

Wnt/ β -Catenin Signaling Pathway in the Development and Progression of Colorectal Cancer

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Abstract: The Wnt/ β -catenin signaling pathway is a growth control pathway involved in various biological processes as well as the development and progression of cancer. Colorectal cancer (CRC) is one of the most common malignancies in the world. The hyperactivation of Wnt signaling is observed in almost all CRC and plays a crucial role in cancer-related processes such as cancer stem cell (CSC) propagation, angiogenesis, epithelial-mesenchymal transition (EMT), chemoresistance, and metastasis. This review will discuss how the Wnt/ β -catenin signaling pathway is involved in the carcinogenesis and progression of CRC and related therapeutic approaches.

Keywords: Wnt pathway, colorectal carcinogenesis, APC, CRC therapy

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in the world. Understanding the mechanisms involved in its development and progression can help to treat it more effectively. The findings of the last more than 20 years show that the main role in this process is played by pathologically increased activity of the Wnt/ β -catenin signaling pathway.¹

Wnt pathway is an intercellular molecular signaling pathway discovered in 1982 by *Drosophila melanogaster*, which performs essential functions throughout the animal kingdom, including humans.² The Wnt signaling pathway participates in embryonic development and physiological homeostasis, but its deregulation has been implicated in tumor initiation and progression.^{3–6} Wnt signals are transmitted through at least three different intracellular pathways, including the canonical Wnt/ β -catenin signaling pathway, non-canonical Wnt/Ca²⁺ pathway, and non-canonical Wnt/PCP (planar cell polarity) pathway. All play important roles in tissue and organ formation and act as regulators of the cytoskeleton. The Wnt/ β -catenin pathway is most recognized for its significance in CRC and will be discussed exclusively in this review.

We summarize the Wnt/ β -catenin signaling pathway and its related regulation process, followed by the discussion of its involvement in CRC carcinogenesis and progression as well as related therapeutic approaches. The hyperactivation of Wnt/ β -catenin signaling is observed in almost all CRC, and plays a crucial role in cancer stem cell (CSC) propagation, angiogenesis, epithelial-mesenchymal transition (EMT), chemoresistance, and metastasis. A number of drugs, phytochemicals, and molecular agents targeting the Wnt pathway for the treatment of colorectal cancer have been developed.⁷ Understanding the roles and mechanisms of Wnt/ β -catenin signaling pathway in CRC will contribute to the development of new targeted drugs.

Wnt/ β -Catenin Pathway

Overview of the Wnt/ β -Catenin Pathway

The Wnt/ β -catenin pathway is a growth control pathway involved in various biological processes, such as embryonic development, maintenance of stem cell state, cell renewal, and adult tissue homeostasis.^{3–6} The β -catenin protein is the central molecule and crucial nuclear effector of the canonical Wnt pathway, which is not only a transcription factor but also an important component of the cytoskeleton.

In the absence of Wnt ligands, ubiquitin-dependent proteasome degradation keeps the β -catenin protein at the basal level.⁸ This process is controlled by a protein complex called the β -catenin destruction complex, which consists of the proteins Axis inhibition protein (Axin), Glycogen synthase kinase 3 (GSK3), Casein kinase 1 (CK1), Adenomatous polyposis coli (APC), and the E3 ubiquitin ligase β -TrCP2. β -catenin is phosphorylated by CK1 and GSK3 sequentially, thus recognized by the E3-ligase β -TrCP2, which drives the ubiquitination and proteasomal degradation of β -catenin.⁹ This process allows the Groucho protein to bind to the T cell factor/lymphoid enhancer factor (TCF/LEF), thereby blocking transcriptional promoters and enhancers of the Wnt/ β -catenin pathway.

There are 19 Wnt ligands (Wnts) known in humans, which are all secretory palmitoleate glycoproteins.¹⁰ The glycosyl and lipid residues (specifically palmitic and palmitoleic acid residues) they contained give them a hydrophobic character which is essential for the secretion and function of Wnt ligands.^{11,12} Wnt ligands must be processed and exported, and then modified by an attachment of a lipid, palmitoleic acid, which is performed by Porcupine O-acyltransferase (PORCN) in the endoplasmic reticulum.¹³ From there, Wnt bound to the Wntless protein (Wls) is transferred to the cell membrane by the vacuolar system of the Golgi apparatus and subsequently secreted. The activation of Wnt/ β -catenin signaling is initiated when Wnt ligands bind to the Frizzled (FZD) receptors and the low-density lipoprotein receptor-related proteins 5/6 (LRP5/6) co-receptors on the target cells, which leads to recruitment of Dishevelled (Dvl), causing the relocalization of the destruction complex to the receptor.^{9,14,15} This process disrupts the activity of the β -catenin destruction complex, leading to an accumulation of β -catenin.^{16,17} β -catenin then translocates to the nucleus, interacting with TCF/LEF and co-activators, such as BCL-9 and Pygopus, to activate the Wnt target genes like c-MYC, CCND1 (the gene encoding cyclin D1), EGFR, and leucine-rich repeat-containing receptor 5 (LGR5) (Figure 1).¹⁸

The Regulation of the Wnt/ β -Catenin Pathway

Several evolutionarily conserved protein families and genes are known to antagonize or regulate Wnt signaling. Wnt inhibitory factor (WIF), a secreted protein that has been recognized as an important Wnt antagonist, inhibits Wnt/ β -catenin

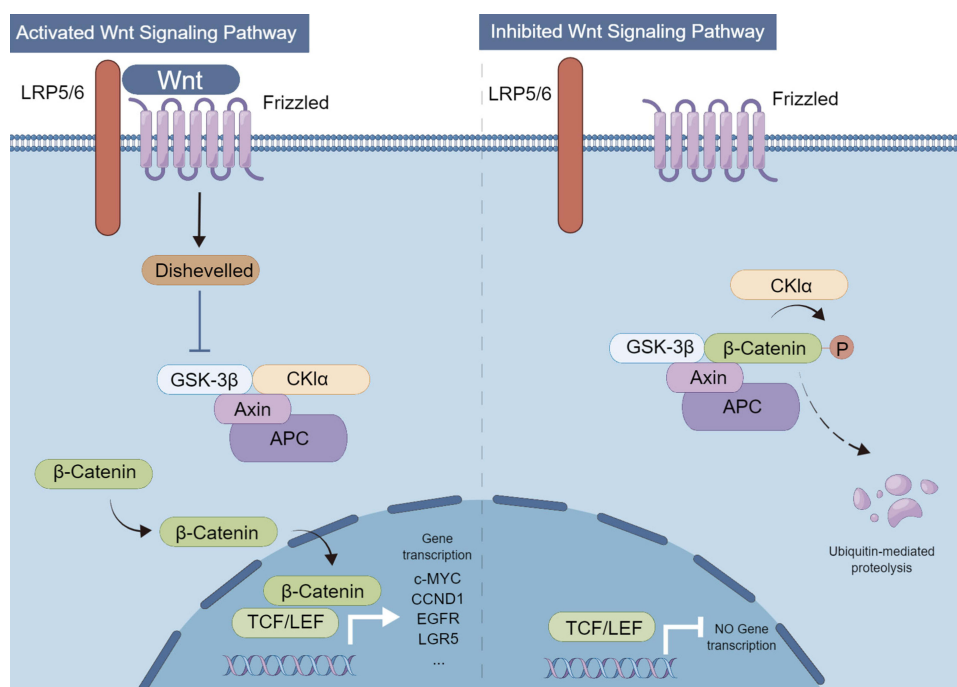


Figure 1 Wnt/ β -catenin signaling in the target cell. In the absence of Wnt ligands, the free cytoplasmic level of β -catenin is reduced by the activity of the β -catenin destruction complex (Axin, APC, CK1 and GSK3). TCF/LEF together with other molecules act as repressors of gene transcription. The Wnt/ β -catenin signaling is activated when Wnt ligands bind to the FZD receptors and the LRP5/6 co-receptors on the target cells, which leads to recruitment of Dishevelled (Dvl), causing the relocalization of the destruction complex to the receptor. β -catenin accumulates and then translocates to the nucleus, interacting with TCF/LEF to activate gene transcription. (By Figdraw).

signaling directly by binding to Wnt proteins.¹⁹ Dickkopf1 (DKK1), a member of the DKK family of proteins, competes with Wnt proteins to bind to LRP5/6 receptors, thus negatively regulating the initiation of signaling.²⁰ Secreted FZD-related proteins (sFRPs), a kind of soluble proteins, block the initiation of Wnt signaling by trapping Wnt proteins directly or by binding to FZD receptors to prevent the interaction between receptors and Wnt ligands.²¹ In addition to these proteins, other inhibitors of Wnt signaling include *wis*/SOST, IGFBP, APCDD1, Cerberus(CER), and so forth.²⁰ ZNRF3 and its homolog RNF43 are transmembrane E3-ubiquitin ligases that act as negative-feedback regulators of Wnt signaling by downregulating FZD receptors through ubiquitylation.²² R-Spondins (RSPO) is a family consisting of four secreted growth factors, of which RSPO2 and RSPO3 are RNF43/ZNRF3 ligands, which can derepress FZD receptors from RNF43/ZNRF3-mediated degradation by ubiquitination and activate Wnt signaling.²³

The effect of the activated Wnt/ β -catenin pathway varies considerably depending on the age of the organism, the type of tissue and cell, its location, functional status, and probably a number of other determinants.²⁴ This indicates that the regulation of the signaling is more complex than just the presence or absence of β -catenin in the nucleus. There are four genes of TCF / LEF transcription factors, but there are many more of their products (different splicing, posttranslational modifications, including phosphorylation, etc.) and they differ in their effects. Some act activating even without β -catenin, others are unable to bind it and are persistent inhibitors (dnTCF / LEF), and others react with different promoters and affect other genes.²⁵ Some are inhibitors due to their ability to export β -catenin from the nucleus such as inhibitor of β -catenin and TCF-4 (ICAT).²⁶ In principle, the effect of the Wnt pathway is influenced by conformational changes in DNA, currently expressed genes, and the activity of other signaling pathways.

Overall, refined regulation of Wnt/ β -catenin signaling can activate or inhibit the expression of specific genes to exert various biological functions. The identification of positive and negative regulators needs further investigation, which would help reveal the potential regulatory mechanism of β -catenin-dependent transcription and be therapeutically useful in a variety of disease settings.

Wnt/ β -Catenin Pathway in Colorectal Carcinogenesis and Progression

Wnt/ β -Catenin Pathway and Colorectal Carcinogenesis

Wnt signaling is an essential factor for normal intestinal function, and in particular, for the maintenance and self-renewal of epithelial stem cells located at the base of intestinal crypts.²⁷ The epithelium of the colon and rectum is renewed in regular cycles of about 3–6 days, which is due to the intestinal stem cells (ISCs), the multipotent cells located at the base of mucosal crypts.²⁸ They live at the crypt base all their lives and have the ability of an unlimited number of divisions, migrating towards the top of the villi while differentiating into mature mucosal cells with absorption functions, mucus-secreting goblet cells, or cells of the diffuse neuroendocrine system producing various hormonal agents.^{29,30} Mature cells cover about the upper third of the crypts. They travel further toward the intestinal lumen, where they undergo apoptosis, separate, and are replaced by new ones. Undifferentiated character, lifetime survival, the ability of unlimited division, and the production of different types of daughter cells are not autonomous properties of ISCs but are largely determined by Wnt signaling. The source of Wnt glycolipoproteins are myofibroblasts located subepithelial at the base of the crypts, which are essential for maintaining the local supply of stem cells, and thus for lifelong epithelial replacement.

Over the past few years, people have conducted extensive research on the ISC population and a number of ISC subpopulations have been identified. One cell type is the fast-cycling crypt base columnar (CBC) stem cells that express a Leucine-rich G-protein coupled receptor 5 (LGR5), which is identified as a direct regulator of Wnt activity subsequently.³¹ Wnt-mediated signaling is maintained at high levels by RSPOs in the LGR5+ intestinal stem cells, for RSPOs bridging an interaction between LGR4/5 and RNF43/ZNRF3 to sequester the ligases activities, leading to Fzd expression and continued signal activation.³²

Alterations in core Wnt regulators were found in a sequencing project of 1134 colorectal cancer samples, noting the incidence of oncogenic Wnt activation in 96% of human colorectal cancers.³³ The associations between hyperactivation of the Wnt/ β -catenin signaling and the progression of colorectal cancer have been long identified. Aberrant activation of the Wnt signaling is mainly due to a disorder (mutation, deletion, attenuation of gene expression) of its three components, APC, β -catenin, and AXIN. The defection of APC and AXIN disrupts the formation and function of

the β -catenin degradation complex, which results in β -catenin being resistant to degradation and overexpression of Wnt pathway genes.

Abnormal regulation of the transcription factor β -catenin, which is the pivotal component of the Wnt signaling pathway, leads to early events in carcinogenesis.^{34–36} As a study of 720 colorectal patient samples shows, the loss of membrane β -catenin is remarkably associated with poor prognosis when using overall survival as the endpoint.³⁷ The loss of membranous β -catenin is particularly prominent in the invasion front of colorectal cancer, and both membrane localization and invasion front are prognostic markers for prolonging disease-free survival. However, high nuclear accumulation in colorectal cancer is associated with worse disease-free survival, overall survival, and a higher probability of lymph node metastasis.^{38,39}

Clinical studies have shown that about 70% of sporadic and hereditary colorectal cancer has APC gene deletion or mutation,⁴⁰ which is recognized as one of the earliest genetic events in the development of CRC. Loss-of-function APC mutations are pathognomonic of familial adenomatous polyposis (FAP) and contribute to the development of the most sporadic colorectal cancer. Since APC is part of the destruction complex, its degradation can result in cytoplasmic accumulation of β -catenin protein, thus abnormally activating the Wnt/ β -catenin pathway, and eventually leads to promoting cell proliferation, differentiation, and inducing carcinogenesis.

Due to elegant work in human cancer cell lines and murine models of intestinal neoplasia, it was highlighted that oncoprotein MYC is an essential downstream driver of Wnt/ β -catenin driven carcinogenicity followed by APC loss.⁴¹ Scholz et al reported that Wnt signaling can further promote MYC accumulation through post-transcriptional mechanisms, thereby enriching the availability of cytoplasmic pools of MYC transcripts.⁴² APC restoration can drive tumor-cell differentiation and sustained regression without relapse in colorectal cancer, as well as reverse the MYC-driven oncogenic state and reestablish tissue homeostasis.⁴³ As mentioned earlier, the interactions between BCL9/9L and nuclear co-receptor complexes help activate the transcription of Wnt target genes. In CRC, BCL9/9L appears to decrease the association of β -catenin in adherens junctions, promoting its nuclear translocation to upregulate Wnt signaling.⁴⁴ Moreover, loss of BCL9/9L suppressed many features of acute APC loss and subsequent Wnt pathway deregulation in vivo, resulting in a level of Wnt pathway activation that favored tumor initiation in the proximal small intestine and blocked tumor growth in the colon.⁴⁵ These all demonstrate the key roles of APC mutations in both initiating and maintaining intestinal neoplasia and emphasize the importance of the Wnt pathway as a potential therapeutic target for CRC.

AXIN, GSK3, CK1, and E3 ubiquitin ligase β -TrCP2 as other main constituents of the β -catenin destruction complex, also play a role in CRC carcinogenesis. While APC is the most frequently mutated tumor suppressor gene in the Wnt pathway, mutations in the AXIN1 or AXIN2 genes have been found in a subset of CRCs as well as in a number of other cancer types.⁴⁶ Some studies indicated that the expression and activity of GSK3 β are both increased in CRC.⁴⁷ GSK3 β was found to contribute to higher cell proliferation and modulate the chemoresistance through the NF- κ B pathway.⁴⁸ It has been found that CK1 mutants affect the growth and proliferation of tumor cells and induce tumor growth in xenografts, and the expression level of CK1 correlates with poor survival in CRC.^{49,50} Increased expression of β -TrCP1 is associated with activation of both β -catenin and NF- κ B, suggesting that the integration of these signaling pathways by increased β -TrCP expression may contribute to the inhibition of apoptosis and tumor metastasis.⁵¹

Wnt/ β -Catenin Pathway and CRC Progression

From its initial function in CRC initiation, the Wnt/ β -catenin pathway has evolved to explain more phenomena during the disease progression including cancer stem cell (CSC) propagation, angiogenesis, epithelial-mesenchymal transition (EMT), chemoresistance, tumor immunomodulation and ultimately responsible for tumor recurrence after therapy, metastasis and poor patient survival.^{52,53}

Cancer Stem Cell (CSC) Self-Renewal and Differentiation

Wnt signaling plays a critical role in CRC stem cell (CSC) self-renewal in the intestinal crypt.⁵⁴ Being activated in CSCs, the Wnt pathway upregulates transcription of genes necessary for proliferation (such as c-myc), cell cycling (such as

cyclin-D), anti-apoptosis (such as survivin), metabolic switching to aerobic glycolysis (PDK1, MCT-1), and invasion and metastasis (SLUG, MMP).^{55–60} Depending on whether the recruited co-activator of β -catenin is CREB-binding protein (CBP) or p300, Wnt activity will favor either differentiation or proliferation of CSCs. p300/ β -catenin binding promotes CSC differentiation, whereas CBP/ β -catenin binding favors the maintenance of CSC potency.⁶¹ In addition, the Wnt/ β -catenin signaling target gene *Lgr5* is a primary marker of CSCs in the intestinal crypt.⁶² CSCs are thought to be resistant to standard chemotherapeutic agents and, therefore, therapies targeting CSCs may provide an effective means of combating CRC.⁶³ CSCs also play a critical role in tumor angiogenesis through their ability to generate functional blood vessels and transdifferentiate into tumor epithelial cells (ECs), which could modify the tumor microenvironment and survive outside of the primary tumor at metastatic sites.^{64,65}

Angiogenesis

As one of the key mechanisms of malignant growth and tumor progression, angiogenesis is critical for colorectal cancer growth and metastasis and is one of the commonly accepted indicators of prognosis.⁶⁶ β -catenin produced by the tumor cell directly induces VEGF production and an increase in vessel density, which was proved in the colon cancer cell lines (HCT116, SW620) and APC-Min/+ mouse model. This indicates the participation of β -catenin in angiogenesis initiation.^{67–69} A positive correlation was also demonstrated between the upregulation of VEGF-A expression and APC mutational status.⁶⁹ Norrin is a non-Wnt ligand of the Fzd receptors, which can selectively bind to Fzd4 and activate Wnt signaling.³ This interaction is modulated via the regulation of Fzd4 expression by Wnt2.⁷⁰ Norrin produced by colorectal cancer cells could increase EC growth and motility, which plays a key role in CRC tumor microenvironment angiogenesis.⁷¹ As a predominant component of tumor microenvironment, cancer-associated fibroblasts (CAFs) play an important role in promoting tumor progression. In CRC, CAFs are highly enriched with the Wnt2 protein, one of the pro-angiogenic Wnt signaling components.^{72,73} The overexpression of Wnt2 protein was initially observed in CRC cells, with a knockdown of Wnt2 downregulating the expression of the Wnt/ β -catenin target gene. In addition, the proliferative properties of Wnt2 were also demonstrated.⁷⁴ Wnt2 overexpression increased tumor volume and vascular density in CRC xenografts. Additionally, the expression of proteins with pro-angiogenic properties, such as ANG-2, IL-6, granulocyte colony-stimulating factor (G-CSF), and placental growth factor, as well as vascular markers in human CRC are correlated with the Wnt2 level. Thus, it is demonstrated that Wnt2 plays a crucial role in the development of the active CAF phenotype in CRC, which increases angiogenesis by shifting the balance in support of pro-angiogenic signals.⁷³

Epithelial-Mesenchymal Transition (EMT)

Overactivation of the Wnt/ β -catenin pathway promotes epithelial-mesenchymal transition (EMT) by inducing the expression of EMT-related transcription factors. In colorectal cancer (CRC), EMT is associated with an invasive or metastatic phenotype in CRC and contributes a lot to chemotherapeutic resistance.⁵³ Enhanced Wnt/ β -catenin signaling in CRC cells increases the level of SNAIL, which represses E-cadherin and regulates EMT, thus promoting local invasion.⁷⁵ Wnt3a protein overexpression in CRC promotes invasion and induces Snail expression, which is concomitant with EMT features, such as reducing the epithelial marker E-cadherin expression, increasing the mesenchymal marker Vimentin expression, and localization of nuclear β -catenin. DKK1 can partially reverse the expression of EMT-associated factors in Wnt3a-overexpressing cells.⁷⁶ Transmembrane-4 L-6 family member-1 (TM4SF1) is a small plasma membrane glycoprotein, which is upregulated in CRC tissues more than in non-tumor tissues and is positively correlated with poor prognosis.⁷⁷ TM4SF1 promotes the EMT and cancer stemness of CRC cells through the Wnt/ β -catenin pathway. It is found that TM4SF1 modulates the Wnt/ β -catenin-mediated regulation of Sox2 expression via c-Myc in CRC.⁷⁸ Runt-related transcription factor 1 (RUNX1), a member of the RUNX family of transcription factors, which is function as critical lineage determinants in various tissues.⁷⁹ RUNX1 expression is upregulated in CRC tissues and is closely correlated with cancer metastasis and EMT of CRC. In addition, RUNX1 can interact directly with β -catenin and target the promoter and enhancer regions of KIT to activate Wnt/ β -catenin signaling in CRC cells, thereby promoting KIT transcription.⁸⁰ KIT signaling may play a growth-stimulatory role in colon cancer.⁸¹

Chemoresistance

Multidrug resistance (MDR) is a major impediment to the successful treatment of CRC, and overcoming MDR becomes a great challenge in fighting against CRC.⁸² ATP-binding cassette (ABC) membrane transporters can pump various chemotherapeutic agents out of cells to reduce intracellular drug accumulation and attenuate drug-induced cytotoxicity, which is one of the most typical mechanisms of MDR.¹ Attenuating the efflux activity of most ABC transporters, which directly contribute to chemoresistance, could largely reverse the resistance.⁸³ P-Glycoprotein (P-gp), also known as multidrug resistance protein 1 (MDR1) or ABCB1, is the most significant ABC transporter in the human gastrointestinal tract.⁸⁴ Nuclear β -catenin has been shown to selectively bind CBP to the promoter region of the MDR1 gene, one of the target genes of TCF/LEF, in the Wnt/ β -catenin signaling cascade.⁸⁵ Many T-cell factor 4 (TCF4) binding sites were discovered in the MDR1 gene promoter, and this gene was shown to be transcriptionally downregulated in CRC following TCF4 inactivation, indicating MDR1 is a direct target of the TCF4/ β -catenin transcriptional complex.⁸⁶ The MDR1 gene's expressed P-gp efflux may be reversed and drug-induced apoptosis could again be sensitive to drugs if endogenous β -catenin was depleted by RNA interference. Sec62 was first discovered as a protein involved in translocation in the endoplasmic reticulum membrane.⁸⁷ By binding to β -catenin and boosting Wnt signaling, Sec62, which is increased by the METTL3-mediated m⁶A modification, aids in the stemness and chemoresistance of CRC. As a result, the m⁶A modification-Sec62- β -catenin molecular axis may serve as a therapeutic target for treating CRC.⁸⁸ It is indicated that exosomal Wnts derived from fibroblasts could induce the differentiation of cancer cells to CSCs thus promoting chemoresistance in CRC, which suggests that interfering with exosomal Wnt signaling may help to improve chemosensitivity and the therapeutic window.⁸⁹

Wnt/ β -Catenin Crosstalk with Other Pathways

Deregulation of the Wnt pathway alone is not enough to cause malignancy. Disorders of multiple signals are involved, such as transforming growth factor β (TGF β) pathway, epidermal growth factor signaling (EGF, with EGFR receptor and cascading interacting kinases Kras, Braf, and MAPK), nuclear factor kappa-B (NF- κ B), Notch, Hippo/YAP, and PI3K/AKT pathway.^{90–92}

APC loss leads to the increase of β -catenin and RAS (especially mutant KRAS), which promotes the synergistic tumorigenesis of CRC.⁹³ Through interaction with β -catenin and forming a complex, EGFR was proved to promote the invasion of cancer cells.⁹⁴ I κ B kinases (IKKs) interact with β -catenin and phosphorylate it, and IKK- α could specifically increase the expression of β -catenin dependent gene in CRC cell lines.⁹⁵ The crosstalk between Wnt and Notch signaling was first reported in fruit fly which showed NICD can bind to Dvl1 and the subsequent Notch signaling and then regulate the Wnt pathway. Wnt/ β -catenin signaling increases the Notch expression and triggers the onset of CRC through activation of Jagged-1.⁹⁶ It has been shown that the Hippo pathway could inhibit Dvl phosphorylation, thus inhibiting β -catenin accumulation in the nuclear and transcription of Wnt/ β -catenin/TCF target genes.⁹⁷ PI3K has a substantial effect in the setting of carcinogenesis through modulating β -catenin signaling. It has been proposed that PI3K can inhibit GSK3 β as a downstream target.⁹⁸ AKT kinase may potentially cause β -catenin to become active. As a result, it has been demonstrated that the Wnt/ β -catenin and PI3K-AKT pathway interact to promote unregulated cell cycle progression, increased cell proliferation, apoptosis escape, increased invasiveness and metastasis, and resistance to cancer treatment.⁹⁹

Wnt/ β -Catenin Pathway and Colorectal Cancer Therapy

The incidence rate of CRC is increasing year by year, early-stage patients can be treated by surgical resection, whereas the treatment of patients with metastasis remains challenging. Chemotherapy is the main treatment for advanced CRC, which significantly prolong the survival time and improve the quality of life. In recent decades, molecular targeted therapy has been progressing, and chemotherapy combined with targeted therapy has further improved the survival of patients with advanced CRC. The significance of the Wnt pathway in CRC is unquestionable. Experiments on human colorectal cancer cell lines show that its inhibition can stop the growth and cause apoptosis of tumor cells even in the presence of mutations in other signaling pathways.^{100,101} However, targeted WNT therapies have not made headway in the clinic.

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are widely discussed drugs for colorectal carcinogenesis, which play an active role in both cancer prevention and cancer treatment.¹⁰² NSAIDs inhibit the enzyme cyclooxygenase (COX), and thus the production of prostaglandins, substances that control a number of biological processes including immune and inflammatory. COX occurs in two isoforms, with COX1 performing several physiological roles, while COX2 is predominantly associated with pathological conditions. COX2 overexpression is present in both colorectal adenomas (40%) and carcinomas (85%) and generally leads to inhibition of apoptosis, angiogenesis, and disease progression. Prostaglandins produced by COX also increase β -catenin transfer to the nucleus and Wnt signaling activity, while NSAIDs can degrade β -catenin in cells and inhibit the Wnt/ β -catenin pathway.¹⁰³ Diclofenac and celecoxib have been proved to reduce the expression of AXIN2, Cyclin D1, and c-myc, which are all target genes of β -catenin. In addition, after treatment with diclofenac and celecoxib, the phosphorylation of β -catenin increased, and the phosphorylation of GSK3 decreased, thus the cytoplasmic accumulation and nuclear translocation of β -catenin were significantly reduced.¹⁰⁴ These studies showed that NSAIDs can affect β -catenin, to reach to its related anti-tumor effect. Bowen et al demonstrated that the combination of an NSAID (sulindac) and a rexinoid (bexarotene) as a durable approach can prevent colorectal carcinogenesis of FAP through the Wnt/ β -catenin pathway.¹⁰⁵ Possibly, there is a role for aspirin or NSAIDs as primary prevention in those patients with a defined hereditary predisposition (eg, Lynch syndrome and FAP).¹⁰⁶ However, the serious adverse reactions of traditional NSAIDs as anti-cancer drugs cannot be ignored. Common side effects include gastrointestinal bleeding, liver and kidney damage, and long-term application of NSAIDs will also cause weight gain, edema formation, and fluid retention. Therefore, it should be viewed on an individual basis, balancing its beneficial effects on both cardiovascular disease and colorectal cancer incidence against the potential harms.

Therapeutic Target Molecules in Wnt Signaling

Targeting Wnt Ligand/Receptor Interface

Porcupine (PORCN), a membrane-bound O-acyltransferase, is involved in an essential step of Wnt ligands secretion.¹⁰⁷ Thereby, targeting PORCN is a way to inhibit Wnt signaling. Inhibitors of Wnt production (IWPs), a class of small molecules that could act as Wnt antagonists by targeting PORCN, was discovered.¹⁰⁸ A more recent study identified another potent porcupine inhibitor LGK974 that inhibits the secretion of Wnt3a and effectively suppresses the growth of murine tumors induced by mouse mammary tumor virus-driven ectopic Wnt1 expression.¹⁰⁹ The data indicate the therapeutic potential of PORCN inhibitors to treat cancers that are exogenous Wnt-dependent, such as LKB1 and RNF43 mutations in CRCs.¹¹⁰ Loss-of-function mutations in the tumor suppressor kinase LKB1 fail to restrain the activity of FZD receptors, whereas RNF43 loss fails to remove Wnt receptors from the cell surface.^{111,112} A recent study found that the new PORCN inhibitor ETC-159 is extremely effective against genetically characterized human CRCs with RSPO2/3 translocations.¹¹³

Targeting particular Wnt ligands or receptors that are identified to be overexpressed in malignancies is a further strategy for Wnt-receptor complex guided treatment. Salinomycin, a new LRP6 small molecule inhibitor, targets CSCs by inhibiting Wnt signaling through LRP6 degradation.¹¹⁴ In patient-derived xenograft mice models, tumor growth has been shown to be inhibited by the monoclonal antibody OMP-18R5 (OncoMed Pharmaceuticals/Bayer) by blocking Wnt ligand engagement.¹¹⁵ The information taken together suggests that Wnt ligand-receptor complexes can be targeted using an antibody or peptide therapy to inhibit Wnt signaling.

However, due to the extensive involvement of Wnt/ β -catenin signaling in regulating various biological processes, it is difficult to separate the role of Wnt signaling in normal tissue homeostasis from its function in cancer growth. Wnt signaling inhibitors may produce harmful side effects because of their on-target impact on normal Wnt pathway signaling.

Targeting β -Catenin Destruction Complex

The destruction complex, composed of APC, AXIN, GSK3, and CK1, is the core network that regulates β -catenin degradation and thereby serves as a cytosolic gatekeeper for β -catenin-mediated transcription. As the destruction

complex enforces endogenous tumor suppression, it represents an attractive target to attenuate WNT signaling. In theory, there are numerous paths to stabilize the destruction complex, but the best explored is the regulation of AXIN function via Tankyrase activity.

Loss-of-function mutations of AXIN or decreased AXIN expression have been found to increase the expression of the Wnt downstream targets.¹¹⁶ Therefore, mutations of the AXIN gene are deemed to be linked to numerous neoplasms, especially CRC.¹¹⁷ Tankyrase enzymes (TNKS1 and TNKS2) are members of the Poly ADP-Ribose Polymerase (PARP) family of proteins that act to PARsylate AXIN, marking it for degradation, thus destabilizing the destruction complex. Numerous studies conducted over the past decade have revealed independent TNKS inhibitors to be powerful suppressors of WNT pathway activity. Some of these studies have shown that TNKS blockade can prevent the proliferation of APC-mutant CRC cell lines in vitro and that it can be effective in vivo with APC-mutant adenomas.^{118,119} Despite the excitement surrounding TNKS inhibition as a potential therapy to cure the majority of APC-mutant CRCs, the field is shrouded in doubt as a result of the inconsistent information provided by numerous research regarding the toxicity of TNKS blocking. XAV939, initially identified as selectively inhibited β -catenin-mediated transcription via AXIN stabilization, stabilized AXIN by inhibiting TNKS1 and TNKS2.¹²⁰ In the APC-mutated CRC cell lines SW480 and DLD-1, XAV939 was also shown to block Wnt/ β -catenin signaling by raising the level of the AXIN-GSK3 β complex.¹²¹ G007-LK and G244-LM are novel tankyrase small-molecule inhibitors, exhibit about 50% inhibition of APC mutation-driven signaling by blocking poly(ADP-ribosylation)-dependent AXIN degradation in most CRC cell lines.¹¹⁸ Pyrvinium suppresses Wnt signaling and proliferation while specifically potentiating the CK1 α kinase activator, which is utilized to treat colorectal cancer HCT116 and SW480 cell lines with mutations of the APC gene or β -catenin.¹²²

However, more comprehensive reviews of these inhibitor analogs revealed intestinal toxicity, which restricts the full extent of the antitumor efficacy of these medications.¹¹⁸ Together, these studies highlight the therapeutic potential of tankyrase inhibitors for malignancies with APC mutations. However, due to the observation of nontumor-specific Wnt inhibition in preclinical models, safety concerns must still be addressed.

Targeting β -Catenin-Mediated Transcription

Gene transcription that is mediated by β -catenin is the final effector of Wnt pathway activation. It follows that suppressing this transcriptional response is the most effective way to prevent Wnt hyperactivation. However, genetic investigations have shown that complete deletion of β -catenin is blatantly harmful to normal intestinal epithelium.¹²³ Several teams have instead developed methods to prevent β -catenin from binding to particular transcriptional co-activators in order to combat this problem. With the description of ICG-001, a small chemical that specifically disrupts the interaction of β -catenin with CREB binding protein (CBP), but not p300, the first instance of this was published more than ten years ago.¹²⁴ This can prevent the essential stem and/or progenitor cell transition points controlled by β -catenin/CBP from switching to β -catenin/p300.¹²⁴

It has been demonstrated that disrupting the TCF/ β -catenin connection efficiently prevents target gene activation and reduces colorectal cancer cell growth in vitro.¹²⁵ The crucial connection between β -catenin and the transcription factor TCF4 is severely disrupted by LF3, a 4-thioureido-benzenesulfonamide derivative. In a mouse colon cancer xenograft model, LF3 also inhibited tumor development and promoted differentiation.¹²⁶ By weakening both Ras and β -catenin, KYA1797K/ KY1220 successfully inhibited the development of colorectal cancer cells.^{127,128} In vitro and in mouse cancer models, MSAB (methyl 3-benzoate), a selective inhibitor of Wnt/ β -catenin signaling, demonstrated a powerful and selective Wnt-dependent antitumor effect by downregulating Wnt/ β -catenin target gene CRC cells.¹²⁹ A further investigation revealed that the catenin responsive transcription inhibitors 3 (iCRT3), 5 (iCRT5), and 14 (iCRT14) similarly interfered with the TCF/ β -catenin connection and reduced the expression of Wnt-targeted genes in CRC cells.¹³⁰ To confirm how selective these compounds are in β -catenin/TCF targeting, to assess their toxicity, and to determine whether they have an impact on typical homeostatic tissue functions at the effective dose, further research into these compounds is necessary.

Chromatin state is also an influencing factor in regulating β -catenin-mediated transcriptional output. Chromatin chemical modifications include acetylation, deacetylation, methylation, demethylation, phosphorylation and dephosphorylation, as well as ubiquitination, ADP ribosylation, etc., through which DNA accessibility to transcriptional regulators

can be increased or decreased, thereby coordinating gene expression. In Wnt signaling, several related chromatin modifiers have been identified as activators or inhibitors of Wnt/ β -catenin transcription.¹³¹

Acetyltransferase p300/CBP-associated factor (PCAF) is a transcription cofactor, which has intrinsic histone acetyltransferase (HAT) activity.¹³² It is found that PCAF could increase the β -catenin transcriptional activity, induce its nuclear translocation, and up-regulate its protein level by inhibiting its ubiquitination and improving its stability.¹³³ Some studies show that Lysine-Specific Demethylase 1 (LSD1/KDM1A) activates the Wnt/ β -catenin signaling pathway by down-regulating the pathway antagonist DKK1, which contributes to colorectal carcinogenesis.¹³⁴ It is reported that the NAD-dependent deacetylase sirtuin-1 (SIRT1) deacetylates β -catenin and suppresses its ability to activate transcription and drive cell proliferation. Moreover, SIRT1 promotes cytoplasmic localization of the otherwise nuclear-localized oncogenic form of β -catenin.¹³⁵

Natural Bioactive Compounds

In recent years, accumulating evidence has revealed that some natural bioactive compounds from different dietary sources may regulate the activity of the Wnt signaling pathway in CRC to exert anti-cancer efficacy.¹³⁶

Flavonoids, a class of plant bioactive compounds comprised of a group of natural polyphenols, may effectively protect against CRC by modulating the Wnt signaling pathway.¹³⁷ For example, (-)-epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, exerts a preventive and anticancer effect on CRC by promoting the phosphorylation and proteasomal degradation of β -catenin through a mechanism independent of the GSK-3 β and protein phosphatase 2A (PP2A).¹³⁸ Another natural flavonoid, taxifolin, was shown to induce cell cycle arrest and tumor regression in CRC cells by activating Wnt/ β -catenin signaling pathway.¹³⁹

Anthocyanins/anthocyanidins (A/A) have emerged as potential phytochemicals capable of promoting relevant health benefits in CRC due to their known antioxidant and anti-inflammatory properties.¹⁴⁰ A/A exert their anti-tumor effects against carcinogenesis via multiple mechanisms, one of which is reducing Wnt signaling and suppressing abnormal epithelial cell proliferation.¹⁴¹ A pentacyclic triterpenoid called ursolic acid (UA) has been discovered in a wide range of fruits, spices, and medicinal plants. UA may suppress the malignant phenotype of CRC, induce apoptosis, and arrest the cell cycle, possibly attenuating the Wnt/ β -catenin signaling axis.¹⁴²

Natural bioactive compounds usually have very low toxicity compared to common chemotherapeutic agents and are easily available through the diet. These properties make them good assistant medicines, which can prevent the risk of cancer progression and reduce the side effects of anti-cancer drugs.

Conclusions and Future Directions

The role of the Wnt/ β -catenin pathway in the development of CRC is indisputable, not only in the initiation of colorectal cancer but also in its progression including cancer stem cell (CSC) propagation, angiogenesis, epithelial-mesenchymal transition (EMT) and chemoresistance. The past twenty years of intensive research have contributed to a deeper understanding of its components, regulations, target genes, functions, as well as the identification of novel activators and repressors. Understanding the potential genetic mutations of these targets is very important for the early diagnosis of CRC and the development of new targeted drugs.

Although numerous modulators of Wnt/ β -catenin signal have made promising progress in the experimental treatment, there is not yet a clinically useful method for its direct inhibition. The reason is the complexity of the signaling systems and their regulation; for the influence of one component could lead to unpredictable and potentially very serious consequences. The inability to selectively affect only tumor tissue is another limitation, as the Wnt signaling performs many essential physiological functions in the body. Additionally, considerable crosstalk between the Wnt/ β -catenin signaling and other signaling are important to designing therapeutic approaches.

Abbreviations

A/A, Anthocyanins/anthocyanidins; ABC, ATP-binding cassette; APC, Adenomatous Polyposis Coli; AXIN, axis inhibition protein; CAFs, cancer-associated fibroblasts; CBC, crypt base columnar; CBP, CREB-binding protein; CER, Cerberus; COX, cyclooxygenase; CRC, colorectal carcinoma; CSCs, cancer stem cells; DKK1, Dickkopf1; DVL, Disheveled; ECs, epithelial cells; EGCG, (-)-epigallocatechin-3-gallate; EMT, Epithelial-mesenchymal transition; FAP,

Familial Adenomatous Polyposis; FZD, Frizzled; GSK3, glycogen synthase kinase 3; HAT, histone acetyltransferase; ICAT, inhibitor of β -catenin and TCF-4; iCRT, catenin responsive transcription inhibitors; ISCs, intestinal stem cells; LEF/TCF, lymphoid enhancer-binding factor/T-cell factor; LGR5, leucine-rich G protein coupled receptor 5; LRP5/6, Lipoprotein receptor-related protein 5/6; LSD1, Lysine-Specific Demethylase; MDR, Multidrug resistance; MDR1, Multidrug resistance protein 1; MSAB, methyl 3-benzoate; Non-steroidal anti-inflammatory drugs (NSAIDs); PACF, p300/CBP-associated factor; PARP, Poly ADP-Ribose Polymerase; PCP, planar cell polarity; PORCN, Porcupine O-acyltransferase; PP2A, protein phosphatase 2A; RNF43, ring finger 43; RSPO, R-Spondins; RUNX1, Runt-related transcription factor 1; sFRPs, secreted FZD-related proteins; SIRT1, sirtuin-1; TM4SF1, Transmembrane-4L-6 family member-1; TNKS, Tankyrase; UA, ursolic acid; VEGF-A, vascular endothelial growth factor-A; WIF, Wnt inhibitory factor; Wls, Wntless protein; ZNRF3, zinc and ring finger 3.

Disclosure

The authors report no conflicts of interest in this work.

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