LGR4

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Contributor: KARLA VAZQUEZ-SANTILLAN , Alejandro Ordaz-Ramos , Victor Hugo Rosales-Gallegos , Jorge Melendez-Zajgla , Vilma Maldonado

Leucine-rich repeats containing G protein-coupled receptor 4 (LGR4) is a receptor that belongs to the superfamily of G protein-coupled receptors that can be activated by R-spondins (RSPOs), Norrin, circLGR4, and the ligand of the receptor activator of nuclear factor kappa-B (RANKL) ligands to regulate signaling pathways in normal and pathological processes.

LGR4 GPR48 cancer

1. Introduction

Cancer is one of the major burdens of disease worldwide. Cancer development involves genetic and epigenetic alterations that allow cells to escape from the mechanisms that control proliferation and survival. Many of these alterations correspond to signaling pathways that regulate multiple cellular processes such as cell growth, cell death, fate, and motility [1]. G protein-coupled receptors (GPCRs) are one of the largest superfamilies of cell-surface receptors involved in membrane-initiated signaling processes. GPCRs share various structural characteristics including an extracellular N-terminal domain, seven transmembrane domains connected with extra- and intra- cellular loops, and an intracellular C-terminal domain. Many types of GPCRs have been described in humans, which have key roles in a variety of physiological and pathological processes [2]. In cancer, GPCRs participate in a plethora of processes such as proliferation, migration, apoptosis, and tumorigenesis [3].

Leucine-rich repeats containing G protein-coupled receptors (LGRs) are a group of transmembrane receptors that belong to the GPCRs superfamily, characterized by a large extracellular domain that recognizes ligands and regulates numerous cellular processes. LGRs are classified into three groups according to their function and structure. Group A receptors include LGR1, which recognizes follicle-stimulating hormone (FSH), LGR2, which recognizes luteinizing hormone (LH), and LGR3, which recognizes thyroid-stimulating hormone (TSH). Group B includes LGR4, LGR5, and LGR6 receptors, which play crucial roles in developmental processes and are involved in several types of cancer. Finally, Group C includes LGR7 (RXFP1 receptor) and LGR8 (RXFP2 receptor) recognizing relaxin and insulin-like peptide 3 (INSL3) [4][5].

LGR4 has a large extracellular Leucine-rich domain that is capable of interacting with its ligands. LGR4 is commonly activated by RSPOs, Norrin, Receptor activator of NF-kappa B ligand (RANKL), and circLGR4 ligands, and its activation results in the signaling of the Wnt/β-catenin and G protein-associated pathways [6][7][8][9][10]

[11]. Accumulating evidence indicates that LGR4 expression is upregulated in cancer tissues and participates in the regulation of various tumorigenic processes.

2. LGR4 Characterization

LGR4 is a transmembrane receptor member of the GPCRs superfamily and belongs to group B of the LGR family [4]. LGR4 was first characterized in 1998 as homologous to the well-known members of the LH/FSH/TSH family of receptors (Group A) [12]. LGR4 is encoded by a highly conserved 106,827 pb gene, located on human chromosome 11 (11p14.1). The genomic organization of the LGR4 gene involves 18 exons that are subjected to alternative splicing resulting in two isoforms, one of them encoding a protein of 951aa [12][13][14].

Similar to all LGRs, LGR4 has a large N-terminal extracellular domain that enables the binding of specific ligands. This extracellular domain is constituted by 17 leucine-rich repeats (LRR) flanked by N-/C- cysteine-rich regions. A common seven-transmembrane helix domain characteristic of all GPCRs is found in LGR4, having three extracellular and three intracellular loops, and a C-terminal intracellular domain [12][14] (Figure 1).

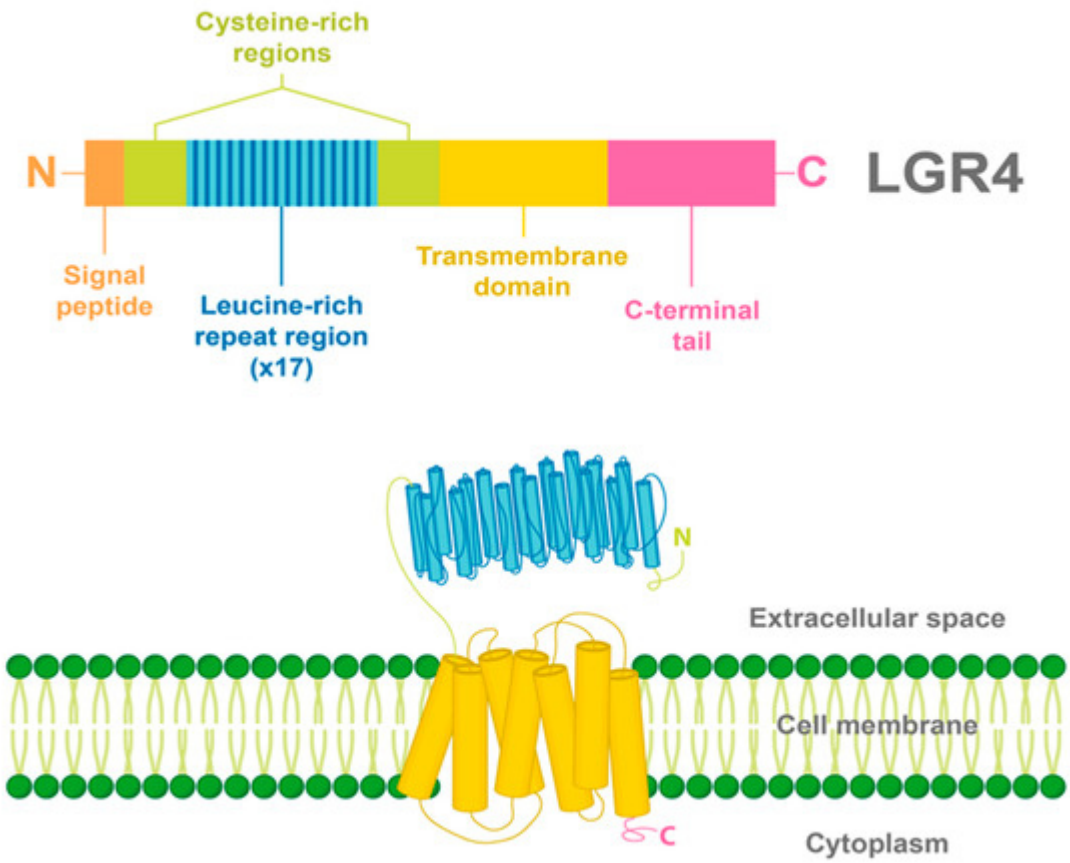

Figure 1. Structure and domains of LGR4. LGR4 is a transmembrane receptor with a long N-terminal extracellular domain constituted by 17 leucine-rich repeats, flanked by cysteine-rich regions. In addition, a seven-transmembrane helix domain and a C-terminal intracellular domain are found in LGR4. The signal peptide of LGR4 is found in the N-terminal region.

LGR4 is widely expressed in several tissues including the mammary gland, bone, prostate, skin, pancreas, ovary, heart, kidney, testis, brain, thymus, among others [12][14][15]. The interaction of this receptor with its ligands modulates signaling pathways associated with physiological and developmental processes.

3. Ligands and Canonical Signaling Pathways Regulated by LGR4

3.1. R-Spondins (RSPOs)

LGR4 was considered an orphan receptor until 2011, when Carmon et al., Glynca et al., and Ruffner et al. performed co-immunoprecipitation, co-immunofluorescence, and binding assays to show that RSPOs bind LGR4 and modulate the Wnt signaling pathway [6][7][11].

R-spondins (roof plate-specific spondin) are a family of four secreted proteins (RSPOs 1-4) that were characterized for the first time in mice. These proteins share about 40–60% of amino acid identity between them and also have homologous structures [16]. Structurally, RSPOs have (1) a signal peptide at the N-terminus for secretion; (2) two furin-like cysteine-rich domains that are necessary for ligand activity; (3) a thrombospondin 1 repeat domain (TSR); and (4) a basic amino acid-rich domain with different lengths at the C-terminus [16][17][18] (Figure 2a). RSPOs can activate three of the eight members of the LGR's family (LGR4/5/6) and can induce a potent and sustained activation of the Wnt pathway, a well-known signaling cascade involved in several physiological and pathological processes including cancer [6][7][11][19].

The Wnt signaling pathway is mainly activated by the frizzled/LRP5-6 receptor complex. LGR4, through its extracellular domain, can interact with any of the four RSPOs and enhance the Wnt signaling pathway. Stimulation of LGR4 by RSPOs stabilizes the Frizzled/Lrp5-6 complex in the membrane, avoiding its degradation by inhibiting the activity of ZNRF3 and RNF43 proteins [20][21][22][23] . Furthermore, LGR4 recruits IQGAP1, a scaffold protein that induces the recruitment of the β-catenin destruction complex to the Fzd/LRP5-6 receptor complex [24][25]. The interaction of the Frizzled/LRP5-6 receptor complex with the Wnt ligands results in the cytoplasmic accumulation of β-catenin and its later nuclear translocation. In the nucleus, β-catenin, in a complex with the TCF transcription factor, acts as a transcription regulator of its target genes (Figure 2b) [26][27]. Interestingly, RSPOs can also enhance Wnt signaling independently of LGR receptors [16][17].

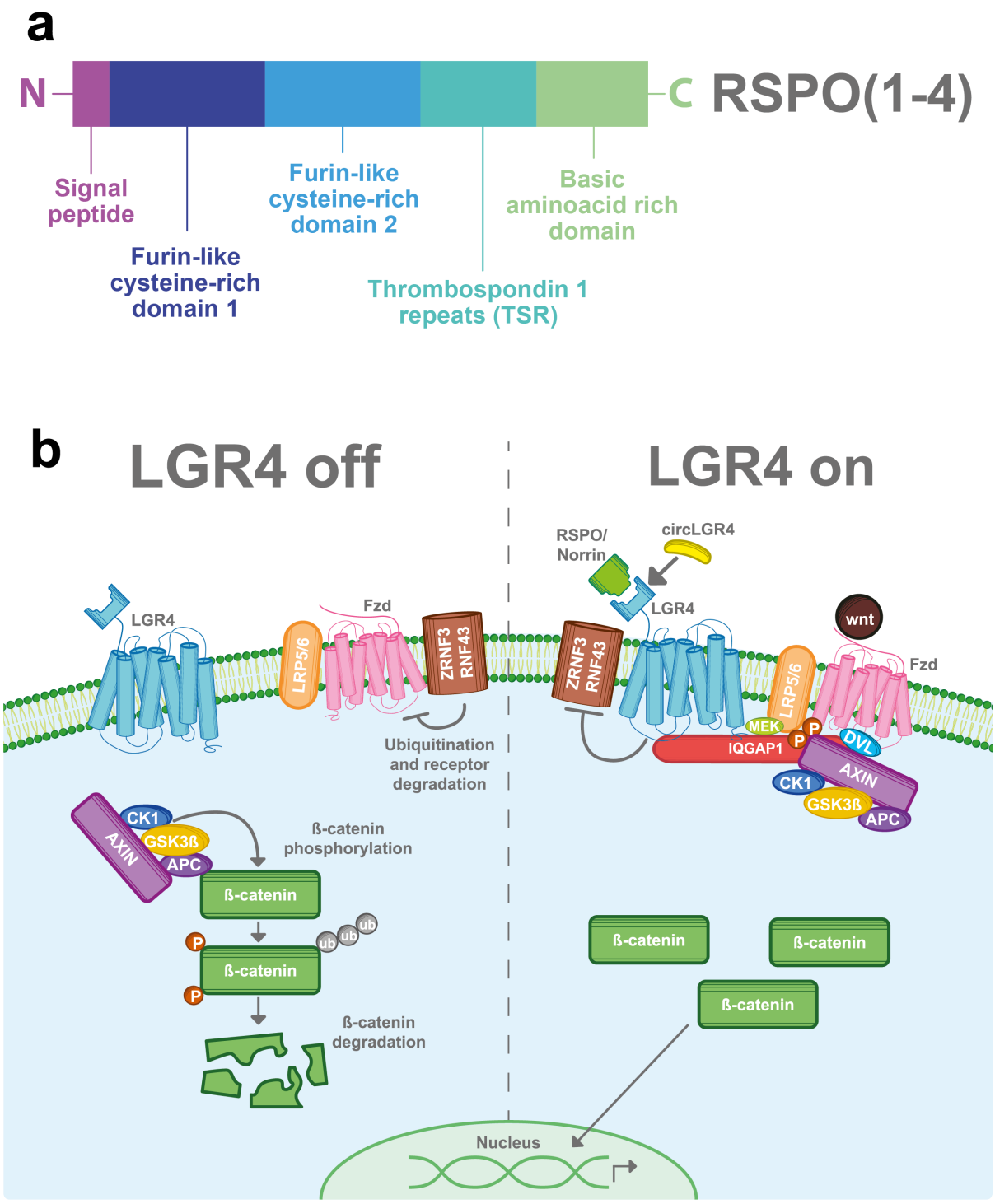

Figure 2. LGR4-induced Wnt/β-catenin signaling pathway. **(a)** RSPOs are a family of secreted proteins that can activate LGR4-induced Wnt/β-catenin signaling. Structurally, all the RSPOs have a signal peptide in the N-terminal domain, two furin-like cysteine-rich domains, a thrombospondin 1 repeat domain (TSR), and a basic amino acid-rich domain, which varies in size according to the RSPO member **(b)** In the absence of RSPO, ZNF3/RNF43 ubiquitinates the frizzled (Fzd)/LRP5-6 receptor complex for degradation. Wnt signal is blocked and the β-catenin destruction complex (formed by CK1, GSK3β, APC, and AXIN) is activated. GSK3β and CK1 phosphorylate β-catenin, inducing its ubiquitination and consequent proteasomal degradation. When LGR4 is activated by RSPOs, Norrin, or circLGR4 ligands, it stabilizes the frizzled/Lrp5-6 complex in the membrane, avoiding its degradation by

inhibiting the activity of ZNRF3 and RNF43 proteins. Furthermore, LGR4 recruits IQGAP1 with an increasing affinity for DVL and recruits MEK, which phosphorylates LRP5/6, leading to the recruitment and inhibition of the β-catenin destruction complex into the Fzd/Lrp5-6 complex receptor.

3.2. Norrin

In 2013, Deng et al. found a second ligand for LGR4 [8]. Norrin is a secreted protein that can promote Wnt signaling and regulate physiological processes. Structurally, Norrin has an N-terminal signal peptide and a cysteine-rich C-terminal domain that allows its homodimerization. Similar to RSPOs, Norrin can also interact with LGR4, stabilize the frizzled/LRP5-6 receptor complex and thus enhance the Wnt/β-catenin signal (Figure 2b). In addition, Norrin can also interact with frizzled receptors and intensify the Wnt pathway in an LGR4-independent way [8][28].

3.3. RANKL

The ligand of the receptor activator of nuclear factor kappa-B (RANKL) is a homotrimeric type II membrane protein with no signal peptide, which belongs to the tumor necrosis factor (TNF) family of cytokines [29]. Three alternative spliced isoforms of RANKL have been detected; the full-length RANKL (RANKL1), a shorter form that lacks part of the cytoplasmic domain (RANKL2), and a soluble form with the N-terminal part deleted (RANKL3)[30]. RANKL was first characterized as a ligand of the RANK receptor, a well-known activator of the NF-κB signaling pathway, and both RANK and RANKL have been associated with bone remodeling by regulating osteoclast differentiation [29].

Since LGR4 has a typical GPCR structure, efforts have been made to search its G-protein-dependent function. In 2016, Luo et al. showed for the first time that RANKL binds to the extracellular domain of LGR4 and activates a Gαq protein that activates the GSK3β signaling pathway, thus suppressing the expression of the NFATC1, a key transcription factor for osteoclastogenesis [9].

It has been shown, in an osteoclast model, that LGR4 competes with RANK to bind RANKL and suppresses canonical RANK signaling, thus exerting an opposing effect on the RANK pathway. Interestingly, LGR4 is a downstream target of RANKL–RANK signaling, suggesting that LGR4 acts as a feedback loop controlling RANKL activities [9]. Furthermore, it has been suggested that RANKL can compete with RSPOs to bind LGR4 and, in this way, the interaction of RANKL/LGR4 can disrupt the Wnt/β-catenin signaling potentiated by RSPOs [9].

3.3. CircLGR4

CircLGR4 is a circular RNA (circ-LGR4) that encodes a 19 amino acid peptide, which is secreted through the Golgi pathway. Recently, in a colorectal cancer model Zhi, et al. showed that the circLGR4 peptide interacts with the extracellular domain of LGR4 and enhances Wnt/ β-catenin signaling (Figure 2b). Disruption of circLgr4 expression resulted in impaired colon cancer stem cell self-renewal, tumorigenesis, and invasion [10].

4. LGR4 in Cancer

Cancer is one of the leading causes of death worldwide. Cancer development involves genetic and epigenetic alterations that allow cells to escape from the mechanisms that control proliferation and survival. Many of these alterations correspond to signaling pathways that control multiple processes such as cell growth, cell death, cell fate, and motility [1]. Accumulating evidence indicates that LGR4 is upregulated in cancer tissues and is associated with the initiation, progression, and metastasis of a variety of cancers.

4.1. Breast Cancer

Breast cancer is one of the most common malignancies worldwide. This disease is the most commonly diagnosed neoplasm and is the leading cause of cancer-related deaths among females around the world [31]. LGR4 is over-expressed in breast cancer and it has been associated with poor prognosis. Patients carrying breast tumors with high expression levels of LGR4 have poor overall survival, decreased post-progression survival, reduced distant metastasis-free survival, and decreased relapse-free survival [32]. Loss of LGR4 results in decreased tumorigenic capacity, reduced cell proliferation, decreased migration, and impaired invasion and metastasis of breast cancer tumors [32][33]. Interestingly, Yuo et al. showed that LGR4 downregulation decreases the self-renewal potential of breast cancer stem cells, by regulating SOX2 expression and disrupting the EMT process through the modulation of the Wnt/β-catenin signaling cascade. In addition, LGR4 can modulate the FAK-SRC pathway and it regulates the actin dynamics and cell adhesion of breast cancer cells, thus promoting cell migration [32].

4.2. Colorectal Cancer

Colorectal cancer is the fourth most common cancer and the third leading cause of cancer-related deaths among both sexes [31]. Accumulating evidence indicates that LGR4 is highly expressed in colorectal tumors, especially in advanced tumors, metastasis, and metastatic lymph nodes [10][34][35]. Remarkably, LGR4 levels correlate with tumor stage and lymph node status, and high expression levels of this molecule are a poor prognosis factor for 5-year overall survival [35]. Moreover, the overexpression of LGR4 has been associated with higher invasion and lung metastatic capacity [34].

Gao et al. showed that LGR4 expression levels are inversely correlated with the levels of p27, a protein that acts as a negative regulator of the E2F transcription factor. They showed that the transcriptional activity of LGR4 is mediated by E2F in colorectal cancer [34]. In addition, LGR4 can mediate the signaling of β-catenin/TCF via regulation of GSK-3β phosphorylation through the MAPK/ERK1/2 and PI3K/Akt pathways in colorectal cancer [35]. A recent study showed that the circLGR4-derived peptide activates LGR4 to enhance WNT/β-catenin in colorectal cancer. Additionally, LGR4 is expressed preferentially in the cancer stem cell subset compared to non-cancer stem cells. CircLGR4, through LGR4, increased colon cancer stem cell self-renewal and enhanced their invasive and metastatic capacity [10]. Taken together, these reports suggest that LGR4 acts as a promoter of invasion and metastasis in colorectal cancer, and it modulates colon cancer stem cells by the activation of the WNT/β-catenin signaling pathway.

4.3. Lung Cancer

Lung cancer is the third most commonly occurring cancer and the first leading cause of cancer-related deaths among both men and women worldwide [31]. LGR4 is abundantly expressed in lung cancer adenocarcinomas [36] [37] and tumors co-expressing both LGR4 and the RSPO3 ligand exhibit high aggressiveness. Interestingly, RSPO3 high expression levels have been associated with poor survival. RSPO3/LGR4 signaling enhances cell migration and invasion and promotes EMT by modulating the function of IQGAP1, a scaffold protein that binds LGR4 and leads the formation of the Wnt signalosome supercomplex [37]. Additionally, Yang et al. showed that LGR4 is targeted by mir-449b, which is downregulated in non-small cell lung carcinomas compared with normal tissues. Overexpression of mir-449b reduced proliferation and the invasive capacity of lung cancer cell lines by decreasing LGR4 expression [39], thus, highlighting the role of LGR4 in migration and invasion processes.

4.4. Oral Cancer

Oral cancer is a broad group of diseases occurring in any oral tissue. Oral cancer represents the seventeenth most common cancer among both sexes all over the world and fourteenth cancer in terms of mortality [31]. LGR4 exerts an influence on the progression of tongue squamous cell carcinoma [38][39]. It has been shown that both LGR4 and its ligand RSPO2 are over-expressed in tongue carcinoma. Notably, the high expression of RSPO2 is positively associated with advanced clinical stages, tumor size, and metastasis. High levels of RSPO2 decrease disease-free survival and increase the recurrence of patients harboring tongue squamous cell carcinomas.

LGR4 activation by RSPO2 binding enhances proliferation, tumorigenesis, invasion, and migration and it also increases EMT and stemness by activating the Wnt/β-catenin signaling pathway. Interestingly, the interaction of RSPO2-LGR4 increases the phosphorylation of LRP6 and DVL3, while it decreases the GSK-3β phosphorylation, leading to β-catenin translocation to the nucleus, thus inducing the expression of CyclinD1, c-Myc, and CD44.

4.5. Prostate Cancer

Prostate cancer is the second most frequently diagnosed cancer among men worldwide [31]. LGR4 plays an important role in prostate cancer progression. Luo et al. showed for the first time that high expression of LGR4 is associated with a shorter time of recurrence in patients with prostate cancer [40]. LGR4 inhibition decreases proliferation, invasion, migration, EMT processes, metastasis, and increases apoptosis of prostate cancer cells. Likewise, LGR4 over-expression increases tumorigenesis and decreases apoptosis. Interestingly, LGR4 inhibition affects tumorigenic capacity and metastasis in vivo in a prostate cancer murine model [37][33][40][41][42].

Liang et al., in 2015, showed that over-expression of LGR4 is associated with the up-regulation of Akt, a key effector of the PI3K/AKT signaling pathway, promoting tumor growth [41]. Recent evidence has shown that LGR4 over-expression increased the expression level of the androgen receptor, a transcription factor that controls PSA transcription and plays essential roles in prostate cancer progression. Interestingly, LGR4 facilitated the interaction of Jmjd2a, a histone demethylase of dimethylated lysine 9 H3, with the androgen receptor (AR) to increase PSA transcription [42].

A recent study showed that radiation treatment enhances the expression of both LGR4 and its ligands in AR-positive and negative prostate cancer cells. Remarkably, LGR4 inhibition confers radiation sensitivity only in AR-positive prostate cancer by regulating the activation of CREB1, a transcription factor that promotes DNA repair [43].

It is interesting to note that LGR4 expression can also be targeted by some microRNAs in prostate cancer cells. Li et al. showed that the expression of miR228 disrupts IL6-mediated prostate cancer tumorigenesis via suppression of LGR4 expression [37]. MiR-137 also directly targets LGR4 and inhibits migration, invasion, and EMT through the EGFR/ERK signaling pathway [38].

4.6. Skin Cancer

LGR4 plays crucial roles in skin carcinogenesis and melanoma development. LGR4 is over-expressed in melanoma cells; however, it is barely expressed in squamous and basal cell carcinomas [44][45]. LGR4-deficient mice show retarded skin tumors and smaller tumor structures compared to wild-type mice. LGR4 deficiency reduces hyperplasia and keratinocyte proliferation by decreasing the Wnt/β-catenin and MEK/ERK signaling pathway [45]. Strikingly, LGR4 activity has also been shown to enhance keratinocyte proliferation via EGFR/ERK/STAT3 signaling [46]. Recent evidence has shown that LGR4 expression is regulated by mir-34a. Overexpression of mir-34a attenuates migration, invasion, and EMT by decreasing LGR4 expression, which regulates the expression of the matrix metalloproteinase 2 (MMP2) in melanoma cell lines [44].

4.7. Other Cancers

Overexpression of LGR4 has also been observed in glioblastoma, osteosarcoma, gastric, ovarian, and thyroid carcinomas [47][48][49][50][51][52]. Interestingly, high levels of LGR4 can promote the proliferation of glioma and gastric cancer cells, probably due to Wnt/β-catenin activity [33][47]. Moreover, high levels of LGR4 have been associated with poor overall survival and recurrence-free survival in ovarian cancer [49]. In thyroid cancer, upregulation of the LGR4/RSPO2 pathway leads to tumor aggressiveness, promoting cell proliferation and migration through the Wnt/β-catenin pathway and MAPK/ERK1/2 signaling [48]. In osteosarcoma, Liu et al. showed that STAT3 binds to the LGR4 promoter region in response to IL-6 and promotes its transcription [50].

A recent study associated the role of LGR4 with the regulation of immune cells in the tumoral microenvironment. LGR4 can promote tumor-associated macrophages (TAMs) M2 polarization due to the activity of RSPOs/LGR4/ERK/STAT3 signaling. TAMs are the largest leukocyte population found in the tumoral microenvironment. These cells have high plasticity and can polarize to M1-macrophages with pro-immunological activity, or M2-macrophages with immunosuppressive activity and a role in tumor immune evasion. Researchers showed that RSPOs/LGR4-inhibition with a soluble LGR4 extracellular domain (LGR4-ECD) or an RSPOs neutralizing antibody attenuates M2-TAMs polarization and it enhances the anti-tumor activity of $CD8^+$ T-cells [53].

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