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Original Article

Impact of LGR5 in colorectal cancer on overall and progression-free survival: a systematic review and meta-analysis

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Abstract: Objective: The prognostic and clinical significance of LGR5 in Colorectal Cancer (CRC) remains controversial. Here, we conducted a systematic review and meta-analysis to evaluate the impact of LGR5 expression on prognosis and clinicopathological features of CRC patients, clarifying a precise determinant of the clinical significance of LGR5. Methods: Pubmed, Web of Science, Medline and Chinese CNKI (from 1982 to December 15st, 2015) were searched for titles and abstracts, using the following keywords: "LGR5", "GPR49", and "colorectal cancer" to identify studies eligible for our analysis. Meta-analysis was performed by Reman5.2, and the used data were extracted from published hazard ratios (HRs), if available, or derived treatment estimates from survival curves. The outcomes included overall survival, progression-free survival and various clinicopathological features. Results: A total of eleven eligible studies were finally included, and our results showed that LGR5 expression in CRC tissues was significantly correlated with overall survival (OR=2.01, 95% CI=1.16-3.47, *P*=0.01, Random model) and progression free survival (OR=2.27, 95% CI=1.37-3.77, *P*=0.002, Fixed model) of CRC patients. With respect to clinicopathological features, LGR5 expression was positively correlated with TNM stage (OR=2.18, 95% CI=0.97-4.92, *P*=0.06), but has no relationship with the differentiation (OR=1.40, 95% CI=0.55-3.57, *P*=0.48) of CRC patients. Conclusion: LGR5 expression trends to correlate with a worse overall survival and progression-free survival in CRC patients, which indicates that LGR5 could be a potential prognostic marker for CRC patients. In addition, a high TNM stage was found in LGR5 positive CRC patients.

Keywords: Colorectal cancer, LGR5, overall survival, meta-analysis

Introduction

Colorectal cancer (CRC) is one of the most common cause of cancer-related death worldwide, and yet a best understood neoplasms [1]. Since the cancer stem cells (CSCs) theory occurred in 1997, tumors can be recognized as a result of abnormal organogenesis driven by a small subpopulation of cancer cells that are able to be self-renewal and to produce the heterogeneous lineages of cancer cells [2, 3]. LGR5 is one of the most common used stem cell markers in colorectal cancer which targets the Wnt signaling [4-7]. Numerous studies have demonstrated that over-expression of LGR5 has close association with initiation and recurrence of colorectal cancer and predicts poor prognosis [7-10], which were hardly concurred by others [11]. This immediately challenges the conventional interpretation and hinders the application of LGR5 as a novel prognostic marker in CRC patients. Here, we performed a systematic review and meta-analysis in the published literatures in order to appraise the prognostic value and the association with clinicopathological features of LGR5 in CRC patients with the goal of gaining insights into the clinical implications.

Materials and methods

Literature search strategy

We adapted the Cochrane Central Register of Controlled Trials, and a comprehensive publication search was performed in the Pubmed, Web of Science, Medline, and Chinese CNKI database using the following search strings: "LGR5" or "GPR49", and "colorectal cancer" from January 1, 1982 to October 15th, 2015 to identify

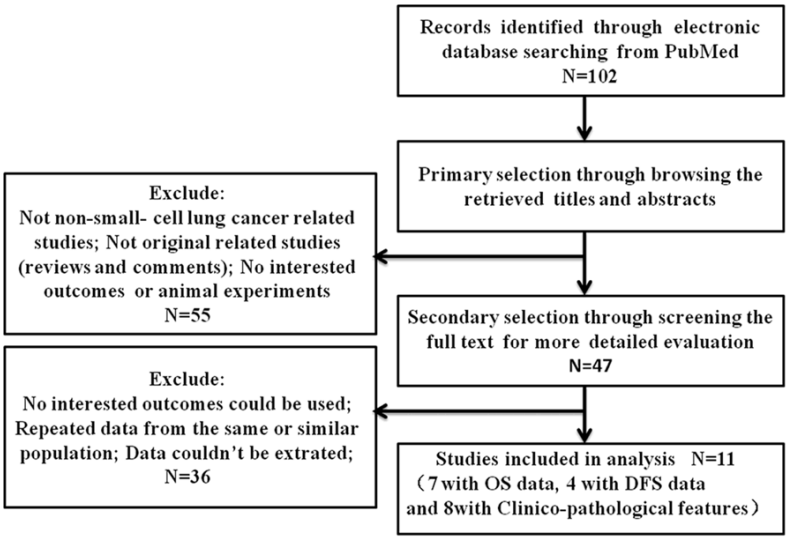

Figure 1. Flow chart for selection of studies.

studies eligible for our analysis. Titles and abstracts were reviewed to identify reports which examined the association of LGR5 expression with clinical outcomes, such as overall survival (OS), progression-free survival (PFS), and clinicopathological features, including age, gender, differentiation, TNM stage and lymph node metastasis in CRC patients.

Selection criteria

The inclusion criteria were listed as follows: (1) studies dealing with CRC patients including the colon and rectum cancer patients; (2) articles concerning about the association of LGR5 expression with either prognosis or clinicopathological features; (3) the expression of LGR5 was detected on cancer tissue, rather than in serum or any other kinds of specimens; (4) the detection method could be variable, including immunohistochemistry (IHC), tissue microarray and real-time PCR; (5) articles providing sufficient data to allow the estimation of an odds ratio (OR) of OS, PFS and clinicopathological features; (6) reviews, duplicated studies, comments, animal or cell experiments, and irrelevant articles were excluded (**Figure 1**).

Data extraction

The valuable data including author, country of study, publication year, number of patients, detection method, cut-off value, TNM stage and positive rates of LGR5 were extracted from the included papers and illustrated in **Table 1**. Data were extracted independently by three authors and discrepancies were discussed among them in cases of conflicting evaluations.

Statistical analysis

Statistical analysis was performed by Review 5.2 software. Hazard ratios (HRs) and the associated 95% confidence intervals (CIs) for OS and PFS outcomes were extracted to assess treatment efficacy between the LGR5 positive and negative subgroups. In order to include the most updated OS data, we extracted the hazard ratios and 95% confidence intervals by the survival curves using the methods of Parmar. Heterogeneity across studies was evaluated by the *Q* test and *P*-values (Cochrans *Q* test, *P*-value >0.05 or I^2>50% indicated the existence of heterogeneity across studies). Fixed or Random model was used depending on heterogeneity analysis. ORs and RRs were calculated by a fixed-effects model if I^2<50%, otherwise a random effects model was used. And *P*<0.05 was considered as a statistical significance.

Results

Included studies

A total of eleven studies including 1725 patients met with our criteria were finally included in this meta-analysis. The main features of each eligible study were extracted and illustrated in **Table 1**. 7 studies were determined with OS, 4 studies indicated PFS and eleven reported clinicopathological details. Two main methods were used to evaluate the expression of LGR5 in CRC patients, including immunohistochemistry (IHC) and real-time PCR (**Table 1**).

Effects of LGR5 on OS in CRC patients

Data of OS extracted from seven studies were included in the meta-analysis. Since the heterogeneity was significant (I^2=78%, *P*=0.0002), a random-effect model was used to evaluate the OR of OS in CRC patients. Meta-analysis sh-

Table 1. General characteristics of included studies

First author	Year	Country	Histology	No. of patients	Method	Cut-off values	TNM stage	NO. of POS. (%)
Liu Z [4]	2014	China	Colon	366	IHC/PCR	-	I--	193 (53%)
Hsu HC [6]	2013	China	Colorectal	296	IHC	Score ≥180	I18	175 (59%)
Simon E [7]	2011	USA	Colon	100	IHC	>1%	I%C	61 (61%)
Saigusa S [8]	2012	Japan	Rectal	52	IHC/PCR	-	I--	-
Wu XS [9]	2012	China	Colorectal	192	IHC	Score ≥5	Ico	108 (56%)
Uchida H [10]	2010	Japan	Colorectal	50	PCR	-	II--	35 (70%)
deSousa EMF [11]	2011	Netherlands	Colorectal	77	IHC	Score ≥100	III10	30 (39%)
Fan XS [26]	2010	China	Colorectal	102	IHC	≥5%	I5%	55 (54%)
Takahashi H [27]	2011	Japan	Colon/Rectal	180	PCR	-	I--	90 (50%)
Valladares AM [28]	2012	Spain	Colorectal	54	PCR	-	I--	48 (89%)
Takeda K [29]	2011	Japan	Colorectal	60	IHC	≥5%	I5%	52 (87%)

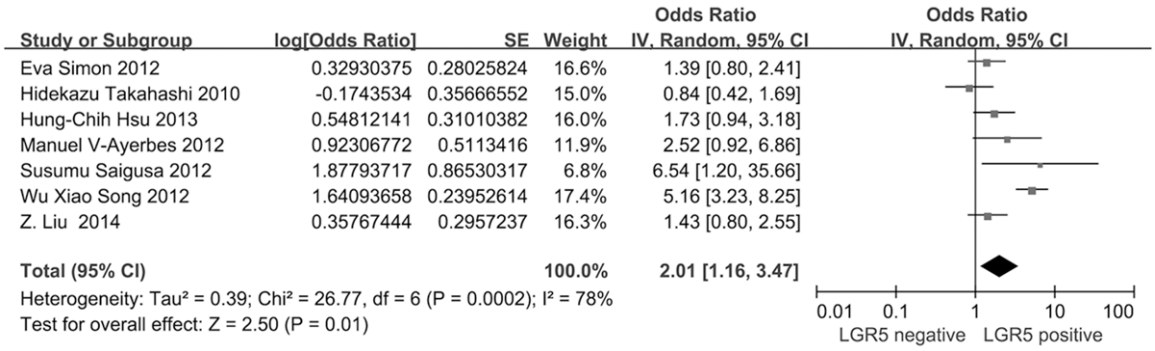

Figure 2. Meta-analysis of overall survival between LGR5 positive and LGR5 negative in CRC patients.

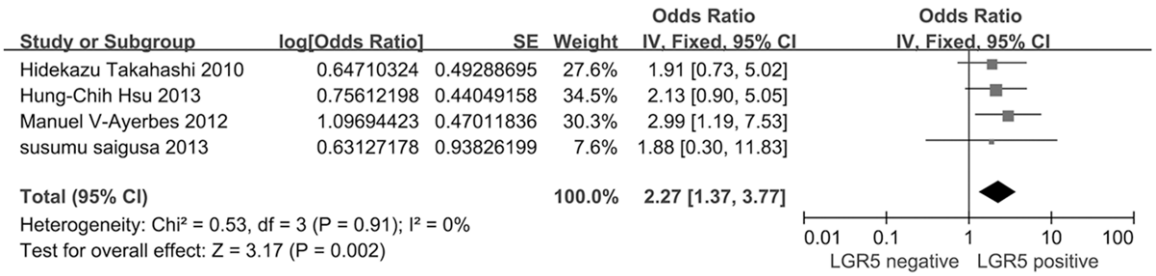

Figure 3. Meta-analysis of Progression-Free Survival between LGR5 positive and LGR5 negative in CRC patients.

owed that LGR5 high expression was correlated with the poor OS of CRC patients (OR=2.01, 95% CI=1.16-3.47, *P*=0.01, Random model), suggesting that LGR5 could be an independent prognostic marker for CRC patients (**Figure 2**).

Effects of LGR5 on PFS in CRC patients

Data on PFS were extracted from four studies including a total of 594 patients. A fixed-effect model (I^2=0%, *P*=0.91) was used to calculate the OR of PFS in CRC patients. The meta-analysis showed that patients with high expression of LGR5have a worse PFS compared with those with low expression of LGR5 (OR=2.27, 95% CI=1.37-3.77, *P*=0.002, the Fixed Model) (**Figure 3**).

LGR5 expression and clinicopathological parameters of CRC patients

All the eleven studies provided the information of various clinicopathological parameters. And their correlations with LGR5 expression were

Table 2. Narrative review of the association between clinicopathological features and LGR5 expression with respect to non-small-cell-lung cancer patients

Items	Significant correlation (P<0.05)	Non-significant correlation (P≥0.05)
Age	[27]	[4], [5], [6], [7], [8], [9], [10], [26], [28], [29]
Gender	[26]	[4], [6], [8], [9], [10], [27], [28], [29]
TNM stage	[4], [6], [7], [9], [27]	[8], [26]
Differential	[8], [9]	[4], [6], [27]
Lymph node metastases	[5], [6], [7], [9], [10]	[8], [27]

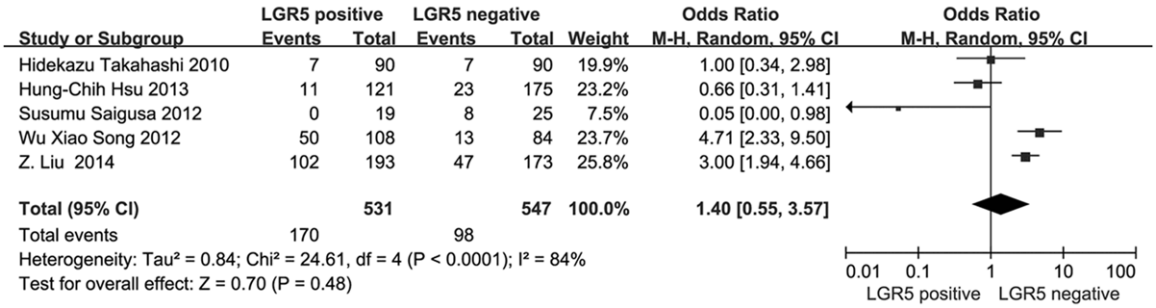

Figure 4. Meta-analysisof correlation between LGR5 expression and differentiation in CRC patients.

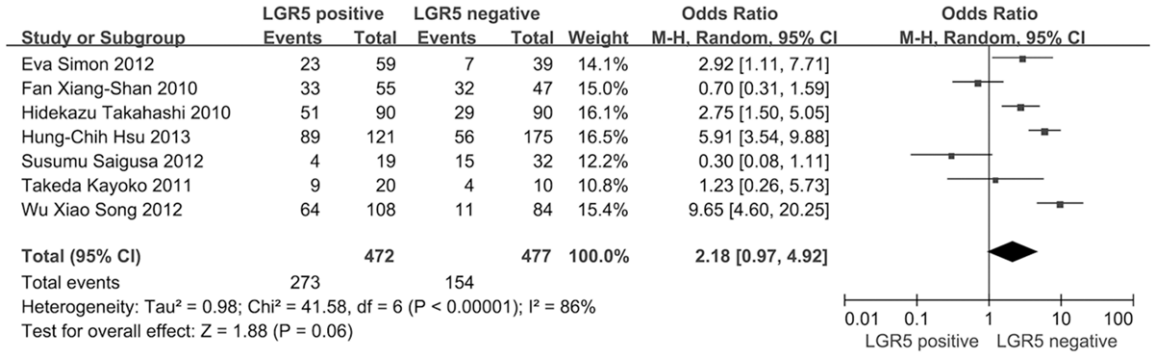

Figure 5. Meta-analysis of correlation between LGR5 expression and TNM stage in CRC patients.

summed in **Table 2**. 8 studies had the statistic data on age of patients, and among them just 1 reported the existence of association between age and LGR5 expression. And 1 of 9 studies demonstrated that correlation was existed between LGR5 expression and gender.

Except these above-mentioned parameters, controversies also existed in the correlation between differentiation, TNM stage and LGR5 expression in these included studies. We conducted a meta-analysis to clarify the association of LGR5 expression and the two clinicopathological parameters. Five studies evaluated the relationship between LGR5 expression and differentiation in CRC Patients. The pooled OR was 1.40, (95% CI=0.55-3.57, P=0.48, random effect), suggesting that LGR5 expression was not associated with differentiation of CRC patients (**Figure 4**). Notably, the meta-analysis suggested that LGR5 expression was positively correlated with TNM stage of CRC patients (OR=2.18, 95% CI=0.97-4.92, P=0.06, random-effect) (**Figure 5**).

Discussion

Colorectal cancer is the most common malignancy of the gastrointestinal tract and causes 60000 deaths worldwide every year [12, 13].

Though many treatment protocols have been applied to deal with colorectal cancer, a complete cure has not been obtained [12-15]. This may due to the existence of colorectal cancer stem cell (CSCs) which is resistant to radiation therapy, chemotherapy and even can enable the recurrence of cancers [16-19]. Therefore, identifying cancer stem cells among the block of cancer cells becomes urgent. Actually, the searches have been intense for the past decades to find a specific biomarker of CSCs [20, 21]. In 2007, Barker et al discovered that the leucine-rich repeat containing G protein-coupled receptor 5 (LGR5) had a restrict expression in the crypt base of small and large intestines, and it could be a valuable stem cell marker for colorectal cancer [22]. This was received by more and more researchers, while still some studies were not in agreement with it [23-26].

LGR5 (leucine-rich repeat-containing G-protein-coupled receptor 5) also known as Gpr49 is a target of Wnt signaling and is associated with carcinogenesis and tumor invasion in colorectal cancer [11, 27, 28]. From the currently completed findings, we hypothesize that LGR5 expression participates in the maintenance and proliferation of cells which was associated with poor survival of colorectal cancer. However, several reports surprisingly found that elevated expression of LGR5 was actually associated with good prognosis [27, 29-31]. Thus, the prognostic and clinicopathological values of LGR5 in colorectal cancer patients become unclear.

The present study included a total of 11 eligible studies with 1725 patients for qualitative analysis. IHC and RT-PCR were the most commonly used for detection. Overall, the finally analysis showed that LGR5 positive expression predicted poor prognosis of CRC patients. However, it should be noted that there are some limitations existed here. First, publication bias should be concerned, because more positive results tended to be published, thus potentially exaggerating the association between LGR5 expression and poor outcomes. Second, most of the HRs and 95% confidence intervals (CIs) for OS and PFS were determined from survival curves in the published papers. This might be less reliable than that from direct analysis of variance. Third, the conditions of patients included in this study were not normalized. The stage of patients varied from I to IV, and the detection methods including immunohistochemistry (IHC) and real-time PCR. Finally, the number of studies included in this analysis is limited. These differences might partly impact the significance of LGR5 in the survival and the clinicopathological analysis.

In summary, the present meta-analysis indicates that LGR5 expression is associated with a poor OS, PFS, and a higher TNM stage. And no correlation was found between LGR5 expression and tumor differentiation. These findings suggest that the cancer stem cells marker LGR5 might be a valuable prognostic marker and a potential new therapeutic target for the treatment of colorectal cancer patients. In addition, co-detection of LGR5 with other CSC markers such as CD133, ALDH1A1, Nanog and OCT4 may be more valuable and helpful in clinical application in CRC patients [32, 33].

Disclosure of conflict of interest

None.

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