LGR-5 as a Biomarker in Colorectal Cancer: A Systematic Review of Clinicopathological Features and Prognostic Value

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ABSTRACT

Colorectal cancer (CRC) had the third highest cancer incidence worldwide. Recent research is targeting specific gene expression as a CRC marker. Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) is one of the regulating genes for tumor metastasis and growth. The aim of this study is to explain the correlation between LGR5, clinical pathology, and prognosis of CRC. This systematic review was arranged using PRISMA for eligible cohort studies and evaluated using the Newcastle-Ottawa scale. From 63 articles found, 6 articles were eligible and selected for data extraction and evaluation. Based on the studies, LGR5 positivity was positively associated with histopathological characteristics, TNM staging, and vascular invasion. LGR5 expressions in those studies were also high and could be positively detected by this marker. LGR5 regulates the Wnt/β-catenin signaling pathway promotion in normal colon stem cells. Methylation of the Wnt target gene promoter is a potent predictor of CRC recurrence; thus, the expression of LGR5 can be used as a CRC marker. LGR5 plays a role as a pro-oncogenic factor in colorectal carcinogenesis via prostaglandin E2 and epidermal growth factor signaling. LGR5 induction also could increase cancer cells chemoresistant ability. Thus, LGR5 could determine the prognosis of CRC patients and was related to certain clinicopathological features and tumor progressions.

Keywords: Colorectal cancer, LGR5, marker, prognosis.

ABSTRAK

Kanker kolorektal (*colorectal cancer/CRC*) merupakan kanker dengan angka kejadian tertinggi ketiga di seluruh dunia. Penelitian terbaru menarget ekspresi gen spesifik sebagai penanda CRC. *Leucine rich repeat-containing G-protein coupled receptor 5* (LGR5) adalah salah satu gen yang mengatur metastasis dan pertumbuhan tumor. Tujuan penelitian ini adalah untuk menjelaskan korelasi antara LGR5, patologi klinis, dan prognosis CRC. Tinjauan sistematis ini disusun menggunakan PRISMA untuk studi kohort yang memenuhi syarat dan dievaluasi menggunakan skala Newcastle-Ottawa. Dari 63 artikel, 6 artikel memenuhi syarat untuk diekstraksi dan dievaluasi. Hasil analisis menunjukkan LGR5 secara positif terkait dengan karakteristik histopatologis, stadium TNM, dan invasi vaskular. Selain itu, ekspresi LGR5 dalam studi tersebut tinggi dan dapat dideteksi secara positif oleh penanda ini. LGR5 mengatur promosi jalur pensinyalan Wnt/β-catenin dalam sel punca usus besar normal. Metilasi promotor gen target Wnt merupakan prediktor kuat untuk kekambuhan CRC sehingga ekspresi LGR5 dapat digunakan sebagai penanda CRC. LGR5 berperan sebagai faktor pro-onkogenik pada karsinogenesis kolorektal melalui sinyal prostaglandin E2 dan *epidermal growth factor*. Induksi LGR5 juga dapat meningkatkan kemampuan kemoresisten sel kanker. Dengan demikian, LGR5 dapat menentukan prognosis pasien CRC dan berhubungan dengan gambaran klinikopatologi tertentu dan perkembangan tumor. **Aiman Hilmi Asaduddin, Roisya Nur Farhania, Namira Putri Imani, Cindy Ayudia Pramaesti, Andayani Yuwana Sari. LGR-5 sebagai *Biomarker* Kanker Kolorektal: Tinjauan Sistematik atas Gambaran Klinikopatologis dan Nilai Prognostik.**

Kata Kunci: Kanker kolorektal, LGR5, penanda, prognosis.

INTRODUCTION

Malignancy in the gastrointestinal tract is the main leading cause of death throughout the world.[1] One of the most frequent malignancies in the gastrointestinal tract is colorectal cancer (CRC). CRC occurs due to clinical significance of a transformed colonic polyp or malignant type of tumor.[2] CRC can be defined as a cancer that develops slowly and starts as a tumor or tissue growth on the inner lining of the rectum or large intestine.[3] Epidemiologically, CRC had the third highest cancer incidence in the world.[4] Male patients were slightly higher than female, with approximately 814,000 cases in men and 664,000 cases in women.[5] In Indonesia, CRC tends to occur at a younger age; the general incidence of CRC increased significantly after the age of 50 years.[1,2]

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CRC occurs through complex interactions among dominant genetic factors such as Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPC), and also environmental factors.[6,7] Genetic changes during the cancer progression are initially started in the normal colon and continue to abnormalities of APC, hMSH2, and hMLH1 (heredity syndrome). This condition leads to the abnormalities in methylation and inactivation of APC, hM3SH2, and hMLH1, which causes epithelial hyper-proliferation. Furthermore, K-ras mutates, and deletions of DCC and p53 occur, which initially causes adenoma. Further accumulation of genetic abnormalities induces colorectal carcinoma.[6–8]

Researchers are targeting specific gene expression as a principal method of CRC therapy. Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) is one of the genes in the form of cancer stem cells that regulate tumor metastasis and growth. LGR5 is a Wnt target in the Wnt/β-catenin signaling pathway whose expression will increase in cases of gastrointestinal cancer, especially in CRC. The binding between LGR5 and the Wnt receptor can induce the Wnt/β-catenin signaling pathway, resulting in the accumulation of β-catenin in the cytosol and activating pro-oncogenes. This process is a trigger for colorectal carcinogenesis.[9]

The expression of LGR5 during the development of CRC could determine the prognosis in CRC patients of cohort studies.[10,11] LGR5 expression was higher in CRC tissues than in normal mucosa and had not been associated with other cancer stem cell markers.[11] Particularly, positive LGR5 was used as an independent prognostic marker for better clinical outcomes in colorectal cancer patients.[11] Overexpression of LGR5 could suppress tumor growth by decreasing ERK phosphorylation along with decreased colony formation and migration ability in DLD1 cells.[10]

Ihemelandu, *et al*, in 2019 assessed the correlation between LGR5 expression and clinical pathology (sex, age at diagnosis, stage of cancer, lymph node status, histopathology, and patient prognosis) by statistical analysis.[11] The clinical pathology characteristics of the cohort showed the role of LGR5 expression in CRC.[11] There were no significant differences observed between the high and low LGR5 cohorts regarding sex, age at diagnosis, cancer stage, lymph node status, distant metastasis, and histopathology results. Cohort studies regarding the correlation between LGR5 with clinical pathology and prognosis had been carried out.[11] The purpose of this systematic review is to explain the correlation between LGR5, clinical pathology, and the prognosis of colorectal cancer.

METHODS

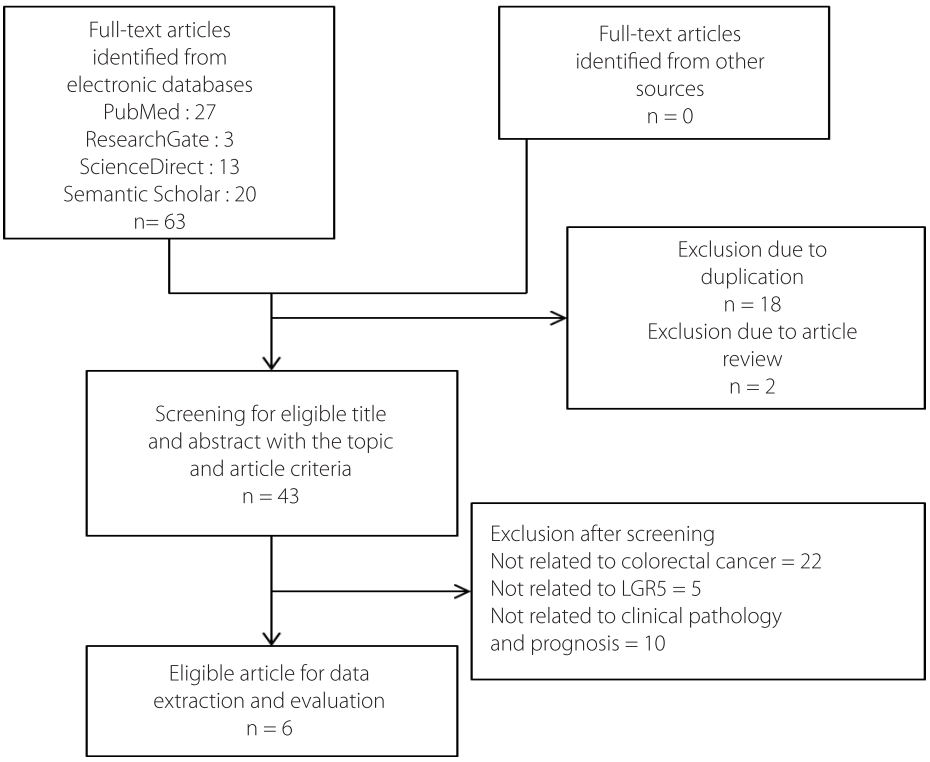
This systematic review was arranged using PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses—Protocols).[12] A total of 12 articles were identified from scientific databases, including PubMed, ScienceDirect, Semantic Scholar, and ResearchGate, with a searching strategy using some keywords that were "clinicopathology AND prognosis AND LGR5 AND colorectal AND (cancer OR tumor OR carcinoma)." The database searching was carried out by combining the keywords using Boolean operations and Medical Subject Headings (MeSH). The eligible articles were selected according to the inclusion and exclusion criteria.

The eligible article criteria used were original/empirical research; cohort studies in patients with colorectal cancer, English language articles, and published in January 2016 - July 2020. After completing the literature search, we obtained full-text articles for all potentially relevant studies and then deduplicated the same references. Screening of the titles and abstracts was identified using Mendeley to exclude major irrelevant studies. Selecting full-text articles for inclusion in this review used eligibility criteria: patients with colorectal cancer (male and female), with an index of prognosis of LGR5, and within study outcomes of LGR5 expression, clinical pathology, metastasis, and survival value in cohort study.

Study quality was assessed by the Newcastle-Ottawa scale (NOS) for the cohort study. Data extraction from selected studies was done by collecting and combining data that related to the research objectives, research designs, settings, subject information, phenotypes, network types, data collection methods, statistics, and results. Then, the extracted data was summarized in a table.

RESULTS

The database search results were 63 articles. After the first exclusion based on duplicated and review articles, a total of 43 articles were



Scheme. Systematic review flowchart diagram.

Table 1. Characteristics of eligible studies.

Author	Year	Country	Method	Number of Patients	Age	Stage				NOS Score
						I	II	III	IV	
Jang, *et al.*[10]	2018	South Korea	IHC	788	<60 years: 58%; ≥60 years : 42%	118	247	288	135	7
Ihemelandu, *et al.*[11]	2019	USA	IHC	49 (60 surgeries)	Median=61,4	5	16	11	28	8
Shekarriz, *et al.*[13]	2019	Iran	IHC	40	Male: 57.8±11.6; Female: 58.4±12.77	N/A				8
Zheng, *et al.*[14]	2018	China	IHC	204	Median=64	24	71	98	11	5
Gzil, *et al.*[15]	2020	Poland	IHC	89	<65 years: 37; ≥65 years : 52	N/A				8
Harb, *et al.*[16]	2016	Egypt	IHC	60	57,46±12.42	20	58	30	12	6

Abbreviations: IHC: immunohistochemistry; NOS: Newcastle Ottawa scale; N/A: not available.

screened as proper studies to be evaluated based on the suitability of the title and abstract with the topic and based on inclusion and exclusion criteria. From those articles, 6 articles were selected as eligible articles for further evaluation and data extraction (**Scheme**).

The six articles were studies from the last 5 years and were conducted in 6 different countries with a cohort study design. CRC tissue samples were taken from patients and analyzed by immunohistochemistry (IHC) methods to determine LGR5 expression in these tissues **(Table 1)**. Data extraction details of studies can be seen in **Table 2**. The NOS score for those cohort studies can also be seen in **Table 1**.

There was no significant correlation between LGR5 expression and age or gender in CRC patients.[10,11,13–16] LGR5 positivity was positively associated with histopathologic characteristics.[10] Ihemelandu, *et al*, (2019) and Shekarriz, *et al*, (2019) showed that there were no significant correlations between LGR5 expressions and clinicopathological features of CRC,[11,13] but the other studies from Zheng, *et al*, (2018) and Harb, *et al*, (2016) showed a significant correlation to TNM staging and tumor grading.[14,16] In the other study, Gzil, *et al*, (2020) revealed that there was no correlation between LGR5 and tumor staging but showed a correlation to vascular invasion.[15]

On the other hand, the majority of LGR5 expression in those studies was high and could be positively detected by this marker **(Table 2)**. Regarding the evaluation of eligible articles about the correlation of LGR5 and CRC prognosis, Ihemelandu, *et al*, (2019) showed that the mean overall survival was 9 years for high LGR5 and 6.3 years for low LGR5 patients.[11] Shekarriz, *et al*, (2019) revealed that overall survival of 1, 3, and 5 years follow-up were, sequentially, 100%, 81%, and 75%, while Harb, *et al*, (2016) only revealed 3 years follow-up with a result of overall survival of 24.1% for high LGR5 expression and 100% for low LGR5 expression (**Table 3**).[15] The remaining articles did not show any information about overall survival.

DISCUSSION

The carcinogenesis of CRC theory is still controversial. Even the initial process of CRC is still unclear; G protein-coupled receptors (GCRs) had been shown to be significantly related to cancer stem cells (CSCs) in colorectal carcinogenesis.16 LGR5, which is one of the GCRs family, was revealed to be a potential marker of cancer cell population.[17] Increased changes in LGR5 expression could represent one of the molecular changes in CRC, which leads to long-term potentiation of Wnt/β-catenin signaling.[17]

LGR5 plays a role in promoting the Wnt/β-catenin signaling pathway in normal colon stem cell.[18] LGR5 has a ligand in R-Spondin (RSPO), which works together with the Wnt receptor (Frizzled/FZD and LRP5/6) to potentiate Wnt/β-catenin signaling.[19–21] RSPO binding to LGR5 leads to Wnt signaling by neutralizing RNF43/ZNF3 ligase, which cannot remove Wnt receptor from the cell membrane.[22] FZD and LRP5/6 are freed to bind Wnt ligands that lead to stabilizing β-catenin and activating the downstream of targeted Wnt genes, such as c-MYC, CyclinD1, and Axin2.[23] Methylation of the Wnt target gene promoter is a potent predictor of CRC recurrence; thus, the expression of LGR5 can be used as a CRC marker.[23]

Other studies also mentioned pro-carcinogenic activities of LGR5 in a functional in vitro study.[25] The study showed that LGR5 plays a role as a pro-oncogenic factor in colorectal carcinogenesis.[26] Although LGR5 is a Wnt/β-catenin signaling target, previous research also revealed that prostaglandin E2 (PGE2) and epidermal growth factor (EGF) signaling could influence LGR5 expression, thus affecting the proliferation and survival ability of colorectal adenomas.[25,26] LGR5 induction also could increase cancer cells chemo-resistant ability.[27]

Other molecular biology approaches have shown that LGR5 overexpression could increase cell adhesion as a consequence of cortical F-actin enhancement.[28,29] Besides, LGR5 in CRC CSC was characterized by higher expression of mesenchymal-associated genes such as Snail, Slug, Zeb1 and 2, and N-cadherin. It was also characterized by lower expression of epithelial-associated genes, such as E-cadherin, occluding, and epithelial cell adhesion molecule (EpCAM).[30] Based on previous studies, this condition explained the sphere formation of CSC is associated with a decreased LGR5 expression because of progressive CpG island methylation during the carcinogenesis process.[10] These events


lead to intracellular mechanisms promoting cell adhesion that may decrease with EMT promotion and induce increased metastatic ability of CSC. This mechanism might explain why low LGR5 in a primary cancer is related to lymph node metastases.[15]

LGR5 expression in CRC tissues is also related to tumor angiogenesis and other clinical outcomes. According to the correlation of LGR5 and vascular invasion in CRC, He, *et al*, (2014) studied this with further research using an anti-human CD34 monoclonal antibody and evaluated angiogenesis by measuring micro-vessel density (MVD).[31] The results showed that LGR5 expression was significantly related to MVD in 25 tissue samples of CRC, which interpreted LGR5 could trigger tumor angiogenesis and neovascularization in CRC. Thus, it could lead to proliferation and metastases of the tumor.[31] This evidence showed that LGR5 has been related to certain clinicopathological features of CRC.

Table 2. LGR5 and clinicopathological features in CRC patients.

Author (Year)	(%) LGR5/Clinical Pathology in Patient Tumor Characteristics								
	Gender		Differentiation/Grade		TNM			Stage	
	High LGR5	Low LGR5	High LGR5	Low LGR5	High LGR5	Low LGR5	Vascular Invasion	High LGR5	Low LGR5
Jang, *et al*, (2018).[10]	Male: 327 (69%); Female: 209 (66%)	Male: 146 (31%); Female: 106 (34%)	Well: 27 (77%); Moderate: 496 (68%); Wild: 13 (52%)	Well: 8 (23%); Moderate: 232 (32%); Wild: 12 (48%)	N/A	N/A	High LGR5: 66 (65%); Low LGR5: 35 (35%)	I: 80 (68%); II: 178 (72%); III: 190 (66%); IV: 88 (65%)	I: 38 (32%); II: 69 (28%); III: 98 (34%); IV: 47 (35%)
Iheme-landu, *et al*, (2019). [11]	Male: 17 (48.6%); Female: 18 (51.4%)	Male: 8 (32.0%); Female: 17 (68.0%)	Well: 1 (3.6%); Moderate: 20 (71.4%); Wild: 7 (25%)	Well: 3 (14.3%); Moderate: 12 (57.1%); Wild: 6 (28.6%)	N/A	N/A	N/A	I: 1 (2.9%); II: 11 (31.4%); III: 8 (22.9%); IV: 15 (42.9%)	I: 4 (16%); II: 5 (20%); III: 3 (12%); IV: 13 (52%)
Shekar-riz, *et al*, (2019). [13]	Male: 17 (54.8%); Female: 14 (45.2%)	Male: 4 (44.5%); Female: 5 (55.5%)	Well: 28 (90.3%); Moderate: 2 (6.5%); Wild: 1 (3.2%)	Well: 8 (88.9%); Moderate: 0 (0%); Wild: 1 (11.1%)	T1, T2: 4 (12.9%); T3: 27 (87.1%); N0: 19 (61.3%); N1, N2: 8 (25.8%); M: 6 (19.35%)	T1, T2: 1 (11.1%); T3: 8 (88.9%); N0: 5 (55.5%); N1, N2: 3 (33.5%); M: 0 (0%)	High LGR5: 4 (12.9%); Low LGR5: 1 (11.1%)	I: 3 (9.7%); II: 14 (45.16%); III: 4 (12.9%); IV: 6 (19.35%)	I: 1 (11.11%); II: 4 (44.5%); III: 3 (33.3%); IV: 0 (0%)
Zheng, *et al*, (2018). [14]	Male: 97 (Positive); 23 (Negative) Female: 71 (Positive); 13 (Negative)		Well/Moderate: 139 (Positive LGR5); 32 (Negative LGR5) Wild: 29 (Positive LGR5); 4 (Negative LGR5)		T1: 0; T2: 23; T3: 130; T4:15; N0: 73; N1: 74; N2: 21; M0:157; M1:11		0: 119; 1: 49	I: 13; II: 57; III: 87; IV: 11	
Gzil, *et al*, (2020). [15]	Male: 52 (58%) Female: 37 (42%)		Well/Moderate: 82 (92%); Wild: 7 (8%)		T2: 16 (18%); T3: 58 (65%); T4: 15 (17%); N0: 43 (48%); N1:31 (35%); N2:15 (17%); M0: 45 (52%); M1: 43 (48%)		16 (18%)	N/A	
Harb, *et al*, (2016).[16]	Male: 19 (67.90%); Female: 9 (32.10%)	Male: 18 (56.30%); Female: 14 (43.80%)	Well: 0 (0%); Moderate: 16 (57.10%); Wild: 12 (42.90%)	Well: 10 (31.30%); Moderate: 22 (68.80%); Wild: 0 (0%)	N/A	N/A	N/A	I: 0 (0%); II: 6 (21.40%); III: 10 (35.70%); IV: 12 (42.90%)	

Abbreviations: LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; N/A: not applicable.

Table 3. LGR5 and prognosis value of CRC patients.

Author (Year)	LGR5 Marker (%)		Survival Rate (%)							
			1 year		3 years		5 years		OS	
	Positive	Negative	High LGR5	Low LGR5	High LGR5	Low LGR5	High LGR5	Low LGR5	High LGR5	Low LGR5
Jang, *et al*, (2018).[10]	68%	32%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Ihemelandu, *et al*, (2019).[11]	6384.09 (3881)	3790.16 (2967)	N/A	N/A	94%	72%	75%	53%	9.0 years	6.3 years
Shekarriz, *et al*, (2019).[13]	High LGR5: 31 (73.8%) Low LGR5: 9 (23.7%)	N/A	100%		81%		75%		N/A	N/A
Zheng, *et al*, (2018).[14]	93.60%	6.40%	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Gzil, *et al*, (2020).[15]	82 (92%)	7 (8%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Harb, *et al*, (2016).[16]	True positive: 22 (36.7%) False negative: 8 (13.3%)	True negative: 24 (40%); False positive: 6 (10%)	N/A	N/A	24,1%	100%	N/A	N/A	N/A	N/A

Abbreviations: LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; N/A: not applicable.

Besides, LGR5 also had been valued as a prognostic indicator of CRC therapy. The previous review mentioned that LGR5 was related to poor prognosis and clinical outcomes.[24] Based on Herb, *et al*, (2016) study of 60 cohort patients, it showed that there was a strong correlation between LGR5 immunoexpression of CRC with tumor location, recurrence, metastases, and poor differentiation.[16] Other studies also revealed LGR5 poor prognosis and short survival rate of CRC23.[31–34]

Melling, *et al*, (2016) reported that the increase of the survival rate was related to high expression of Ki-67 in CRC patients.[35] Ki-67 had been shown a positive correlation with LGR5 expression in the G1 to M transition phase in the cycle of cell proliferation.[36] Ki-67 is an independent prognostic factor that maintains cell survival via proportional regression of multivariable Cox.[35] But overexpression of LGR5 was related to poor prognosis and other risk factors in CRC cells.[36]

This study has several limitations, including a lack of eligible studies and existing studies that are limited to six countries. Among those studies, there were differences in participant numbers, broad staging of CRC, follow-up duration, and outcome data. Thus, further research is needed to validate the data homogeneity.

CONCLUSION

The expression of LGR5 during the development of CRC could determine the prognostic value in a CRC patient because it could trigger tumor angiogenesis and neovascularization in CRC. LGR5 has been related to certain clinicopathological features and tumor progressions, such as location, measure, lymph-vascular invasion, lymph node metastases, further tumor stadium, overall survival, tumor angiogenesis, poor prognosis, and other clinical outcomes. High LGR5 patients had longer survival than low LGR5 patients. Nevertheless, there was no significant correlation between LGR5 expression and age or gender in CRC patients. There should be a systematic review and further research on the correlations between LGR5 expressions and the clinicopathological features of CRC. Further research related to LGR5-targeted therapy is also suggested.

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