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The Review of Contribution of LGR5 towards Cancer Stem Cells and Its Signal Pathway

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Abstract: *In recent years, the research on stem cells has broadened people's knowledge of the occurrence and development of tumors. Nearly all tumors are related to stem cells，and stem cell markers have become the focus of current research. LGR5 is a type of leucine-rich repeat receptor (LGR), and studies confirm that LGR5 is expressed at various levels in the crypts of the floor of the small intestine, the crypts of the large intestine, the basal column cells of the gastric glands, and hair follicles. Therefore, LGR5 is one of the potential markers for cancer stem cells. LGR5 is a Wnt signaling ligand that plays an active role in ontogeny. LGR5's binding partner, R-spondin (RSPO), can control Wnt signaling along with LGR5. This article analyzes the signaling pathways of LGR5 in tumor stem cells, summarizes the role of LGR5 there, and points out future research directions.*

Keywords: *Cancer stem cells, LGR5, Wnt pathway, RSPOs*

1. Introduction

The discovery of cancer stem cells (CSCs) suggests that not every cancer cell has the potential to cause cancer *in vivo.* The main reason for tumors being stimulated is that they have a subset of tumor cells that resemble stem cells [1]. Cancer stem cells require two types of regulatory factors: ① act as pluripotent, differentiated, and self-renewal regulators; ②Migration, invasion, implantation, and communication regulators [2].In previous CSC studies, only a few cancer cells could proliferate indefinitely, and those cells are classified as CSCs. Colorectal malignant tumors, brain tumors and breast cancer have been proved to contain CSCs [3-6].

LGR5 is widely distributed in human tissues, such as the intestine, colon, and liver [5-7]. It belongs to the superfamily of G-protein-coupled, 7-transmembrane receptors (GPCR). During late embryonic development, this protein helps to create and maintain adult intestinal stem cells by encoding receptors for R-spondins, which function in the normal Wnt signaling pathway [8]. Notably, more and more data show that cancer cells labeled by LGR5 have tumor-initiating ability [9]. Previous studies have shown that $LGR5^+$ cells have better tumor-originating ability than $LGR5^-$ cells [10]. In addition, Yang L et al. said it was shown that LGR5 is highly expressed in stem cells and maintains breast CSC [9].

Carmon et al. demonstrated that the family protein R-spondins (RSPOs) is currently discovered to be a physiological ligand of orphan receptors LGR4 and LGR5, which can enhance the Wnt /β signaling pathway, and it is also essential in embryonic development as well as the self-renewal and maintenance of adult stem cells [11]. The Wnt/β-catenin pathway has the ability to induce LGR5 [9]. Furthermore，LGR5 cells are located at the bottom of the colon mucosa and have the ability to self-renew and differentiate into various types of mucosal cells. Their products constitute R-spondins of Wnt receptors, suggesting that LGR5 is an important part of the positive feedback loop [12]. Almost all tissue and organ systems depend on signaling cascades initiated by the Wnt/β-catenin pathway to grow normally throughout their lives. Given the importance of Wnt signaling pathways in stem cell biology, it is no wonder that stem cell tumorigenicity is associated with abnormal Wnt signaling [13]. In all these tissues, cell proliferation and tissue homeostasis are regulated by the Wnt signaling pathway. As a stem cell marker, LGR5 has a wide range of uses in tissues where regulation of the Wnt signaling plays an important role [14,15]. Based on this, we investigated the signaling pathways of LGR5 in tumor stem cells, summarized the beneficial role of LGR5 in tumor stem cells, reviewed the discovery and development of LGR5, and laid a solid foundation for the subsequent development.

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2. The primary function of LGR5

2.1 The classification and structure of LGR5

The most prevalent and varied class of membrane protein receptors are G protein coupled receptors (GPCRs) [16]. GPCRs are important signal sensors for physiological processes such as immunological response, hormone and enzyme release from neurotransmitter glands, cardiac, endocrine, and exocrine glands, smooth muscle contraction, and blood pressure regulation [17]. Nonsensory GPCR (excluding GPCR of light, smell, or taste receptors) is based on known drug characteristics and is classified as follows: Class A receptor resemble rhodopsin, Class B receptors resemble secretory glands, class C receptors resemble metabolic glutamate receptors/pheromones, and Class IV receptors are frizzled/smoothened. The largest category is A, which is divided into four groups: a, b, g, and d [18]. In Group d, there are purine and olfactory receptors, MAS- related and leucine-rich repeat receptors (LGR) [19].

No distinct structure of a similar protein has been discovered in the current investigation. However, earlier research has shown the C2 crystal structure of the LGR5 and R-spondin 1 outer domain complex (fu1fu2). With a resolution of 3.2 A, Weng et al. discovered dimers in four crystal structures, which are primarily organized in a 2:2 complex. 17 leucine-rich repeat sequence (LRR) units make up the horseshoe-shaped outer domain of LGR5. The extracellular loop of the 7TM area and the lengthy loop with a helical shape in C-Cap may combine to generate a stable structure, however the majority of it is disordered [20].

2.2 Functions and mechanism of action of LGR5

LGR5 usually regulates standard Wnt signaling intensity through binding to its ligand [11]. LGR5 activates the Wnt/β-catenin signaling pathway and promotes self-renewal and proliferation of cancer stem cells [9,21]. On the other hand, LGR5 may negatively regulate Wnt / β-catenin signaling [22]. In particular, many studies using gene phylogenetic analysis or antibody detection of LGR5 have identified LGR5 as a cancer stem cell marker for various human cancers and are closely associated with tumorigenesis. Specific tumors include adenocarcinoma, glioblastoma, colorectal cancer, and breast cancer [23,24].

The negative effect of LGR5 on Wnt labeling is mainly through the LGR5 overexpression or LGR5 reduction connectivity through RNAi, which has been further confirmed in colorectal disease cell lines. Walker et al. concluded that overexpression of LGR5 in colon cancer cell lines might inhibit the response to Wnt markers, expand intercellular adhesion, further reduce clonality and tumorigenicity. Conversely, LGR5 migration leads to the renewal of Wnt signal characteristics, such as prolonged invasion time, gateless development and increased tumorigenicity [22].

2.3 How LGR5 works as an Adult Stem Cell Marker

Baker et al. first identified LGR5, which works as an adult stem cell marker in the intestine [7]. In addition, LGR5 can also be used as a marker for adult stem cells in the stomach, breast, and hair follicles [25]. These results come from a series of pedigree tracking experiments. By using LGR5+cells, a previous study has demonstrated that LGR5+cells have stem cell activity [28]. For example, through tracking experiments, it was found that small intestinal stem cells proliferate through division. Studies have shown that the self-renewal epithelium of the small intestine of rats consists of crypts and small intestinal villi. Intestinal crypts are monoclonal, and each crypt originates from its own intestinal stem cells. Stem cells regularly produce transient proliferating cells through asymmetric division, so as to differentiate into various types of small intestinal epithelial cells. In the past decade, many small intestinal stem cell markers have been proposed, and LGR5 is the latest small intestinal stem cell marker. Morphologically, LGR5+crypt basal columnar cells (CBC) have sparse cytoplasm and wedge-shaped nuclei facing the crypt cavity, which are easily distinguished from adjacent pan cells. The proliferation marker Ki-67 was almost positive in CBC, and the M-phase marker phosphor histone H3 was occasionally expressed, indicating that these cells showed typical periodic changes. Therefore, Barker et al. believe that LGR5+CBC has the characteristics of maintaining epithelial self-renewal for a long time, can differentiate into all cell types in the intestinal epithelium, and has apoptosis inhibition and high proliferative activity [29].

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2.4 LGR5 overexpression level and unique role

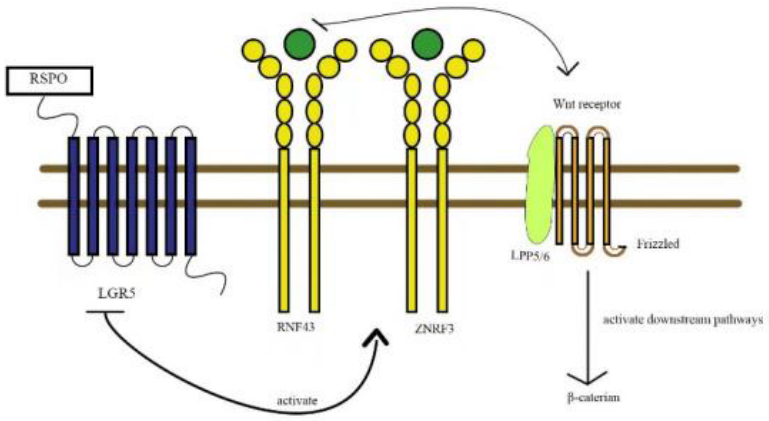
IHC staining was used to detect LGR5 protein levels in tumor tissue and adjacent normal tissue in 66 patients with hepatocellular carcinoma (HCC). The results showed that the expression level of LGR5 in HCC tissue was significantly higher than that in other normal stem cells compared to other tissues. Patient survival decreased with increased LGR5 expression in tumor tissue. Therefore, it is more noteworthy that LGR5 expression in tumors is positively correlated with different stages, suggesting that LGR5 is not only a tumor biology, but also an excellent predictor of HCC disease progression [30]. Through the experimental results and these indirect experimental results, it can be found that LGR5 overexpression can promote the viability of HCC cells, enhance clone formation, lead to cell proliferation, and enhance chemotherapy resistance, which is consistent with previous research results [31]. In addition, LGR5 has also been found in many cancers, including breast cancer, basal cell carcinoma, and liver cancer [9,16,32].

3. LGR5 signal pathway

3.1 Binding partner

In mammals, R-spondins (RSPOs) are a family of four proteins, which contain specific domains of neural tube spongiform proteins (RSPO1, RSPO2, RSPO3 and RSPO4). The main feature of RSPO protein is that it plays a regulatory role in the classical Wnt signaling pathway. In Xenopus laevis, RSPO2 was found to be a positive Wnt signal stimulator encoding this gene. Subsequent studies showed that RSPOs could increase the typical Wnt signal activity caused by Wnt protein, confirming the relationship between RSPOs and Wnt signal [33]. Through a candidate adhesive method, Carmon et al. found that R-spondins protein is a high-affinity adhesive of LGR5 through a series of functional bonds and analysis [11]. In total crypt culture, the addition of RSPO1 promoted the proliferation of LGR5+ cells. In vivo overexpression of RSPO1 leads to extensive specific proliferation of LGR5+ crypt stem cells [34]. Importantly, it was found that RSPO1 is expressed in Paneth cells in the nest, surrounding and forming the niche of LGR5 + stem cells [35].

3.2 The relationship between LGR5, RSPO, and Wnt signaling



LGR5 / RSPO complex can activate two transmembrane E3 ligases RNF43 and ZNRF3 to enhance Wnt signaling. RNF43 and ZNRF3 can activate Wnt receptors (Frizzled and LRP5/6) to enhance Wnt signaling and trigger downstream β-catenin activation.

Figure 1: One of the upstream activation of Wnt signaling pathway

The Wnt signaling system is essential in tissue regeneration, disease, and embryonic development, including cancer. Some adult stem cell niches, including the intestinal gland, skin hair follicles, hematopoietic tissue, and mammary glands, require standard Wnt / β-catenin signaling to regulate stem cells, proliferation, and differentiation. On the other hand, standard Wnt signaling is involved in the development and progression of various human malignancies [15].

Although LGR5 is considered an "orphan" receptor, it currently has an established ligand for R-spondin (RSPO) and works with Wnt receptors (Frizzled and LRP5 / 6) to provide Wnt / β-catenin.

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Enhances signaling. The LGR5 / RSPO complex enhances Wnt signaling by neutralizing two transmembrane E3 ligases, RNF43 and ZNRF3 [14]. Inhibition of Wnt signaling by LGR5 was eliminated in the presence of RSPO and increased cellular response to Wnt. In stem cell biology, understanding the critical molecular pathways involved in RSPO: LGR5 regulation of Wnt signaling is an significant goal (Figure 1). It is also important to investigate whether the RSPO-LGR5 complex functions independently of the Wnt-FZD complex and activates intracellular signaling pathways [19]. But right now, no studies have shown this.

4. Discussion

LGR5 enhances Wnt/β-catenin signaling through R-spondins(RSPOs), Rnf43, and Znrf3. In many vertebrate tissues, LGR5 marks are homeostasis and facultative stem cells. LGR5+ stem cells rely on RSPO ligands from the stem cell niche. LGR5+ cells have a role in tumor genesis, development, and metastasis, but not tumor survival, according to recent breakthroughs in lineage tracking and control in human and animal tissues. Additionally, medicines that target anti-LGR5 antibodies can be used to target LGR5+ CSCs [36]. LGR5 is a surface-expressed biomarker that might be used to target anti-tumor treatment. LGR5 also has a varied and intricate involvement in tumor growth. Diverse forms of cancer might well have signalling pathways connected to LGR5 that play different or even opposing functions. consequently, therapeutic modulation of LGR5-related signaling pathways might open up new avenues for anticancer treatment. Single-targeted LGR5+CSCs have shown specific anticancer effects in solid cancer using the antibody binding drug delivery system. However, while selectively targeting LGR5+CSCs by gene ablation can be temporarily effective in treating tumors, untargeted cancer cells will eventually overcome their antitumor effects. Therefore, combination therapy for LGR5+ and LGR5 cancer populations may be worth further investigation. Comprehensive studies on the prevalence of cancer cells and the transfer of LGR5 and LGR5+ between cancer cells are needed [15].

Currently, the research on LGR5 has shifted from the basic understanding of the structure and related signaling pathway to the study of LGR5 in various human cancers, especially colorectal cancer, breast cancer, and gastric cancer. However, how to identify and treat LGR5 as a potential marker is still a key and urgent point. Future studies should focus on how to use LGR5 for tumor prevention and how to achieve tumor treatment by regulating Wnt signaling pathway, among which, it is also crucial to give people better insights into the deeper functions of LGR5 in CSCs.

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