



Review Article

Oncogenic Role of Cancer Stem Cell *LGR5* in Colorectal Cancer Progression

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ABSTRACT

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Colorectal Cancer (CRC) is the second most common cancer in women and the third most common cancer in men. There is strong evidence for the role of Cancer Stem Cells (CSCs) in cancer progression. The identification and understanding CSCs genes such as *LGR5* involved in the induction of cancer development is crucial in the prognosis of CRC. *LGR5* is a membrane protein involved in several molecular signaling pathways, such as the Wnt signaling and NOTCH pathways. It has been shown that the *LGR5* gene was overexpressed in CRC and is associated with the worst outcomes in patients with CRC, but molecular mechanisms of *LGR5* in CRC development have been poorly identified. This review has summarized current studies about the role of stem cell marker of *LGR5* in CRC progression. Future research in this area may improve the early detection of CRC, new therapies, and monitoring of CRC.

Introduction

Cancer Stem Cells (CSCs) are a subpopulation of neoplastic cells with self-renewal potential responsible for cancer progression, metastasis, invasion, and pharmacological resistance. These cells express many cancer stem cell markers, which are used to identify cancer cells. Furthermore, many investigations proved the association of these markers with clinicopathological features in patients who had cancer [1, 2].

CRC is introduced as the third most common cancer in males and the second in females; an increasing body of evidence indicates that CRC incidence and mortality rates are rising in the world [3, 4]. Many specific gene alterations, such as Adenomatous Polyposis Coli (APC), *KRAS* (Kirsten rat sarcoma two viral oncogene homolog), and *P53* (Tumor protein p53), result in CRC initiation and progression. *APC* acts as a tumor suppressor gene, and its defect interrupts the Wnt signaling pathway; in the following gain-of-function (GOF), mutations of the *KRAS* and loss of *P53* can lead to normal mucosa into cancerous tissue [5-9]. Many cancer stem cell markers are involved in colorectal development [10]. One of the CSCs is Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), a membrane protein involved in many pathways, such as the Wnt signaling and NOTCH pathway [10, 11]. Generally, many studies have demonstrated that the *LGR5* gene has an oncogenic role in numerous cancers, such as neuroblastoma, thyroid cancer, glioma, and CRC. As well, *LGR5* can improve metastasis, tumor growth, epithelial-mesenchymal transition (EMT), and drug resistance [12-14]. LGR5 protein has been identified as an intestinal marker, i.e., a pro-oncogene, and a target of Wnt/ β -catenin signaling pathway. This signaling acts a critical action in cell proliferation, cancerous cell stability, and tumor progression in CRC [15-17]. Wnt receptors are induced by the binding of R-Spondin (RSPO) to LGR5 and joining Wnt ligands; furthermore, induced Wnt signaling can stabilize β -catenin and stimulate downstream targeted genes, such as *c-MYC*, *AXIN2*, *CyclinD1*, resulting in transformation of a normal tissue to CRC (Figure 1) [17-19]. It has been indicated that *LGR5* gene was meaningfully upregulated in CRC compared with normal mucosa, resulting in worst outcomes in patients with CRC (Figure 2) [19].

Figure 1 indicates that R-spondin stimulates LGR5. Then, LGR5 protein activates the LPR-frizzled receptor complex that joins WNT ligands and increases WNT signaling activation via phosphorylation of LRP5/6. In the following, β -catenin is translocated to the nucleus, binds to transcription factor LEF/TCF, and enhances the expression of WNT target genes, such as *c-MYC*, *LGR5*, and *CyclinD1*. Upregulation of these genes leads tumor to cell proliferation, EMT, stemness of CSCs, and tumor progression.

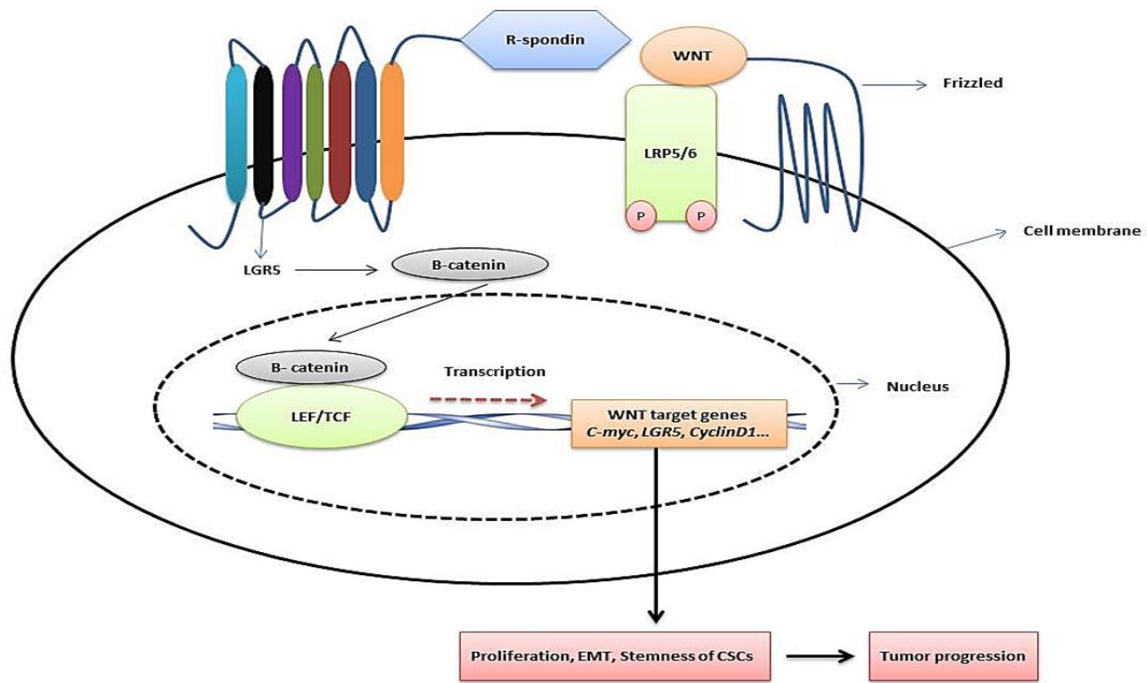


Figure 1. A schematic pattern of the *LGR5*-mediated activation of colorectal tumor growth through interaction with the WNT/ β -catenin signaling pathway.

Figure 2 illustrates that overexpression of the *LGR5* gene can affect colorectal carcinogenesis and lead normal mucosa to advanced cancer.

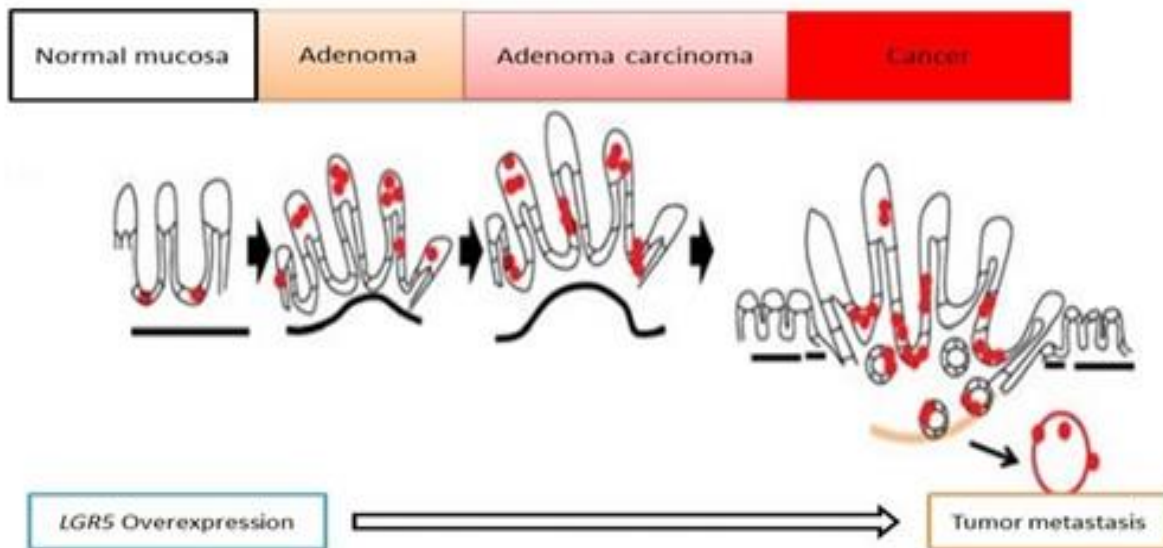


Figure 2. The effect of *LGR5* upregulation on colorectal carcinogenesis.

Although several investigations indicated the role of the *LGR5* gene in CRC (Table 1), molecular mechanisms of *LGR5* and CRC have been poorly understood. In this review, we discuss the oncogenic role of *LGR5* in CRC progression.

Table1

Summary of Several Investigations about the Role of LGR5 Gene and Protein in CRC

References	Samples	Method	Conclusion
Shin et al. (2021)	Mouse model	Real-time PCR	DDK2 knockdown increased <i>LGR5</i> expression and CRC [39].
Xu et al. (2020)	human CRC tissues	Western blot	Overexpression of <i>LGR5</i> protein was correlated with advanced stage, cell differentiation, depth of infiltration, poor overall survival. Metastasis, vascular invasion, liver metastasis, and LNM. <i>LGR5</i> was an independent risk factor [40].
Gzil et al. (2020)	human CRC tissues	Immunohistochemical staining	<i>LGR5</i> up-regulation increased lymph-node metastases, vascular invasion, and poor survival [50].
Ihemelanda et al. (2019)	Paraffin-embedded tissue blocks	Tissue microarray	Overexpression of <i>LGR5</i> was an independent biomarker for overall survival [41].
Jange et al. (2018)	Paraffin-embedded tissue blocks and cell line	Immunohistochemistry, tissue microarray, and Real-time PCR	Upregulation of <i>LGR5</i> was correlated with adenoma-carcinoma transition [32].
Jang et al. (2016)	Human colonic polyps	Reverse transcription-polymerase chain reaction	Overexpression of <i>LGR5</i> was related to a precancerous lesion of CRC and tubular adenoma [51].
Szkandera et al. (2015)	Paraffin-embedded tissue blocks	PCR	The <i>LGR5</i> rs17109924 T>C wild-type genotype was associated with <i>LGR5</i> upregulation and increased resistance to 5-FU-based chemotherapy [44].
Wang et al. (2015)	Primary CRC tissues from patients, mouse model, human adenomas cells	Immunohistochemistry	Up-regulation of <i>LGR5</i> and CRC progression [37]
Lin et al. (2015)	Human CRC, tissues, CRC cell line	Real-time RT-PCR	Reduction of proliferation, growth, migration, and invasion using knock-downing of <i>LGR5</i> [52]
He et al. (2014)	Primary tumors from CRC patients	Real-time RT-PCR	The expression level of <i>LGR5</i> estimated a poor prognosis [38].
Chen et al. (2014)	Meta-analysis	Meta-Analysis	The expression level of <i>LGR5</i> showed a poor prognosis [53].
Choi et al. (2014)	Mice model	Side-view confocal endomicroscopy	Upregulation of <i>LGR5</i> in tumors and adenomas was observed and considered as a prognostic marker for CRC [43].
Al-Kharusi et al. (2013)	Adenomas cell line	Western blot, quantitative real-time PCR	Decrease in cell proliferation, growth, migration, and invasion by knock-downing <i>LGR5</i> [54].
Hsu et al. (2013)	Paraffin-embedded tissue blocks	Immunohistochemistry	Up-regulation of <i>LGR5</i> expands cancerous cells proliferation and chemotherapy resistance [54].
Becker et al. (2008)	Paraffin-embedded tissue blocks	Immunostaining	Upregulation of <i>LGR5</i> in adenomas compared with normal group imaging [55].

Methods

In the current investigation, we obtained many articles published in English using electronic databases, including PubMed and Google Scholar, Scopus, and Web of Sciences. The following keywords were used in the search strategy: colorectal cancer, cancer stem cell, *LGR5*, and adenoma-carcinoma sequence.

Structure of LGR5

LGR5 is a member of the G protein-coupled receptors (GPCRs) family with a large extracellular domain counting multiple leucine-rich repeats (LRRs). R-spondin proteins are the biological ligands of *LGR5*. The *LGR5* is a member of GPCR class A receptor proteins that are located on chromosome 12q22 with ~ 144kb long [20]. *LGR* family proteins have

been divided into three categories, including A, B, and C. LGR5 belongs to the B receptor group and has 13-18 LRR-containing domains and a linker area between the last LLR domain and the first transmembrane [21].

Furthermore, LGR5 is a member of the Wnt signaling pathway; co-stimulation of LGR5 ligand along with R-spondin 1 and Wnt-3a increased induction of *LGR5* gene expression and initiation of related signaling pathways. LGR5 also cooperates with LRP6 and FZD5 via a clathrin-dependent pathway to form a ternary complex upon Wnt ligand binding. Although the *LGR5* gene can promote different types of cancers via the regulation of Wnt and NOTCH signaling pathways, it can be specifically expressed in the small intestinal crypt and introduced as a potential marker for CRC [21, 22].

The Role of *LGR5* in Cancer Progression

Identifying cancer biomarkers can provide relevant and effective ideas for developing new anti-cancer treatment strategies [12]. An abnormal increase in *LGR5* may be one of the most common changes at the molecular level in some human cancers, leading to long-term enhancement of Wnt / β -catenin signaling [23]. *LGR5*-mediated suppression of Wnt / β -catenin signaling has also been reported in certain cancers such as B cell malignancies. To date, a therapy targeting the LGR5 signaling pathway in clinical approaches is not available. However, evidence suggests that the endogenous *LGR5*⁺ cell population plays key roles in tumor onset, progression, and metastasis [24]. Thus, targeted therapeutic modulation of the *LGR5*⁺ cancer cell population by targeting Wnt/ β -catenin signals through the targeted drug delivery system or genome modification may be promising for new anticancer therapies [25]. In addition, a combination of therapeutic drugs targeting both *LGR5*⁺ and *LGR5*⁻ cancer cells may receive more attention due to the plasticity of the cancer cells [13, 26]. Also, targeting more cancer cells using dual biomarkers may be much safer and more effective for anti-cancer treatment [27]. The role of this biomarker in the treatment of cancers in other tissues of the body is also being investigated, where it is found to be more effective in identifying gastrointestinal cancers. Available evidence suggests that high-grade serous ovarian carcinoma originates most often from stem cells in the fallopian tube epithelium, and the Wnt signaling pathway augmented by *LGR5* supports tumor development and progression [28]. Additionally, T regulatory cells in the immune system modulation can increase the *LGR5* expression in gastric cancer cells through the TGF- β 1 gene and signaling pathway. This pathway may include activation of the Wnt signaling, and the expression of this biomarker via TGF- β gives a prognosis in gastric cancer [29]. Another study has reported that suppression of this marker is implicated in the treatment or prevention of breast cancer [30]. Also, *LGR5* may serve as a future potential biomarker for patient risk stratification and metastasis in papillary thyroid cancer [31]. Therefore, identifying these biomarkers in the treatment of various cancers can greatly benefit.

The Effect of *LGR5* in CRC

Overexpression of the *LGR5* gene in CRC patients was related to the canonical Wnt signaling activation and poor outcomes of patients [10, 32]. Researchers indicated that β -catenin is induced by Wnt signaling activation, resulting in *LGR5* upregulation and carcinogenesis. So, they suggested that *LGR5* gene upregulation is an important factor in CRC progression [33,

34]. LVI was found to be associated with malignant features, including inflammation, epithelial-mesenchymal (EMT), angiogenesis, and advanced stages [35]. Gzil et al. demonstrated that *LGR5* gene expression was significantly linked to CRC patients with positive LVI and invasion; in addition, *LGR5* gene expression could be able to activate some downstream signaling Wnt/ β -catenin pathways, such as c-MYC, CDKN1A, and NOTCH [36].

Wang et al. reported that *LGR5* overexpression was correlated with tumor size, malignant features, and Hepatocellular carcinoma progression [37]. Also, previous studies illustrated that the expression level of the *LGR5* gene was a novel prognostic biomarker for CRC [10, 25, 38].

Recently, Shin et al. have shown that knock out of *DDK2N* reduced the expression level of the *LGR5* gene and increased CRC progression in a mouse model. They suggested that the expression levels of *LGR5* could be controlled with targeted therapy [39].

Results obtained from Xu et al. showed that upregulation of LGR5 protein in CRC tissue was linked to advanced stages, cellular differentiation, depth of infiltration, lymph nodes metastasis (LNM), vascular invasion, liver metastasis, and distant metastasis. In contrast, a significant correlation of decreased expression of LGR5 protein with better overall survival in patients with CRC was observed. Furthermore, they mentioned that LGR5 may be an independent risk factor for CRC prognosis [40].

In another study, Ihemelanda et al. revealed the prognostic role of *LGR5* as a potential biomarker for the detection of CRC [41]. Jang et al. identified a significant association of the *LGR5* gene and protein with clinicopathological features, including older age, well-differentiated tumors, and nuclear β -catenin expression. Furthermore, the overexpression level of *LGR5* was found during the adenoma-carcinoma sequence [42].

Choi et al. reported LGR5 upregulation in colonic tumors and its association with malignancy risk of adenomas using side-view confocal endomicroscopy in mice model. Besides, they suggested LGR5 protein as a potential biomarker for the prognosis of colon cancer [43].

Szkandera et al. found that the *LGR5* rs17109924 T>C wild-type genotype was correlated with *LGR5* upregulation and enhanced resistance to 5-FU-based chemotherapy. Furthermore, they suggested the *LGR5* rs17109924 T>C can be a prognostic marker for time recurrence in patients with colon cancer treated with 5-FU-based chemotherapy [44].

LGR5 Targeted Therapy for CRC Treatment

Although the structural feature of LGR5 is known, there are no approved LGR5 inhibitors. Junttila et al. utilized anti-LGR5-vc-MMAE antibody-drug conjugate (ADC) for targeted therapy of CRC, where LGR5-MMAE was capable of decreasing tumor size and growth in a mouse model [45]. Also, researchers used the LGR5-iCaspase9 knock-in organoids with cetuximab (anti-EGFR antibody) and oxaliplatin (platinum-based chemotherapeutic), where the potential of combined chemotherapy potentiates *LGR5*⁺ CSCs targeting for CRC cells [46]. The functional role of miR-100 as the *LGR5* gene inhibitor was demonstrated by Zhou et al., where miR-100 was found to reduce *LGR5* expression in colon cancer via joining 3'-untranslated regions [47]. Some new methods, such as CRISPR/Cas9 could be utilized for *LGR5* therapy investigations [48, 49].

Conclusions

In summary, this review indicated that *LGR5* gene upregulation is an independent prognostic biomarker for aggressive clinicopathological features in CRC. The high expression level of *LGR5* can lead normal tissue to adenoma-carcinoma sequence; in addition, its expression was associated with chemoresistance. *LGR5* interacts with many signaling pathways like Wnt/ β -catenin. An increasing body of evidence suggests an important role of *LGR5* in CRC carcinogenesis. Moreover, finding the exact mechanisms of *LGR5* and its effect on CRC progression needs more investigations. Therefore, the utilization of new methods for *LGR5* detection and targeted therapy might become novel research approaches for CRC treatment.

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