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Genomic insights into WNT/ β -catenin signaling

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Abstract

The canonical WNT pathway regulates the stability of the proto-oncogene β -catenin and is aberrantly activated in many cancer types. Studies in a wide range of experimental models confirm that β -catenin activity is required for tumor initiation in cancers where this pathway is deregulated. However, to date this pathway has proven to be challenging to target therapeutically. Moreover, several lines of evidence suggest that other components and regulators of β -catenin exist. Here we will describe recent structural and functional studies describing genomic alterations and new regulators of β -catenin that lead to aberrant activation of the WNT/ β -catenin pathway. These findings provide new insights into the biology of WNT/ β -catenin signaling and suggest potential therapeutic opportunities.

Keywords

WNT; cancer; β -catenin; YAP1; synthetic lethality

The canonical WNT pathway

WNT signaling is involved in diverse processes including embryonic development, maintenance of tissue homeostasis and cancer pathogenesis. The Wingless (*WNT*) gene was first identified in a random mutagenesis screen in *Drosophila melanogaster* [1]. Mutations in *WNT* resulted in loss of wing development and defects in larval segmentation. Subsequent experiments demonstrated that mutations in *WNT* or *ARM* (the *D. melanogaster* β -catenin ortholog) result in a similar segmentation phenotype [1]. Genetic complementation screens and biochemical studies led to the finding that WNT signaling inactivates a cytosolic protein complex (the destruction complex) that regulates β -catenin stability [1].

Binding of WNT ligands to the FZD/LPR6 receptor inactivates the destruction complex, which stabilizes β -catenin [2]. The destruction complex, composed of APC, AXIN1, GSK3 β and CSNK1A1, phosphorylates serine residues in β -catenin leading to its ubiquitination by β TRCP and degradation by the proteasome. Activation of the WNT pathway was previously

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thought to result in disassembly of the destruction complex. However, recent work has shown that upon binding of WNT to the FZD/LPR6 receptor, the destruction complex remains intact and bound to AXIN1. In this state, newly synthesized β -catenin is not recognized by the destruction complex [3]. Stabilized β -catenin directly binds to nucleoproteins Nup62, Nup153 and RanBP2 facilitating its nuclear translocation [4]. In the nucleus, a β -catenin TCF/LEF complex regulates transcription of specific target genes [2] (Figure 1).

WNT in Development

Early studies implicated WNT signaling as a critical regulator of early vertebrate development [5]. Injection of *Xenopus* embryos with mRNA encoding positive regulators of the WNT pathway such as *WNT1*, β -catenin or *LEF1* inhibited formation of the anterior posterior axis and resulted in body axis duplication [5]. In consonance with these observations, β -catenin deletion results in early lethality due to defects in the formation of the anterior posterior axis [5]. Studies in flies revealed that a gradient of WNT ligand found throughout the developing embryo determines the anterior-posterior axis by differential activation of β -catenin [6]. The WNT pathway also regulates cell fate and is essential for differentiation of embryonic stem cells into the endoderm and mesoderm lineages [5]. In adult animals and humans, WNT signaling is also essential to maintain the stem cell compartment in self-renewing organs such as the intestine and hair follicle [5].

During development β -catenin regulates transcription by forming a complex with *TCF7L2* (also known as *TCF4*) or *LEF1* [2]. Indeed, *TCF7L2* null mice fail to develop the small intestine and die within 24 hours of birth [7]. Moreover, both *TCF7L2* and β -catenin have been shown to co-occupy many promoters [8].

WNT activity in cancer pathogenesis

WNT signaling plays an important role in the pathogenesis of several types of human cancers. In a seminal paper, Nusse and Varmus showed that integration of the mouse mammary tumor virus (MMTV) in the mammary epithelium induces mammary tumors by forcing the expression of the proto-oncogene *Wnt1* [9]. Moreover, individuals carrying a germline *APC* mutation develop familial adenomatous polyposis (FAP). Patients affected by FAP develop hundreds of colonic polyps, which progress inevitably to malignant colon cancer [10]. Subsequent studies identified recurrent mutations in components of the WNT signaling pathway in sporadic colon cancers [11].

Recent large-scale sequencing efforts have identified several new recurrent mutations in components of the WNT signaling pathway [11]. Surprisingly, these efforts have identified co-occurrence of mutations in positive and negative regulators of the WNT pathway, suggesting that WNT signaling in cancer is more complex than was previously appreciated. In this review, we will describe emerging evidence suggesting that β -catenin is a modular transcription factor activating distinct context dependent transcriptional programs.

Genomic alterations in components of the WNT/ β -catenin pathway

WNT ligands

WNTs are an evolutionary conserved family of secreted glycoproteins [12]. There are 19 distinct human WNTs that bind specific receptors and activate β -catenin-dependent and independent pathways [12], in part explaining the diverse pathways and biological processes regulated by WNT signaling [12]. Indeed, forced expression of 14 of the 19 WNT ligands in human cell lines stabilizes β -catenin [13].

Several WNTs have been reported to be involved in cancer initiation and progression through autocrine or paracrine mechanisms. For example, expression of Wnt1 in murine mammary tissue leads to development of mammary tumors [9]. *WNT3A* and *WNT10B* are overexpressed in triple negative breast cancer [14]. In multiple myeloma, secreted WNT3A has been reported to induce migration and invasion through activation of RhoA kinase and PKC [15].

In contrast to these WNTs, other WNT ligands do not appear to contribute to cancer initiation. A commonly used model to assess WNT transforming activity is the mouse mammary cell line C57MG. When transformed, C57MG cells undergo morphological changes and are able to form foci (clusters of cells) when seeded at low densities. A transformed phenotype was observed in C57MG cells following expression of Wnt1, 2, 3A and 5B but not Wnt6, 4 or 5A [16].

Why do some WNT ligands promote β -catenin stabilization yet not promote cancer? One possible explanation is that, in addition to control of β -catenin signaling, specific WNTs in particular contexts activate or inhibit pathways that contribute to or inhibit transformation. In support of this view, Green *et al.* reported that *WNT3A* promotes or inhibits tumor growth in a context dependent manner [17]. Specifically, co-injection of fibroblasts overexpressing *WNT3A* and patient-derived triple negative breast cancer into immunodeficient mice resulted in accelerated growth of some cancers, whereas others were inhibited. It is clear that further work will be necessary to identify the key factors that specify the response to WNT signaling.

R-Spondin (RSPO)

Using *Xenopus*-derived cDNA expression libraries, Kazanskaya *et al.* identified *RSPO2* as a potent activator of WNT activity [18]. The human *RSPO* family consists of four members (*RSPO1-4*) that encode proteins with unique developmental functions [19]. Cells treated with both WNT and *RSPO1-4* show a synergistic activation of WNT signaling [18] suggesting that all four *RSPO* family members potentiate WNT signaling. Furthermore, the expression pattern of *RSPO* closely resembles that of *WNTs* in mouse development [20], indicating a physiological role for *RSPO* in WNT signaling.

Two models have been proposed to explain the synergistic activity of WNTs and *RSPO*. The first model postulates that *RSPO* tethers the LGR4/5 and LPR6 receptors enabling more efficient activation of FZD receptors by WNTs [21]. The second model instead suggests that binding of *RSPO* to the LGR4/5 receptor stabilizes LPR6 by inhibiting the ubiquitin ligases RNF43 and ZNRF3 from targeting LPR6 to proteasomal degradation [22]. Treatment of cell lines with *RSPO1* increased the interaction between ZNRF3 and LGR4 and suppressed degradation of the LPR6/FZD receptor [23]. Furthermore, colonic organoids derived from RNF43 and ZNRF3 compound knockout mice can be maintained in culture without addition of *RSPO1*, further supporting the second model [22].

Using RNA sequencing to analyze 70 tumor-normal primary colon cancers, Seshagiri *et al.* identified recurrent *RSPO2* and *RSPO3* fusions in 10% of colon cancer samples [24]. These fusions were mutually exclusive with *APC* mutations, suggesting that these two alterations activate the same pathway. Indeed, *RSPO2* and *RSPO3* fusions expressed in 293T cells were secreted into the culture media and synergized with *WNT3A* to stimulate β -catenin signaling [24].

β-catenin (CTNNB1)

The destruction complex phosphorylates serine and threonine residues on β-catenin leading to its degradation (Figure 1). Specifically, GSK3β phosphorylates Ser33 and Ser37 and CSNK1A1 phosphorylates Thr41 and Ser45 [25]. β-catenin mutations affecting these residues render such mutants refractory to regulation by the destruction complex and lead to constitutive β-catenin signaling. β-catenin mutations are found in various types of cancers, including 30% of hepatocellular and endometrial cancers [11, 26].

Experiments in a wide range of model systems have shown that both activating β-catenin mutations and inactivating *APC* mutations lead to increased β-catenin transcriptional activity (Figure 1). However, several lines of evidence suggest that *APC* and β-catenin have distinct functions in tumor development. Re-expression of WT *APC* in cell lines with inactivating *APC* mutations results in widespread apoptosis [27]. In contrast, suppression of β-catenin expression in the same cell lines results in a G1 cell cycle arrest and does not induce an apoptotic response [28]. Furthermore, although many tumor types harbor mutations in components of the WNT pathway, the prevalence of these mutations is organ specific [11]. For example, colon cancers harbor frequent *APC* but not β-catenin mutations, whereas in gastric and endometrial cancers, β-catenin mutations are more common, suggesting that these mutations have additional tissue specific functions.

APC

Germline *APC* mutations are found in individuals affected by FAP, and the majority of sporadic colon cancers harbor inactivating *APC* mutations [11]. Although less frequent, *APC* focal copy number loss is found in a subset of ovarian, lung, breast and colon cancers. *APC* homozygous deletions are rare (1–3% in colon cancer) suggesting a gain of function role for mutant *APC*. In support of this notion, Rai *et al.* reported that *APC* also regulates cell fate by inhibiting the activity of the DNA demethylase CtBP1 independent of its role in regulating β-catenin [29].

Massively parallel sequencing of 212 primary colorectal cancers identified reoccurring loss of function mutations in *AMER1* (also known as *FAM123B* or *WTX*), a negative regulator of *APC* [11]. *AMER1* mutations are found in 30% of Wilm's tumors (a rare childhood kidney cancer) and have been shown to negatively regulate WNT signaling [30]. Interestingly, in colon cancer, *APC* and *AMER1* mutations are not mutually exclusive, suggesting these proteins have distinct roles that are not related to WNT signaling. Future studies are necessary to determine the roles of *AMER1* and *APC* in tumor progression.

AXIN1, CSNK1A1 and GSK3β

Deletion of *APC* or *GSK3β* in the murine intestine activates the WNT/β-catenin pathway, resulting in rapid development of adenomas and early lethality [31]. Similarly, intestine-specific deletion of *Csk1a1* in mice leads to nuclear accumulation of β-catenin and increased transcription of WNT target genes [32]. However, in contrast to *Apc* null mice, these mice display normal gut homeostasis and do not exhibit an increased incidence of adenomas or carcinomas. Acidic β-gal staining, a commonly used marker of senescence [33], was observed in the intestine of *Csk1a1* null mice, suggesting that activation of the WNT pathway by deletion of *Csk1a1* resulted in oncogene-induced senescence. However, deletion of *Csk1a1* failed to induce colon cancer unless accompanied by co-deletion of *Trp53* [32], suggesting that inhibition of CSNK1A1 in tumors with wild-type p53 may induce senescence.

Unlike *APC*, recurrent *AXIN1*, *CSNK1A1* and *GSK3 β* mutations have not been identified in human cancers. *AXIN1* deletions are found in a subset of cancers including colon, breast ovarian and melanoma [34].

TCF7L2 (TCF4)

Genomic characterization of human colorectal tumors identified recurrent inactivating mutations and focal copy number losses involving *TCF7L2* that often co-occur with *APC* mutations [11]. Specifically, 26 of 212 colorectal cancers (12%) harbored *TCF7L2* mutations. Surprisingly, of these 26 mutations, five samples had inactivating *TCF7L2* mutations (frameshift or non-sense mutations) that result in truncation of *TCF7L2* DNA binding domain and would be predicted to fail to activate β -catenin [35]. In addition, co-occurrence of *TCF7L2* homozygous deletion and *APC* inactivating mutations was found in 4 tumors. These observations demonstrate that in a significant fraction of WNT deregulated colon cancers *TCF7L2* is deleted or functionally inactivated.

Whole genome sequencing analysis on a subset of these cancers identified recurring translocations (3 cases) between *TCF7L1* (also known as *TCF3*), a well-known β -catenin transcription regulator, and *NAV2* [11]. These translocations are similar to a recurring translocation identified between *TCF7L2* and *Viii* [36]. Surprisingly, these translocations result in the expression of *TCF7L1* or *TCF7L2* that lack a β -catenin binding domain and are predicted to act as dominantly interfering alleles [35]. Indeed, suppressing the expression of the *TCF7L2-Viii* fusion in colon cancer cell lines that harbor this translocation inhibited anchorage independent growth [36].

In consonance with these observations, colon specific, heterozygous deletion of *TCF7L2* in *Apc* null mice [37] led to the unexpected finding that these mice developed aggressive colon cancers, compared to *Apc* null mice, which only developed colonic adenomas. Analysis of the aggressive tumors demonstrated loss of heterozygosity (LOH) of the remaining *TCF7L2* allele. Together these observations suggest that although β -catenin and TCF family members are required for adenoma formation, loss of TCF family members is associated with tumor progression.

Functional genomics and WNT signaling

Functional studies in model organisms and cultured cells have been instrumental in deciphering the WNT/ β -catenin pathway. The use of large scale loss of function approaches in cancer cell lines have enabled a detailed view of the components directly involved in regulation of the WNT/ β -catenin pathway and the various pathways that interact with the WNT/ β -catenin pathway in tumor development.

Cyclin-dependent kinase 8 (CDK8)

The serine-threonine kinase *CDK8* was originally identified as a Cyclin C binding partner [38]. *CDK8*, together with *Cyclin C*, *MED12*, and *MED13*, form the kinase module of the Mediator complex, a large protein complex involved in transcription regulation [39]. Although *CDK8* was initially described as a transcription repressor, further work showed that *CDK8* may also activate transcription [40]. Furthermore, biochemical fractionation of *CDK8* from cultured human cell lines identified a second *CDK8* complex that is not associated to the mediator complex, suggesting that *CDK8* may also have functions independent of its role as part of the mediator complex [41].

CDK8 was identified as a gene that regulates β -catenin driven reporter activity in a loss of function RNAi screen [42]. *CDK8* is essential for both proliferation of β -catenin-dependent cancer cell lines and for β -catenin transcriptional activity. *CDK8* is amplified and

overexpressed in 60% of colorectal cancers. Furthermore, high levels of *CDK8* are associated with nuclear β -catenin and colon cancer mortality [43, 44]. Together these observations suggest that *CDK8* is an amplified oncogene. Recent studies have suggested that in addition to *CDK8*, co-amplification of *CDX2* may also contribute to colon cancer progression [45].

Several roles have been suggested for *CDK8* regulation of β -catenin activity. Studies in *Drosophila* flies suggest that *MED12* and *MED13* regulate β -catenin transcriptional activity through interactions with Pygopus (*PYGO*) a well-annotated β -catenin cofactor [46]. In addition, inhibition of WNT activity blocks *CDK8*-mediated cell transformation [42].

CDK8 has also been reported to attenuate *E2F1* inhibition of β -catenin [47]. Inactivation of the RB pathway induces transcription of *E2F1*, a potent β -catenin inhibitor [47]. Studies in flies and human cultured cells demonstrated that *CDK8* inactivates *E2F1* by phosphorylation of *E2F1* on serine 375 relieving the repression of *E2F1* on β -catenin [47].

YAP1 and TAZ

Using genome scale loss-of-function screens, the transcriptional modulator *YAP1* has been shown to bind β -catenin and modulate its function [48]. The *YAP1*- β -catenin complex also includes the SRC tyrosine kinase *YES1* and the transcription regulator *TBX5*. This complex drives the expression of survival genes such as *BCL2L1* and *BIRC5* (Figure 1). Dasatinib, a small molecule inhibitor of *YES1*, was shown to inhibit the proliferation of β -catenin-dependent cell lines and tumors.

Over-expression of a mutant constitutively active form of *YAP1* (*YAP1* S127A) in the murine intestine leads to early morbidity due to loss of intestinal crypts [49], associated with inhibition of β -catenin signaling. Deletion of *YAP1* in the murine intestine resulted in hyperproliferation of intestinal stem cells and increased WNT signaling following injury. Mechanistically, *YAP1* was shown to inhibit WNT signaling by cytosolic retention of *DVL2*. These observations are consistent with the dual role β -catenin has in gut homeostasis and tumor progression. As discussed above, *TCF7L2* is essential for the functions of β -catenin in gut development and interferes with β -catenin activity in tumorigenesis. Similarly, these observations suggest that *YAP1* interferes with β -catenin functions in homeostasis and promotes β -catenin activity in tumor pathogenesis.

Similar to *YAP1*, a homolog *TAZ*, has been shown to be required in β -catenin driven cancers [50]. Phosphorylated β -catenin binds to *TAZ*, leading to proteasomal degradation of the two proteins (Figure 1). Mutations that inactivate the destruction complex stabilize *TAZ*, which subsequently activates transcriptional programs that lead to proliferation and survival of cancer cells.

Mutations in *YAP1* or *TAZ* have not been identified in cancer. *YAP1* amplifications are found in a subset of hepatocellular and ovarian cancers, and hepatocellular cancer cell lines that harbor *YAP1* amplifications depend on *YAP1* for survival [51].

MAPK pathway (KRAS and BRAF)

Activating *KRAS* and *BRAF* mutations are often found in cancers that harbor mutations that activate the WNT pathway [11]. Furthermore, functional studies suggest a synergistic interaction between the WNT and MAPK pathways.

Genetically engineered mice that harbor only loss of function *APC* mutations or gain of function *KRAS* mutations fail to develop malignant colon adenocarcinomas. Specifically, *APC* mutant mice develop adenomas that rarely progress to full adenocarcinoma, and

expression of mutant *KRAS* (*KRAS*^{V12G}) in the murine intestine results in oncogene-induced senescence with no occurrence of cancer [52]. In contrast, compound genetically engineered mice harboring *APC* mutations and *KRAS*^{V12G} develop invasive colon adenocarcinoma accompanied by enhanced morbidity and mortality [53]. Tumors from these mice show increased nuclear β -catenin [52] and enhanced WNT reporter activity [53], suggesting an interaction between *KRAS* effector pathways and WNT signaling. Consistent with these observations, Jeong *et al.* [54] demonstrated that in murine and human colon cancers activation of the WNT pathway leads to activation of the MAPK pathway.

Further supporting the connection between WNT and MAPK signaling, Singh *et al.* [55] reported that *MAP3K7* (*TAK1*) is upregulated and essential in WNT-deregulated colon cancer cell lines that are dependent on *KRAS*. Both *KRAS* and *TAK1* regulate WNT signaling in *KRAS*-dependent colon cancer cell lines. In these cell lines, *KRAS* up-regulates the expression of *BMP7* resulting in activation of the *BMPRIA* receptor. This feedback loop activates both *TAK1* and β -catenin resulting in amplification of β -catenin activity (Figure 1). Furthermore, *TAK1* upregulates NF- κ B activity, resulting in additional proliferative and survival signals. In consonance with these findings, NF- κ B signaling is increased in genetically engineered mice expressing a hyper-activated β -catenin allele, which promoted the proliferation of colonic stem cells [56] (Figure 1). Because *TAK1* phosphorylates *TBK1* [57], a serine-threonine kinase, that activates NF- κ B and interferon signaling and is required for the survival of *KRAS*-driven cancers [58], these observations suggest that *KRAS* and WNT signaling cooperate at several levels to drive colon cancer progression.

Pharmacological targeting of the WNT pathway

The WNT/ β -catenin pathway plays a key role in colon cancer pathogenesis, yet pharmacological targeting has proven to be challenging. Because β -catenin, the major effector of the WNT pathway, drives various context dependent transcriptional outputs that contribute to diverse phenotypes, effective targeting of β -catenin driven cancers will most likely require inhibition of multiple β -catenin-driven pathways.

Several new compounds have recently been described as inhibitors of different nodes of the β -catenin signaling pathway (Table 1). One class of β -catenin inhibitors restores the activity of the destruction complex (Figure 1). Using a WNT reporter screen Chen *et al.* identified IWR1-4 as a class of WNT inhibitors that enhance the activity of the destruction complex by stabilizing AXIN1 [59]. Using a similar approach Cong *et al.* reported that XAV939, a tankyrase 1/2 inhibitor, stabilized AXIN1, which led to β -catenin degradation [60]. Detailed genetic and biochemical studies demonstrated that tankyrase 1/2 stabilize AXIN1 through ADR ribosylation [61]. Recently, a more potent tankyrase inhibitor (G007-LK) has been reported to inhibit the growth of tumors in mice [62]. However, these inhibitors target β -catenin also in normal tissues such as the intestine and may lead to dose-limiting toxicity [62].

A second class of WNT inhibitors target specific nodes within the WNT signaling pathway. Large scale profiling of small molecule inhibitors in a large panel of cancer cell lines identified Navitoclax, a BCL2L1 inhibitor, as having activity in β -catenin mutated cancers [63]. Other compounds such as dasatinib [48] or 5Z-7-oxozeaenol [55] inhibit other nodes of the WNT/ β -catenin pathway (Table 1). Future studies using combination therapies that target multiple nodes of this pathway are likely to result in more potent and durable inhibition of WNT dependent cancers.

Concluding remarks

Although the WNT/ β -catenin pathway has been extensively studied both in development and in cancer, recent genomic studies in colon cancer have identified new components and regulators of the canonical WNT/ β -catenin pathway. These studies suggest that WNT/ β -catenin signaling is more complex than anticipated and may be influenced by context. Further studies will be necessary to understand how WNT/ β -catenin signaling contributes to both tumor initiation and progression.

Indeed, the identification of additional components and regulators of WNT/ β -catenin signaling may also provide new approaches to therapeutically target WNT/ β -catenin deregulated cancers. The identification of genetic alterations in both canonical and new components of WNT/ β -catenin will inform studies to develop new modulators as well as identify cancers that are likely to respond to such manipulations.

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Highlights

Mutations in the WNT/ β -catenin pathway occur in many types of human cancers

New components of the WNT/ β -catenin pathway have been identified

β -catenin forms several transcriptional complexes

β -catenin and RAS signaling interact

Figure 1.

Multiple pathways regulate β -catenin signaling. In normal homeostasis, the destruction complex regulates β -catenin stability (top). Mutations in components of the destruction complex lead to stabilization of β -catenin and activation of various context-dependent transcriptional complexes.

Table 1

Genomic alterations in pathways that regulate β -catenin activity

Pathway	Biological function	Components	Genomic alterations	Tissue	Compounds
Pathways that regulate β -catenin stability					
Destruction complex	Targets β -catenin for proteasomal degradation	APC	LOF Mutations and Deletions	Colorectal, Gastric, Liver,	
		GSK3B			
		AXIN1	Deletions	Melanoma, Liver	XAV939, IWR, G007-LK
		AXIN2	LOF Mutations		
		CSNK1A1			
WNT ligand signaling	Inhibits the destruction complex and activates β -catenin	WNTs	Amplifications	Breast, Ovarian, Pancreas	
		PORCN			IWP, LGK974
		RNF43	LOF mutations	Endometrial, Gastric,Colorectal, Pancreas	
		ZNRF3			
		RSPO	Translocations		
		LRP5/6			
		FZD5/6			Neutralizing antibody
Pathways that interact with β -catenin signaling					
MAPK	Interacts with the WNT pathway and enhances the activity of WNT signaling	KRAS	GOF mutations and Amplifications	Colon, Pancreas, Gastric, Lung	
		BRAF	GOF mutations	Melanoma, Colon	Vemurafenib
		TAK1			5Z-7-oxozeaenol
		YES1/SRC			Dasatinib
YAP1/TAZ	Forms a transcriptional complex with β -catenin	YAP1	Amplification	Liver, Medulloblastoma	
		TAZ			
		TBX5			
		BCL2L1			Navitoclax
		BIRC5			YM-155
TCF/LEF	Development and adenoma formation	TCF7L2	LOF mutations, Deletions, Translocation	Colorectal	
		TCF7L1	LOF Translocations	Colorectal	

Pathway	Biological function	Components	Genomic alterations	Tissue	Compounds
		TCF7			
		LEF1			