Online Information at www.celljournal.org], age [pooled OR (95% CI): 1.26 (0.98, 1.62), Fig.S2A, See Supplementary Online Information at www.celljournal.org], tumor grade [pooled OR (95% CI): 1.42 (0.40, 5.03); Fig.S2B, See Supplementary Online Information at www.celljournal.org] and tumor size [pooled OR (95% CI): 1.05 (0.74, 1.48), Fig.S3, See Supplementary Online Information at www.celljournal.org]. According to results of the study, high expression of *LGR5* is significantly correlated with the advanced stage of tumor [pooled OR (95% CI): 1.91 (1.31, 2.79), Fig. S4A, See Supplementary Online Information at www.celljournal.org], distant metastasis [pooled OR (95% CI): 1.80 (1.15, 2.83), Fig.S4B, See Supplementary Online Information at www.celljournal.org] and presence of lymph node metastasis [pooled OR (95% CI): 1.37 (1.02, 1.85), Fig.S5, See Supplementary Online Information at www.celljournal.org]. The study did not identify any publication bias (P for Egger's test >0.05).

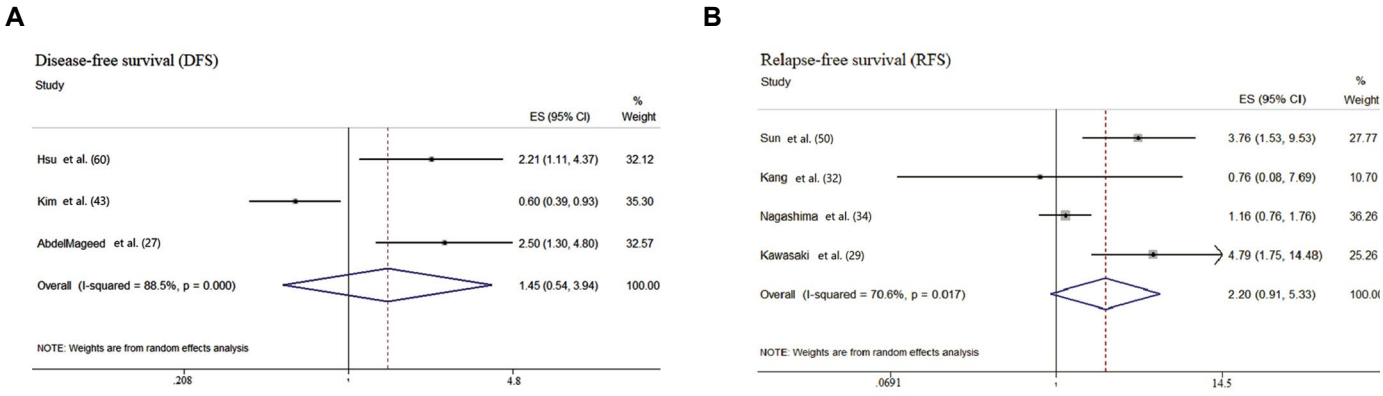

Fig.3: Forrest plot of HR. **A.** Forest plot for relationship between the expression of *LGR5* gene and DFS. **B.** Forest plot for relationship between the expression of *LGR5* gene and RFS. HR; Hazard ratio, ES; Effect Size, and CI; Confidence interval.

Table 3: Meta-analysis of *LGR5* expression and clinicopathological characteristics

Characteristics	Number of studies	Number of patients	Pooled OR (95%CI)	P value	Heterogeneity		Publication bias (Egger's test)
					I^2 (%)	P value	P value
Gender (male vs. female)	37	6340	1.10 (0.97, 1.25)	0.134	5.40	0.377	0.768
Age (old vs. young)	11	2472	1.26 (0.98, 1.62)	0.076	46.4	0.045	0.551
Tumor grade (high vs. low)	7	586	1.42 (0.40, 5.03)	0.584	84.70	<0.001	0.506
Tumor size (large vs. small)	21	2888	1.05 (0.74, 1.48)	0.786	67.3	<0.001	0.393
Tumor stage (high vs. low)	32	5929	1.91 (1.31, 2.79)	0.001	87.3	<0.001	0.936
Distant metastasis (present vs. absent)	15	2241	1.80 (1.15, 2.83)	0.011	57.4	0.003	0.089
Lymph node metastasis (present vs. absent)	32	5951	1.37 (1.02, 1.85)	0.036	78.6	<0.001	0.671

OR; Odds ratio and CI; Confidence interval.

Discussion

As an enhancer of the Wnt signaling pathway, *LGR5* has a crucial functional role in both normal development and cancer (71). *LGR5* has been identified as a marker of CSCs in colorectal, ovarian, esophageal, hepatocellular, and gastric cancers (72, 73). There has been extensive research on the role of *LGR5* in development of tumors, and its correlation with patient survival has been investigated in numerous studies. *LGR5* is overexpressed in gastric cancer, brain cancer, ovarian cancer, and esophageal cancer (74). Several studies have explored the connection between *LGR5* expression and cancer patient outcomes. Although high *LGR5* expression is generally associated with poor prognosis, conflicting findings in some cancers suggested the need for further research. These findings suggested that increased expression of *LGR5* is a negative prognostic factor in multiple types of human cancers (30, 33). On the contrary, some other reports suggested no significant correlation between *LGR5* expression and tumor outcomes (23, 75).

LGR5 is now regarded as a recognized marker for breast, and pancreatic CSCs (51, 76). Based on the gradually accumulating scientific evidences on various organs, limited population of stem cells started to demonstrate overexpression of *LGR5*, which may gain other prerequisites and complete their developmental steps to become CSCs (51). It has been shown that in squamous cell carcinoma of the skin, *LGR5* interacted with R-spondin in canonical Wnt receptors and modulated Wnt/β-catenin. Additionally, *LGR5*-Wnt receptor complex internalization caused a delay in endosomal degradation processes (76). In breast cancer, activation of Wnt/β-catenin signaling pathways by *LGR5* promoted growth and invasion in stem-like cells and it

was necessary for maintaining CSCs (77). In contrast, *LGR5* expression did not substantially correlate with tumor characteristics in patients with triple-negative breast cancer (78). Moreover, the results of Kim et al. (79) showed that expression of *LGR5* in ovarian cancer patients during disease progression to invasive cancer was significantly associated with improved outcomes.

Previous studies on gastric cancers have yielded inconsistent results. *LGR5*-positive patients showed considerably shorter survival periods than *LGR5*-negative patients (59). *LGR5* mRNA expression is not regarded as a prognostic predictor in GC, despite the increased *LGR5* expression in tumors following neoadjuvant chemotherapy. These findings demonstrated that *LGR5* expression was not a reliable prognostic indicator for GC. However, it was a negative prognostic marker when restricted to GC with nuclear catenin expression (80).

For many tumors, chemotherapy is the initial line of treatment that kills cancer cells. Recent research demonstrated a correlation between chemotherapy resistance and *LGR5* expression. *LGR5* has been linked to outcome and therapy resistance in GC. It is well documented that experimental overexpression of *LGR5* in spheroids developed from the GC cell line, caused proliferation, enhanced migration as well as development of resistance towards chemotherapy drugs (81). High *LGR5* expression in GC patients was a marker of bad prognosis and demonstration of resistance to the platinum drugs and 5-FU (16). Clark-Corrigall et al. (82) reported correlation between *LGR5* expression and neuroblastoma (NB) resistance to chemotherapy. Ma et al. (39) reported that *LGR5* acted as a tumor initiator to increase cell migration and induced epithelial mesenchymal transitions (EMT) in HCC cells, thereby increasing resistance to doxorubicin.These results showed that *LGR5* was involved in tumorigenesis.

LGR5 expression was increased with glioma progression and it was connected to negative outcomes (83). Canonical Wnt target genes were overexpressed in the NB tumor taken from patients with advanced disease. High level of Wnt target genes in these tumors accompanied by *LGR5* overexpression was interpreted as Wnt dysregulation in NB (84). Vicari et al. (85) studied *LGR5* activity in NB and concluded that *LGR5* acted as a main hub for Wnt and MEK/ERK signalling regulation in NB. In papillary thyroid carcinoma, there has been a correlation between tumor aggressiveness indicators and *LGR5* overexpression (86).

Substantial evidence highlighted important role of *LGR5* in the pathogenesis of CRC (27, 30). *LGR5* expression is closely associated with tumorigenesis, chemotherapy resistance, and CRC recurrence. However, conflicting results were reported by Jang et al. (42), who found that *LGR5* overexpression reduced proliferation, migration, and colony formation in the late stages of CRC progression. A recent study demonstrated that loss of *LGR5* expression was associated with enhanced resistance to therapy (87). Therefore, conclusions regarding *LGR5* expression and its clinical outcomes are debated and controversial. To address this issue, we performed a comprehensive meta-analysis of eligible studies to assess prognostic value and clinicopathological characteristics of *LGR5* expression in the various cancers.

Our findings revealed a positive association between *LGR5* expression and OS, with *LGR5* overexpression commonly indicating poor prognosis in cancers. The sub-group analysis result based on cancer type found a significant correlation between expression of *LGR5* and OS in the groups of patients with colorectal cancer, including 2482 patients, which is consistent with the previous study (88). However, this relationship was not observed in another cancer type that was included in the study. This result may show different clinical characteristics and biological behaviors of the *LGR5* in different types of cancer. Relationships between high *LGR5* expression and poor OS were significant in the studies that employed the qRT-PCR method but not IHC, according to the results of a subgroup analysis by this method. These results showed that use of different methods to measure expression of *LGR5* was effective in the final result (23). However, our result found no significant relationship between *LGR5* expression with DFS and RFS. It could be due to the small number of samples. Similar to this result, Ihemelandu et al. (35) observed a negative association between *LGR5* expression and patient survival outcomes, and the small sample size was cited as a limitation of the study. Expression of *LGR5* was influenced by various factors in different cancers. *LGR5* was also present in normal stem cells which governs tissue homeostasis. Potentially these *LGR5*-positive cells are amenable to oncogenic transformation (89). The positive results of *LGR5* expression in cancer are related to its basal level in different organs. *LGR5* is very low in breast and stomach tissue and it may not be expressed at all. But, healthy tissue of the colorectum is more expressive. Probably, lack of *LGR5* in homeostatic state of the cells can lead to its low positivity in some cancers (28). *LGR5* expression may vary in different tumor stages. In Kim et al.'s (90) study, GC was divided into three categories based on the expression pattern of several stem cell markers: basic, focal and scattered patterns. The findings demonstrated that *LGR5* expression was elevated during the baseline state and sustained during the initial phases of GCs. Furthermore, presence of different molecular subgroups in the tumor can affect expression of *LGR5*. In the study of Hagerling et al. (31), it was shown that prognostic value of *LGR5* in patients with ER positive BC patients was different compared to ER negative BC patients, and *LGR5* in BCER negative type has the prognostic value.

Correlation between increased expression of *LGR5* and clinical implications was investigated in several different cancers. In epithelial ovarian cancer, there was a correlation of the positive rate of tumor stage and lymph node metastasis with an elevated expression of *LGR5* (15). A study by Rot et al. (40) on oral squamous cell carcinoma

showed that *LGR5* expression was associated with lymph node metastasis. Abdelrahman et al. (30) showed that there was a relationship of the increased expression of *LGR5* and lymph node metastasis with advanced stage of the tumor in colon cancer. In another study, Liu et al. (13) showed no correlation between *LGR5* expression and age, gender, tumor stage, and lymph node metastasis in gastric cancer. Our findings indicated that *LGR5* expression was linked to pathological variables including tumor stage, distant metastasis and lymph node metastasis, which was consistent with the previous studies and emphasized its potential as a prognostic factor. Our study found no significant association between *LGR5* expression and patient age, sex, tumor size, or grade, contradicting the previous research (37).

This meta-analysis was the first to assess predictive importance of *LGR5* expression in different cancer types. The present study found no evidence of publication bias. However, the study does have limitations. Firstly, it should be noted that all studies included were in English, potentially introducing selection bias. Secondly, the included studies were those which used different cut-off values and detection methods for measuring *LGR5* expression. Thirdly, the limited number of studies evaluating RFS and DFS may have led to further bias. Fourthly, our finding might be most relevant to Asian patients, as the majority of the included studies were conducted in Asia. Fifthly, to ensure the credibility of our findings, we only considered publications providing HR with 95% CI directly and did not estimate HR through Kaplan-Meier curves.

Conclusion

Our study demonstrated a strong correlation between high *LGR5* expression and poor OS, distant metastasis, tumor stage, and lymph node metastasis in cancer patients. These findings suggested that *LGR5*, a marker for CSCs, could serve as a valuable prognostic indicator and a promising therapeutic target for cancer treatment. However, to verify these findings, well-designed studies with larger populations and more diverse ethnic groups are necessary.

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Authors' Contributions

R.S., S.J.; Conceptualization. S.Gh., S.J.; Methodology and Data curation. S.Gh., A.N.; Investigation and Visualization. S.J., R.N., N.V.; Validation of data. M.Kh., S.Gh., S.J.; Formal analysis. S.Gh., N.V., S.J.; Writing the original draft. R.S., S.Gh.; Review and editing. R.S.; Supervision. All authors read and approved the final manuscript for submission.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021; 71(3): 209-249.
2. Li Y, Wang Z, Ajani JA, Song S. Drug resistance and cancer stem cells. Cell Commun Signal. 2021; 19(1): 19.
3. Kelley RK, Wang G, Venook AP. Biomarker use in colorectal cancer therapy. J Natl Compr Canc Netw. 2011; 9(11): 1293-1302.
4. Hung KF, Yang T, Kao SY. Cancer stem cell theory: are we moving past the mist? J Chin Med Assoc. 2019; 82(11): 814-818.
5. Marzagalli M, Fontana F, Raimondi M, Limonta P. Cancer stem cells-key players in tumor relapse. Cancers (Basel). 2021; 13(3): 376.
6. Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CL, Rich JN. Cancer stem cells in glioblastoma. Genes Dev. 2015; 29(12): 1203-1217.
7. Carnero A, Garcia-Mayea Y, Mir C, Lorente J, Rubio IT, LLeonart ME. The cancer stem-cell signaling network and resistance to therapy. Cancer Treat Rev. 2016; 49: 25-36.
8. Xu H, Li W, Song X, Zhang H, Wang H, Wang J, et al. Expression and prognostic value of Lgr5 in patients with recurrent nasopharyngeal carcinoma. Int J Gen Med. 2023; 16: 2023-2034.
9. Walcher L, Kistenmacher AK, Suo H, Kitte R, Dluczek S, Strauß A, et al. Cancer stem cells-origins and biomarkers: perspectives for targeted personalized therapies. Front Immunol. 2020; 11: 1280.
10. Haegebarth A, Clevers H. Wnt signaling, lgr5, and stem cells in the intestine and skin. Am J Pathol. 2009; 174(3): 715-721.
11. de Lau W, Peng WC, Gros P, Clevers H. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. Genes Dev. 2014; 28(4): 305-316.
12. Chen W, Fu Q, Fang F, Fang J, Zhang Q, Hong Y. Overexpression of leucine-rich repeat-containing G protein-coupled receptor 5 predicts poor prognosis in hepatocellular carcinoma. Saudi J Biol Sci. 2018; 25(5): 904-908.
13. Liu XS, Lin XK, Mei Y, Ahmad S, Yan CX, Jin HL, et al. Regulatory T cells promote overexpression of Lgr5 on gastric cancer cells via TGF-beta1 and confer poor prognosis in gastric cancer. Front Immunol. 2019; 10: 1741.
14. Xu N, Gao K, Luo H, Wu Y, Shen B, Liu K. Correlation of Lgr5 expression with clinicopathological features of colorectal cancer and its diagnostic and prognostic values. J BUON. 2021; 26(1): 87-92.
15. Yu L, Mao X, Wu S, Zhou L, Song W, Gong X, et al. The correlation of the expressions of WWOX, LGR5 and vasohibin-1 in epithelial ovarian cancer and their clinical significance. Int J Clin Exp Pathol. 2019; 12(1): 327-336.
16. Ehara T, Uehara T, Nakajima T, Kinugawa Y, Kobayashi S, Iwaya M, et al. LGR5 expression is associated with prognosis in poorly differentiated gastric adenocarcinoma. BMC Cancer. 2021; 21(1): 228.
17. Sato K, Uehara T, Nakajima T, Iwaya M, Miyagawa Y, Watanabe T, et al. Inverse correlation between PD-L1 expression and LGR5 expression in tumor budding of stage II/III colorectal cancer. Ann Diagn Pathol. 2021; 52: 151739.
18. Kuraishi Y, Uehara T, Kobayashi Y, Nakajima T, Watanabe T, Shimizu A, et al. Correlation of clinicopathological features and leucine-rich repeat-containing G-protein-coupled receptor 5 expression in pancreatic ductal adenocarcinoma. Pathol Res Pract. 2019; 215(11): 152623.
19. Ogasawara S, Uehara T, Nakajima T, Iwaya M, Maeno K, Tsuchiya S, et al. Correlation of clinicopathological features and LGR5 expression in triple-negative breast cancer. Ann Diagn Pathol. 2020; 46: 151491.
20. Yoshizawa T, Uehara T, Iwaya M, Asaka S, Kobayashi S, Nakajima T, et al. Correlation of LGR5 expression and clinicopathological features in intrahepatic cholangiocarcinoma. Pathol Res Pract. 2022; 232: 153832.
21. Nagata H, Ishihara S, Kishikawa J, Sonoda H, Murono K, Emoto S, et al. CD133 expression predicts post-operative recurrence in patients with colon cancer with peritoneal metastasis. Int J Oncol. 2018; 52(3): 721-732.
22. Cao W, Li M, Liu J, Zhang S, Noordam L, Verstegen MMA, et al. LGR5 marks targetable tumor-initiating cells in mouse liver cancer. Nat Commun. 2020; 11(1): 1961.
23. Sato K, Uehara T, Iwaya M, Nakajima T, Miyagawa Y, Ota H, et al.

Correlation of clinicopathological features and LGR5 expression in colon adenocarcinoma. Ann Diagn Pathol. 2020; 48: 151587.
24. Shekarriz R, Montazer F, Alizadeh-Navaei R. Overexpression of cancer stem cell marker Lgr5 in colorectal cancer patients and association with clinicopathological findings. Caspian J Intern Med. 2019; 10(4): 412-416.
25. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009; 339: b2700.
26. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010; 25(9): 603-605.
27. AbdelMageed M, Ismail HTH, Olsson L, Lindmark G, Hammarström ML, Hammarström S, et al. Clinical significance of stem cell biomarkers EpCAM, LGR5 and LGR4 mRNA levels in lymph nodes of colon cancer patients. Int J Mol Sci. 2021; 23(1): 403.
28. Lee HJ, Myung JK, Kim HS, Lee DH, Go HS, Choi JH, et al. Expression of LGR5 in mammary myoepithelial cells and in triple-negative breast cancers. Sci Rep. 2021; 11(1): 17750.
29. Kawasaki K, Kuboki S, Furukawa K, Takayashiki T, Takano S,Ohtsuka M. LGR5 induces β-catenin activation and augments tumour progression by activating STAT3 in human intrahepatic cholangiocarcinoma. Liver International. 2021; 41(4): 865-881.
30. Abdelrahman AE, El-Azony A, Elsebai E, Ibrahim HM. Prognostic impact of LGR5, Prox1, and Notch1 biomarkers in stage II to III colon cancer. Appl Immunohistochem Mol Morphol. 2022; 30(2): 126-135.
31. Hagerling C, Owyong M, Sitarama V, Wang C-Y, Lin C, Van Den Bijgaart R J, et al. LGR5 in breast cancer and ductal carcinoma in situ: a diagnostic and prognostic biomarker and a therapeutic target. BMC Cancer. 2020; 20(1): 542.
32. Kang XL, He LR, Chen YL, Wang SB. Role of doublecortin-like kinase 1 and leucine-rich repeat-containing G-protein-coupled receptor 5 in patients with stage II/III colorectal cancer: cancer progression and prognosis. World J Gastroenterol. 2020; 26(43): 6853-6866.
33. Zhang X, Yuan A, Zhao X, Li Z, Cui G. Tumoral expression of CD166 in human esophageal squamous cell carcinoma: implications for cancer progression and prognosis. Cancer Biother Radiopharm. 2020; 35(3): 214-222.
34. Nagashima T, Oshima T, Hiroshima Y, Yokose T, Woo T, Rino Y, et al. Clinical significance of tumour CD44v and MIST1 expression in patients with non-small-cell lung cancer. Anticancer Res. 2020; 40(11): 6407-6416.
35. Ihemelandu C, Naeem A, Parasido E, Berry D, Chaldekas K, Harris BT, et al. Clinicopathologic and prognostic significance of LGR5, a cancer stem cell marker in patients with colorectal cancer. Colorectal Cancer. 2019; 8(4): CRC11.
36. Shen R, Wu T, Huang P, Shao Q, Chen M. The clinicopathological significance of ubiquitin-conjugating enzyme E2C, leucine-rich repeated-containing G protein-coupled receptor, WW domain-containing oxidoreductase, and vasculogenic mimicry in invasive breast carcinoma. Medicine (Baltimore). 2019; 98(16): e15232.
37. Freiin Grote A, Halske C, Behrens HM, Krüger S, Wilhelm F, Egberts JH, et al. Expression of LGR5, FZD7, TROY, and MIST1 in perioperatively treated gastric carcinomas and correlation with therapy response. Dis Markers. 2019; 2019: 8154926.
38. Ko CJ, Li CJ, Wu MY, Chu PY. Overexpression of LGR-5 as a predictor of poor outcome in patients with hepatocellular carcinoma. Int J Environ Res Public Health. 2019; 16(10): 1836.
39. Ma Z, Guo D, Wang Q, Liu P, Xiao Y, Wu P, et al. Lgr5-mediated p53 repression through PDCD5 leads to doxorubicin resistance in hepatocellular carcinoma. Theranostics. 2019; 9(10): 2967-2983.
40. Rot S, Kaune T, Taubert H, Greither T, Kotrba J, Güttler A, et al. Prognostic impact of mRNA levels of LGR5 transcript variants in OSCC patients. BMC Cancer. 2019; 19(1): 155.
41. Hou MF, Chen PM, Chu PY. LGR5 overexpression confers poor relapse-free survival in breast cancer patients. BMC Cancer. 2018; 18(1): 219.
42. Jang BG, Kim HS, Chang WY, Bae JM, Kim WH, Kang GH. Expression profile of LGR5 and its prognostic significance in colorectal cancer progression. Am J Pathol. 2018; 188(10): 2236-2250.
43. Kim YJ, Kang DH, Song GJ, Ahn TS, Son MW, Lee MS, et al. Clinical relevance of Lgr5 expression in colorectal cancer patients. Korean J Clin Oncol. 2018; 14(2): 76-82.
44. Harada Y, Kazama S, Morikawa T, Murono K, Yasuda K, Otani K, et al. Leucine-rich repeat-containing G protein-coupled receptor 5 and CD133 expression is associated with tumor progression and resistance to preoperative chemoradiotherapy in low rectal cancer. Oncol Lett. 2017; 14(6): 7791-7798.
45. Lv Z, Yu JJ, Zhang WJ, Xiong L, Wang F, Li LF, et al. Expression and functional regulation of stemness gene Lgr5 in esophageal squamous cell carcinoma. Oncotarget. 2017; 8(16): 26492-26504.
46. Liu J, Yu GZ, Cheng XK, Li XD, Zeng XT, Ren XQ. LGR5 promotes hepatocellular carcinoma metastasis through inducting epithelial-mesenchymal transition. Oncotarget. 2017; 8(31): 50896-50903.
47. Wu Z, Song W, Cheng Z, Yang D, Yu L. Expression of LGR5 in oral squamous cell carcinoma and its correlation to vasculogenic mimicry. Int J Clin Exp Pathol. 2017; 10(11): 11267-11275.
48. Wu W, Cao J, Ji Z, Wang J, Jiang T, Ding H. Co-expression of Lgr5 and CXCR4 characterizes cancer stem-like cells of colorectal cancer. Oncotarget. 2016; 7(49): 81144-81155.
49. Jang BG, Lee BL, Kim WH. Prognostic significance of leucine-rich-repeat-containing G-protein-coupled receptor 5, an intestinal stem cell marker, in gastric carcinomas. Gastric Cancer. 2016; 19(3): 767-777.
50. Sun B, Ye X, Li Y, Zhang W. Lgr5 is a potential prognostic marker in patients with cervical carcinoma. Int J Clin Exp Pathol. 2015; 8(2): 1783-1789.
51. Yang L, Tang H, Kong Y, Xie X, Chen J, Song C, et al. LGR5 promotes breast cancer progression and maintains stem-like cells through activation of Wnt/β-catenin signaling. Stem Cells. 2015; 33(10): 2913-2924.
52. Gao F, Zhou B, Xu JC, Gao X, Li SX, Zhu GC, et al. The role of LGR5 and ALDH1A1 in non-small cell lung cancer: Cancer progression and prognosis. Biochem Biophys Res Commun. 2015; 462(2): 91-98.
53. Sun Y, Jia X, Wu X. High expressions of Lgr5 and ALDH1 in primary epithelial ovarian cancer correlate with advanced tumor stage and grade as well as poor prognosis of the patients. Gynecol Obstet Invest. 2015 (ahead of print)
54. Wang Y, Jiang CQ, Fan LF. Correlation of Musashi-1, Lgr5, and pEGFR expressions in human small intestinal adenocarcinomas. Tumour Biol. 2015; 36(8): 6075-6082.
55. Gao FJ, Chen JY, Wu HY, Shi J, Chen M, Fan XS, et al. Lgr5 overexpression is positively related to the tumor progression and HER2 expression in stage pTNM IV colorectal cancer. Int J Clin Exp Pathol. 2014; 7(4): 1572-1579.
56. Liu Z, Dai W, Jiang L,Cheng Y. Over-expression of LGR5 correlates with poor survival of colon cancer in mice as well as in patients. Neoplasma. 2014; 61(2): 177-185.
57. He S, Zhou H, Zhu X, Hu S, Fei M, Wan D, et al. Expression of Lgr5, a marker of intestinal stem cells, in colorectal cancer and its clinicopathological significance. Biomed Pharmacother. 2014; 68(5): 507-513.
58. Chen WW, Wang F, Zhang DS, Luo HY, Wang ZQ, Wang FH, et al. Primary small cell carcinoma of the esophagus: clinicopathological study of 44 cases. BMC Cancer. 2014; 14: 222.
59. Xi HQ, Cai AZ, Wu XS, Cui JX, Shen WS, Bian SB, et al. Leucine-rich repeat-containing G-protein-coupled receptor 5 is associated with invasion, metastasis, and could be a potential therapeutic target in human gastric cancer. Br J Cancer. 2014; 110(8): 2011-2020.
60. Hsu HC, Liu YS, Tseng KC, Hsu CL, Liang Y, Yang TS, et al. Overexpression of Lgr5 correlates with resistance to 5-FU-based chemotherapy in colorectal cancer. Int J Colorectal Dis. 2013; 28(11): 1535-1546.
61. Jang BG, Lee BL, Kim WH. Distribution of LGR5+ cells and associated implications during the early stage of gastric tumorigenesis. PLoS One. 2013; 8(12): e82390.
62. Zheng ZX, Sun Y, Bu ZD, Zhang LH, Li ZY, Wu AW, et al. Intestinal stem cell marker LGR5 expression during gastric carcinogenesis. World J Gastroenterol. 2013; 19(46): 8714-8721.
63. Ryuge S, Sato Y, Jiang SX, Wang G, Kobayashi M, Nagashio R, et al. The clinicopathological significance of Lgr5 expression in lung adenocarcinoma. Lung Cancer. 2013; 82(1): 143-148.
64. Bu Z, Zheng Z, Zhang L, Li Z, Sun Y, Dong B, et al. LGR5 is a promising biomarker for patients with stage I and II gastric cancer. Chin J Cancer Res. 2013; 25(1): 79-89.
65. Wu XS, Xi HQ, Chen L. Lgr5 is a potential marker of colorectal carcinoma stem cells that correlates with patient survival. World J Surg Oncol. 2012; 10: 244.
66. Ziskin JL, Dunlap D, Yaylaoglu M, Fodor IK, Forrest WF, Patel R, et al. In situ validation of an intestinal stem cell signature in colorectal cancer. Gut. 2013; 62(7): 1012-1023.
67. Takahashi H, Ishii H, Nishida N, Takemasa I, Mizushima T, Ikeda M, et al. Significance of Lgr5(+ve) cancer stem cells in the colon and rectum. Ann Surg Oncol. 2011; 18(4): 1166-1174.

68. Takeda K, Kinoshita I, Shimizu Y, Matsuno Y, Shichinohe T, Dosaka-Akita H. Expression of LGR5, an intestinal stem cell marker, during each stage of colorectal tumorigenesis. Anticancer Res. 2011; 31(1): 263-270.
69. Becker L, Huang Q, Mashimo H. Lgr5, an intestinal stem cell marker, is abnormally expressed in Barrett's esophagus and esophageal adenocarcinoma. Dis Esophagus. 2010; 23(2): 168-174.
70. Fan XS, Wu HY, Yu HP, Zhou Q, Zhang YF, Huang Q. Expression of Lgr5 in human colorectal carcinogenesis and its potential correlation with beta-catenin. Int J Colorectal Dis. 2010; 25(5): 583-590.
71. Hsu SY, Liang SG, Hsueh AJ. Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region. Mol Endocrinol. 1998; 12(12): 1830-1845.
72. Leng Z, Xia Q, Chen J, Li Y, Xu J, Zhao E, et al. Lgr5+CD44+EpCAM+ strictly defines cancer stem cells in human colorectal cancer. Cell Physiol Biochem. 2018; 46(2): 860-872.
73. Wang X, Wang X, Liu Y, Dong Y, Wang Y, Kassab MA, et al. LGR5 regulates gastric adenocarcinoma cell proliferation and invasion via activating Wnt signaling pathway. Oncogenesis. 2018; 7(8): 57.
74. Xu L, Lin W, Wen L, Li G. Lgr5 in cancer biology: functional identification of Lgr5 in cancer progression and potential opportunities for novel therapy. Stem Cell Res Ther. 2019; 10(1): 219.
75. Vaquero J, Guedj N, Clapéron A, Nguyen Ho-Bouldoires TH, Paradis V, Fouassier L. Epithelial-mesenchymal transition in cholangiocarcinoma: From clinical evidence to regulatory networks. J Hepatol. 2017; 66(2): 424-441.
76. Liu S, Gong Z, Chen M, Liu B, Bian D, Wu K. Lgr5-positive cells are cancer stem cells in skin squamous cell carcinoma. Tumour Biol. 2014; 35(11): 11605-11612.
77. Trimboli AJ, Fukino K, de Bruin A, Wei G, Shen L, Tanner SM, et al. Direct evidence for epithelial-mesenchymal transitions in breast cancer. Cancer Res. 2008; 68(3): 937-945.
78. Montazer F, Boozari B, Alizadeh-Navaei R. Evaluation of LGR5 cancer stem cell marker expression in breast cancer and its relationship with hormonal profile and clinical pathological features. Asian Pac J Cancer Prev. 2023; 24(2): 467-470.
79. Kim H, Lee DH, Park E, Myung JK, Park JH, Kim DI, et al. Differential epithelial and stromal LGR5 expression in ovarian carcinogenesis. Sci Rep. 2022; 12(1): 11200.
80. Bauer L, Langer R, Becker K, Hapfelmeier A, Ott K, Novotny A, et al. Expression profiling of stem cell-related genes in neoadjuvant-treated gastric cancer: a NOTCH2, GSK3B and β-catenin gene signature predicts survival. PLoS One. 2012; 7(9): e44566.
81. Jia Y, Li Z, Cheng X, Wu X, Pang F, Shi J, et al. Depletion of death-associated protein-3 induces chemoresistance in gastric cancer cells through the β-catenin/LGR5/Bcl-2 axis. J Investig Med. 2019; 67(5): 856-861.
82. Clark-Corrigall J, Myssina S, Michaelis M, Cinatl J Jr, Ahmed S, Carr-Wilkinson J. Elevated expression of LGR5 and WNT signaling factors in neuroblastoma cells with acquired drug resistance. Cancer Invest. 2023; 41(2): 173-182.
83. Nakata S, Phillips E, Goidts V. Emerging role for leucine-rich repeat-containing G-protein-coupled receptors LGR5 and LGR4 in cancer stem cells. Cancer Manag Res. 2014; 6: 171-180.
84. Liu X, Mazanek P, Dam V, Wang Q, Zhao H, Guo R, et al. Deregulated Wnt/beta-catenin program in high-risk neuroblastomas without MYCN amplification. Oncogene. 2008; 27(10): 1478-1488.
85. Vicari L, Colarossi C, Giuffrida D, De Maria R, Memeo L. Cancer stem cells as a potential therapeutic target in thyroid carcinoma. Oncol Lett. 2016; 12(4): 2254-2260.
86. Michelotti G, Jiang X, Sosa JA, Diehl AM, Henderson BB. LGR5 is associated with tumor aggressiveness in papillary thyroid cancer. Oncotarget. 2015; 6(33): 34549-34560.
87. Posey TA, Jacob J, Parkhurst A, Subramanian S, Francisco LE, Liang Z, et al. Loss of LGR5 through therapy-induced downregulation or gene ablation is associated with resistance and enhanced MET-STAT3 signaling in colorectal cancer cells. Mol Cancer Ther. 2023; 22(5): 667-678.
88. Yang L, Lin Y, Liu Z, Li Y, Liu Y, Liang R. Impact of LGR5 in colorectal cancer on overall and progression-free survival: a systematic review and meta-analysis. Int J Clin Exp Med. 2016; 9(9): 10537-10543.
89. Sigal M, Rothenberg ME, Logan CY, Lee JY, Honaker RW, Cooper RL, et al. Helicobacter pylori activates and expands Lgr5(+) stem cells through direct colonization of the gastric glands. Gastroenterology. 2015; 148(7): 1392-1404. e21.
90. Kim HS, Song HJ, Kim HU, Jeong IH, Koh HM, Shin JH, et al. Expression profile of intestinal stem cell and cancer stem cell markers in gastric cancers with submucosal invasion. Pathol Res Pract. 2021; 218: 153336.