Review Article

The role of the class I Wnt pathway antagonist sFRP4 in colorectal cancer

Yuting Liu[1#], Jianfeng Li[1#], Jian Qi[2]

[1]The Seventh Affiliated Hospital, Sun Yat-sun University, Guangzhou 510000, China; [2]Zhongnan Hospital of Wuhan University, Wuhan 430000, China

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[#]These authors contributed equally to this work.

Correspondence to: Jian Qi. Zhongnan Hospital of Wuhan University, Wuhan 430000, China. Email: qiqidelizi@163.com.

Abstract: Colorectal cancer is a common malignancy in humans. Studies have shown that the development and progression of 90% of colorectal cancers are related to abnormal activation of the Wnt signaling pathways. Secreted frizzled-related protein 4 (sFRP4) is a class I antagonist of the Wnt signaling pathways, and inhibition of sFRP4 expression effectively reduces Wnt signaling activity. DNA hypermethylation of *sFRP4* has been found in the early stage of colorectal cancer. However, there is still controversy surrounding the expression of sFRP4 in colorectal cancer. sFRP4 expression is related to the malignant degree, drug resistance, and invasiveness of tumors. The present article reviews the role of the *sFRP4* gene, a class I antagonist of the Wnt signaling pathway, in colorectal cancer.

Keywords: Wnt pathway; colon cancer; *sFRP4*; review

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Colorectal cancer is a common malignancy in humans. The occurrence of colorectal cancer involves abnormal mutation of multiple genes. A large number of cellular signal transduction pathways are related to colorectal cancer. Among these pathways, mutations and epigenetic alterations in the Wnt/β-catenin, Hedgehog, transforming growth factor β (TGF-β)/Smads and phosphoinositide 3-kinase (PI3K)/Akt signaling pathways are important molecular factors in the occurrence of colorectal cancer. The Wnt signaling pathways play an important role in the development and progression of colorectal cancer, and the early development of 90% of colorectal cancers has been reported to be related to abnormal activation of the Wnt pathways (1,2). Secreted frizzled related protein 4 (sFRP4) is a class I antagonist of the Wnt signaling pathways and may serve as a genetic marker for early diagnosis and treatment of colorectal cancer.

Abnormal activation of the Wnt pathways leads to colorectal cancer development and progression

The Wnt pathways, which are important for regulation of cell growth, development and differentiation, form a highly complex protein interaction network. Abnormal activation of Wnt pathways is closely related to the occurrence of numerous tumors [such as colorectal, ovarian, lung, breast, cervical and prostate tumors (1,3,4)], especially colorectal cancer (1,2).

The Wnt pathways are classified into the canonical (Wnt/β-catenin) pathway and the noncanonical (Wnt/planar cell polarity or Wnt/Ca^{2+}) pathway. All Wnt signaling are activated by the binding of a Wnt-protein ligand to a frizzled family receptor (Frz/Fz), co-receptor low-density lipoprotein (LDL) receptor related protein 5/6 (LRP5/6) are required for mediating the interaction between Wnt

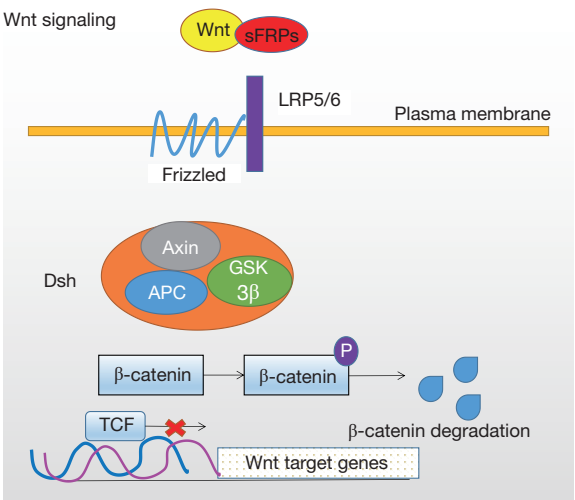

Figure 1 sFRPs and Wnt signaling. sFRPs are a family of secreted proteins (sFRP1–5), and were identified as inhibitors of Wnt signaling pathway. Several members, including sFRP1, sFRP2, and sFPR4, possess a conserved Fzd type cysteine-rich domain that binds Wnts and typically antagonize the Wnt signaling, presumably by preventing Wnt/Fzd interactions. sFRP, secreted frizzled-related protein; Dsh, dishevelled; TCF, T cell factor; APC, adenomatous polyposis coli; GSK-3β, glycogen synthase kinase-3β; Axin, axis inhibition protein; LRP5/6, low-density lipoprotein receptor related protein 5/6.

and Fz. Binding of the Wnt protein to the receptors allows transduction of signals to the second messenger protein dishevelled (Dsh). Activated Dsh protein inhibits the activity of the axis inhibition (Axin)/adenomatous polyposis coli (APC)/glycogen synthase kinase-3β (GSK-3β) complex composed of the scaffold protein Axin, APC protein and glycogen synthase kinase-3β (GSK-3β), preventing β-catenin from being degraded. A large amount of free β-catenin enters the nucleus through the nuclear membrane and acts on T cell factor (TCF)/lymphoid enhancing factor (LEF), which induces transcriptional activation, initiates expression of the downstream target genes C-myc and cyclin D1, and eventually results in malignant transformation of cells and tumorigenesis (5,6) (*Figure 1*).

Mutations in the APC and β-catenin genes are widely present in colorectal cancer and lead to abnormal activation of the canonical Wnt signaling pathway and nuclear accumulation of β-catenin protein (6,7). To date, a variety of Wnt/β-catenin pathway-related target genes have been discovered. These target genes, such as the matrix metalloproteinase-7 (MMP-7), survivin, C-myc, cyclin D1, peroxisome proliferator activated receptor γ (PPAR-γ), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF) genes, are related to colon cancer cell apoptosis, growth, angiogenesis, invasion and metastasis (8,9). Blocking Wnt signaling pathway activity inhibits colon cancer cell proliferation (10). In addition, abnormal activation of the Wnt signaling pathways is related to the sensitivity of rectal cancer to radiochemotherapy (11). Thus, in-depth study of Wnt signaling pathways is conducive to the development of new methods for prevention and treatment of colorectal cancer.

The sFRP gene family antagonizes the Wnt pathway

Abnormal activation of Wnt pathways is often related to inactivation and downregulation of Wnt antagonists. The Wnt pathways are regulated extracellularly by secretory proteins classified as either class I or class II antagonists. Class I antagonists include Cerberus, sFRPs, and Wnt inhibitory factor 1 (WIF-1). Class I antagonists directly bind to Wnt, thereby antagonizing both the canonical and noncanonical Wnt pathways. Class II antagonists include the Dickkopf (DKK) family. DKKs inhibit Wnt signaling through binding to LRP5/6. Therefore, DKKs only antagonize the canonical Wnt pathway.

The sFRP gene family is the first discovered antagonist of the canonical Wnt pathway. sFRPs are secreted protein antagonists that bind directly to Wnt proteins.

sFRP1, sFRP2, and sFPR4, possess a conserved Frz-type cysteine-rich domain that binds Wnts and typically antagonize the Wnt signaling, presumably by preventing Wnt/Frz interactions. Thus SFRPs, except sFRP3, can function as antagonists of Wnt signalling by competing with Wnt proteins for binding to their receptor, Frz. These interactions prevent Wnt proteins from binding to Frz or lead to the formation of nonfunctional complexes with Frz, thereby blocking Wnt signaling. In colorectal cancer cell lines, sFRPs can attenuate Wnt signaling even in the presence of downstream gene mutations (12). The sFRP4 protein is composed of 349 amino acids and has a molecular weight of 39.9 kDa, which is the highest molecular weight among the members of the sFRP family. sFRP4 is expressed in the intestinal tract, endometrium, pancreas, stomach, liver, heart and mammary gland (13). Numerous studies have shown that sFRP4 regulates apoptosis and differentiation of tumor cells (14) and is an important antagonist of the Wnt pathway. However, there is a lack of

systematic studies of sFRP4 in the literature.

The biological functions of sFRP4

SFRP4 is abnormally expressed in malignant tumors and differentially expressed in colorectal cancer

Loss of sFRP4 expression has been observed in most tumors, including ovarian cancer, malignant pituitary tumors, endometrial cancer, and acute myeloid leukemia (14). However, sFRP4 expression is upregulated in chemotherapy-sensitive ovarian cancer (15) and breast cancer (16). In addition, the expression of sFRP4 in these tumors is affected by varying degrees of methylation at its promoter region. Hypermethylation of the *sFRP4* promoter occurs in ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, and colorectal cancer (15).

The occurrence of colorectal cancer is an extremely complex process that involves polygenic changes. In addition to gene mutations, epigenetic alteration-induced silencing of tumor suppressor genes is also an important tumorigenic mechanism. A high degree of DNA methylation at the CPG islands of the promoter regions of tumor suppressor genes is a common feature of human tumors and may occur at various tumor stages. Hypermethylation of the sFRP family of genes, which are class I antagonists of the Wnt pathways, occurs in the early stages of colorectal tumors. *SFRP4* contains abundant CPG islands in its exon regions. Therefore, gene silencing caused by DNA hypermethylation of *sFRP4* may be an important reason behind the development and progression of colorectal tumors.

Colorectal cancer studies have yielded controversial results regarding the expression of sFRP4 in colorectal cancer. Our previous study found that sFRP4 expression was downregulated in 61.1% of colorectal cancer tissues and 9.1% of colorectal adenomas in comparison with normal tissues. In colon cancer, the incidence of DNA methylation of the *sFRP4* gene was found to be 36.1% (26/72), which was significantly different from that in normal mucosa (17). In contrast, Huang *et al.* employed fluorescence-based quantitative polymerase chain reaction (PCR), immunoblotting and immunohistochemical techniques to systematically investigate the expression of sFRPs in large intestinal carcinoma (18). The results showed that the immunostaining level of sFRP4 was rather low in the cytoplasm of normal mucosal cells and adenoma cells, while sFRP4 was highly expressed in high-grade intraepithelial neoplasms and large intestinal carcinoma tissues. sFRP4 was overexpressed in 80% of the patients with large intestinal carcinoma and 42% of the patients with adenomas. These two contradictory results may be explained by pathological differences among the collected colorectal cancer specimens, the number of colorectal cancer specimens and the choice of experimental methods.

In addition, the expression of sFRP1, sFRP2 and sFRP5 has been reported to be elevated in SW480 colorectal cancer cells after downregulation of β-catenin expression, while the expression of sFRP4 was not significantly altered (13). However, one study showed that treatment to induce DNA demethylation of *sFRP4* resulted in inhibition of Wnt signaling pathways (15). Thus, controversies still exist surrounding the role of sFRP4 in the early stage of colorectal cancer development and the factors correlated with sFRP4 expression levels. Epigenetics is a reversible process. Defining the roles of *sFRP4* DNA methylation and gene expression in colorectal cancer may provide a new breakthrough point for treatment of colorectal cancer.

sFRP4 is a potential target for tumor gene therapy

sFRP4 expression may be correlated with the prognosis of tumor patients. Prostate cancer patients with high *sFRP4* mRNA content show a better prognosis and a lower recurrence rate (19). In addition, sFRP4 may promote apoptosis and differentiation of tumor cells (15). sFRP4 expression is also related to the degree of chemosensitivity. Warrier *et al.* reported that the *sFRP4* gene induced apoptosis of cancer stem cells (CSCs) in head and neck squamous cell carcinoma (20) and reduced the expression of the stem cell markers cluster of differentiation 44 (CD44) and aldehyde dehydrogenase (ALDH), thereby inhibiting the pluripotent differentiation of CSCs, increasing the sensitivity of tumors to chemotherapeutic drugs, and reducing drug resistance. In glioblastoma multiforme, introduction of the *sFRP4* gene in combination with anti-tumor drugs increased the expression of the apoptotic markers Bcl-2-associated X (BAX) and p21, downregulated the expression of the differentiation-promoting protein cyclin D1, and effectively inhibited the activity of the Wnt pathway (21). Introduction of the *sFRP4* gene in endometrial cancer effectively inhibited activation of the Wnt pathways through regulation of Wnt7a protein (22). Saran *et al.* proposed that *sFRP4* is an important gene for evaluating the sensitivity of ovarian cancer to chemotherapeutic drugs and that sFRP4 expression was directly proportional to

the degree of chemotherapeutic drug sensitivity (23,24). Therefore, upregulation of sFRP4 expression might be conducive to treatment of chemotherapeutic drug-resistant ovarian cancers.

On the other hand, sFRP4 may be a key molecule at the intersection of the Wnt pathways and other pathways and be closely related to tumor formation, invasion and infiltration. The expression of sFRP4 is negatively correlated with expression of the P53 tumor suppressor gene. Loss of sFRP4 expression induces silencing of the *COX2* gene and the mismatch repair gene *MLH1* and upregulates the expression of MMP2 and MMP9, which are capable of promoting tumor cell invasion and infiltration; thus, loss of sFRP4 expression promotes tumor cell invasion and infiltration. In addition, sFRP4 activates the anti-apoptotic Akt/protein kinase B (PKB) signaling pathway (25,26).

The role of blocking sFRP4 expression in treatment of colorectal tumors

Conventional chemotherapeutic drugs for colorectal cancer include oxaliplatin and 5-fluorouracil (5-FU). Most chemotherapeutic drugs cause serious side effects, such as decreased white blood cell count, loss of appetite, severe hair loss, and liver/kidney toxicity. In recent years, as molecular biology has advanced, researchers have gradually focused on gene therapy. Blocking of the Wnt pathways is a very important step in treatment of colorectal cancer, and for this purpose, the genes in the sFRP family are considered potential targets. A study conducted by Chen *et al.* showed that downregulation of sFRP4 expression decreased the proliferation rate of colorectal cancer cells (27). Miao *et al.* proposed that methyl CpG binding protein 2 (MeCP2) effectively regulates the methylation level of *sFRP4* (28) and found that interference with MeCP2 expression increased sFRP4 expression, which led to a reduction in expression of the downstream protein β-catenin. As a result, activation of the Wnt pathway was blocked.

As a class I antagonist of the Wnt pathways, *sFRP4* displays DNA hypermethylation in the early stages of colorectal cancer. sFRP4 is differentially expressed in colorectal cancer. However, the expression of sFRP4 is controversial in CRC. But blocking sFRP4 expression effectively reduces the proliferation rate of colorectal cancer cells. In addition, sFRP4 is related to chemoresistance in a variety of tumors and may serve as a potential prognostic marker. Epigenetics is reversible, and reversal of *sFRP4* methylation is expected to antagonize Wnt pathway activation, thereby exerting a colorectal tumor-preventing effect.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/dmr.2019.08.01). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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