Wnt signaling and cancer development: therapeutic implication
Minireview

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Wnt proteins are a large family of secreted glycoproteins that activate signal transduction pathways to control a wide variety of cellular processes such as determination of cell fate, proliferation, migration, and polarity. Wnts are capable of signaling through several pathways, the best-characterized being the canonical beta-catenin/Tcf-mediated pathway. Canonical Wnts stabilize β-catenin protein, which has implications in the genesis of many human cancers like non-small cell lung cancer, colorectal carcinoma, prostate cancer, breast cancer and many others. In all of these cancers the common denominator is the activation of target genes. Although detailed mechanisms are not well understood of why Wnts are overexpressed in one tumor and down regulated in another, the pleiotropism of Wnt signaling is evident. The pathway itself offers ample targeting nodal points for cancer drug development. The identification of many important regulatory genes and the mechanism of their function offer an opportunity to develop new therapies targeting this pathway.

In this review, we describe the roles of several oncogenes of the Wnt/β-catenin signaling pathway in the development of tumorigenesis and discuss few strategies that are already developed or can be explored to target key components of the Wnt/β-catenin signaling pathway in finding of anti-cancer drugs.

Key words: Wnt signaling, Oncogene, β-Catenin, Neoplasm, Tumorigenesis, Colorectal cancer, Prostate cancer, Anti cancer drug.

The term "Wnt" was coined from a combination of the Drosophila segment polarity gene *Wingless* and the mouse proto-oncogene *Int-1* [1]. The Wnt extracellular signaling pathway (wingless in *Drosophila*) is one of a handful of evolutionarily conserved signal transduction pathways used extensively during animal development, from Hydra to humans [2, 3]. Wnt proteins constitute a large family of secreted proteins that act as extracellular signaling factors. They function in cell fate determination, patterning in embryogenesis, in the regulation of cell growth and differentiation in a variety of organ systems. There are 19 human WNT genes [4], few of which encode additional, alternatively spliced isoforms [5]. Historically, Wnt proteins have been grouped into two classes-canonical and noncanonical-on the basis of their activity in cell lines or *in vivo* assays.

Wnt signaling has been studied primarily in developing embryos, in which cells respond to Wnts in a context-dependent manner through changes in survival and proliferation, cell fate and movement. But Wnts also have important functions in adults, and aberrant signaling of Wnt pathways is linked to a range of diseases. Altered function or levels of components of the Wnt/β-catenin pathway are associated with proliferative diseases including cancer, as well as Alzheimer disease, osteoarthritis, tooth development, and diseases of the bone, eye and heart [6] and that is why the Wnt/β-catenin signaling cascade increasingly attracts considerable attention of cancer researchers and pharmacologists**.** The number of identified Wnt-associated genes has increased dramatically in recent years. Dysfunctional Wnt/β-catenin signaling, which creates continuous transcription of many target genes sup-

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Abbreviations: Wnt: Wingless-type MMTV integration site family member, APC: Adenomatous polyposis coli, COX-2 : Cyclooxygenase 2, FAP: Familial adenomatous polyposis, Dkk: Dickkopf, Dsh: Dishevelled, LEF: Lymphocyte enhancer factor, Fz: Frizzled, GSK-3: Glycogen synthase kinase-3, ICAT: Inhibitor of β-catenin and Tcf-4, PP2A: Protein phophatase 2A, Pygo: Pygopus, sFRP: Secreted frizzled-related protein, siRNA: Small interfering RNA, Tcf: T-cell factor, LRP: Lipoprotein Receptor Related Protein

porting cell proliferation, has now been documented in a wide range of cancers, including colorectal cancer, melanoma, gastric cancer, and tumors derived from hepatic, breast, and prostate tissue. [7–13]. Numerous studies suggest that activation of the Wnt/β-catenin signaling pathway plays an important role in human tumorigenesis [14–16].

A hallmark of Wnt signaling pathway activation is the enhanced level of cytoplasmic β-catenin protein. Stabilization of cellular β-catenin, leading to elevated protein levels and constitutive gene activation, has been supposed as an important step in many human cancers [12]. The common finding of tumorigenesis is the inhibition of forming right β-catenin degradation complex and the consequences is the stabilization of cytoplasmic β-catenin protein, which translocates to the nucleus and consequently express Wnt target genes. Mutant β-catenin protein becomes more stable because it is capable of avoiding APC-mediated degradation by cellular proteasomes. Stabilization of β-catenin can occur through mutation of the β-catenin gene itself or the genetic defects in other protein members in the Wnt signaling pathway like Axin, APC and T-cell factor (Tcf). Molecular studies have pinpointed activating mutations of the Wnt signaling pathway as the cause of approximately 90% of colorectal cancer (CRC) which is a global concern since it accounts for over 50,000 cancer-related deaths every year in the United States alone [17]**.** Colorectal cancer developed many mutations of key oncogenes including APC (adenomatous polyposis coli) and p53**.** Prostate cancer is another one of the most frequently diagnosed cancer among men in the U.S. and, after lung cancer, is their second leading cause of cancer death.

Research progress in the Wnt–Frizzled area and the deepening appreciation of its fundamental importance and complexity over this past decade have coupled with dramatic technological advances to transform Wnt signaling into a crucial area of active research specifically for the finding of anti-cancer drugs. Investigators are working to identify and understand the differences within the Wnt and Fz family members, all of the elements in the known Wnt pathways, and the complex mechanisms by which the Wnt signal acts and it's dysfunction. The most recent focus–spurred by the Wnt/β-catenin pathway's prominent involvement in a widening range of malignancies–is on this pathway's therapeutic potential, with the goal of blocking β-catenin's nuclear function or inhibing it's expression or enhancing the proteasomal degradation of this protein.

Although during the last few years several novel molecular data have contributed to the understanding of the complexity of the Wnt signaling pathway, many of the underlying mechanisms still remain unknown. Both genetic, epigenetic and expression alterations of molecules in the Wnt signaling pathway are characteristic for human solid tumors. Therefore, a future perspective, when it comes to anti-cancer therapeutics, would be to block the β-catenin-Tcf complex and thereby transcription of Wnt target genes.

In this review we discuss the most studied canonical signaling mechanism in Wnt/β-catenin pathway and describe how the stabilization of β-catenin protein leads to cause different kinds of human tumorigenesis. We also discuss about different oncogenes/protooncogenes that have been identified in the Wnt/β-catenin signaling pathway and few strategies for developing anti-cancer drugs particularly biological based agents and report the name of recently discovered few important drugs that act against several kinds of cancer developed due to dysfunction of Wnt signaling.

Oncogenes and tumor suppressors genes in Wnt signaling. More than 20 Wnt/β-catenin target genes have been identified so far and the list is still growing. Among them many are regulators of cell proliferation, developmental control and genes involved in tumorigenesis, such as Axin-2, Tcf, *c-myc*, *cyclin D1*, *metalloproteinases*, or *VEGF* (a complete list of target genes is available on http://www.stanford.edu/~rnusse/) [18, 19] and many others. Among them many genes were reported to be oncogenes/protooncogenes or tumor suppressors. Significant progress in understanding mechanisms of carcinogenesis has developed from the discovery of oncogenes and protooncogenes and the subsequent discovery of tumor suppressors and mutator genes. Although reports of mutations in Wnt itself in human cancers are rare, yet mutations in its downstream targets are frequent [20, 21]. Oncogenes are cellular or viral (i.e., inserted into the cell by a virus) and their expression can cause the development of a neoplasm [22]. Protooncogenes are normal cellular genes; their conversion to oncogenes can occur via several mechanisms such as amplification or modification [22]. Tumor suppressors (anti-oncogenes, recessive tumor genes) are cellular genes; their inactivation increases the probability of tumor formation, whereas restoration of their functioning may suppress the growth of tumor cells [22]. Few oncogenes/protooncogenes are listed below.

β-catenin. The oncogenic role of β-catenin was prominent by the discovery in which activating β-catenin mutations were detected in approximately 50% of the colorectal cancers that contained wild type APC [1, 8, 23, 24]. β-catenin mutations were detected in 5 of the 104 prostate cancer tissue samples. Four of the five mutations involved serine or threonine residues implicated in the degradation of β-catenin. Mutational analysis of multiple regions from several tumor samples showed that the β-catenin mutations were present focally and therefore may occur during tumor progression [12]. Oncogenic mutations of β-catenin have been found in many other human tumors also. In fact, the critical role of β-catenin in tumorigenesis has recently been demonstrated in a variety of animals models [23, 25, 26], whereas mutations in β-catenin gene have been frequently demonstrated in tumors induced by either carcinogens or activated oncogenes [15].

APC. The most well known dysfunctional gene is *APC* in the Wnt/β-catenin pathway, responsible for the inherited disease familial adenomatous polyposis colonis and also mutated in 70% to 80% of sporadic colon tumors [27]. The *APC* gene was first found as the genetic cause for familial adenomatous polyposis (FAP). FAP patients develop large numbers of

colorectal polyps in early adulthood [28]. Without any interventions, many of the polyps can further develop into carcinomas and metastatic colorectal cancers. Further studies have strongly established that APC tumor suppressor gene functions as a gatekeeper in colorectal tumorigenesis.

Axin. The tumor suppressor Axin is an intracellular protein that binds to the APC/GSK3β/CKIα complex (Fig.1) and plays a central role in regulating β-catenin degradation [29-31]. Hence, the loss of function of Axin results in elevated nuclear β-catenin and consequently increases expression of the target genes such as Cyclin D1 and c-Myc. Mutations of the Axin gene and/or its loss of expression have been found in numerous human neoplasms such as colorectal cancer, esophageal squamous cell carcinoma, and medulloblastoma, hepatocellular carcinomas and a variety of pediatric neoplasm [32-36]. The common finding among these neoplasms is an increase in nuclear β-catenin because it is able to escape the ubiquitin-dependent degradation pathway.

Tcf/LEF. Although activating mutations in Tcf/LEF genes are rare in human cancers, a frequent frameshift mutation in Tcf-4 has been found in both human colorectal cell lines and primary tumors [37,38]. Kramps *et al.* have identified two new components, BCL9 and pygopus (Pygo), in the β-catenin-Tcf/LEF nuclear complex where it was reported that Pygo, linked to β-catenin by BCL9, permits the β-catenin-Tcf/LEF complex to transcriptionally activate Wnt target genes [39].

Wnt5A. Wnt 5A can act to counteract signaling by other Wnts and it was reported to act as a tumor suppressor also, as mice that are mutant for Wnt-5A develop lymphoid malignancies [40]. Studies reported that Wnt-5A is involved in metastatic melanoma progression and invasive ductal breast cancer via adhesion and migration alterations [41]. Evidence has been found that members of the sFRP family are epigenetically inactivated in colon cancer [42]. Because the sFRPs may sequester and inactivate Wnt, loss of sFRP expression may contribute to Wnt activation in cancer.

β-TrCP. β-TrCP is the F-box protein that control degradation of phosphorylated β-catenin. Recently, this gene was found mutated in a human prostate cancer cell line and in gastric cancers [43,44]. Both alterations were heterozygous, but *in vitro* studies showed that they rendered the β-TrCP protein deficient in β-catenin binding and accumulation of nuclear β-catenin was observed. Wild type *APC* and *CTNNB1* were seen in both cases suggesting that β-*TrCP* might substitute for *APC* and *CTNNB1* mutations in prostate cancer. Interestingly, increased expression of β-TrCP is detected in cells with an activated Wnt signaling pathway, indicating that β-TrCP is involved in a negative feedback regulation mechanism. Recently five missense mutations (5.3%): A99V, H342Y, H425Y, C206Y and G260E have been found in the development in gastric cancer [44].

PP2A. The *PP2R1B* gene, which encodes the β isoform of the A subunit of PP2A is mutated in 15% of human primary colon tumors [45]. Mutations of the *PPP2R1B* gene, which encodes the Abeta scaffolding subunit of serine/threonine protein phosphatase 2A (PP2A), have been identified in several types of cancer including lung and breast carcinoma [45,46]. These mutations might destabilize the holoenzyme complex and thus abolish its effect on the Wnt signaling pathway.

α-catenin. The *CTNNA1* gene encodes α-catenin, a protein involved in cell adhesion by anchoring the β-catenin-E-cadherin complex to the actin cytoskeleton. *CTNNA1* has so far been found mutated only in some lung, prostate, ovarian, and colon cancer cell lines [47]. Homozygous deletion of *CTNNA1* in a human lung cancer cell line lead to loss of cell adhesion, whereas introduction of the wild-type *CTNNA1* restored normal adhesion. However, an effect of α-catenin inactivation on Wnt signaling has not been reported.

Bcr. Recent findings shown Bcr as a putative tumor suppressor that negatively regulates the expression of proliferation-promoting genes. Bcr has been described as a negative regulator of β-catenin pathway [48]. Bcr can form a complex with β-catenin and negatively regulate expression of c-Myc. Expression of Bcr in the human colon carcinoma cell line HCT116, which has a high level of endogenous β-catenin, leads to reduced c-Myc expression. Bcr leads to downregulation of β-catenin/Tcf-dependent transcription [48].

The Wnt signaling mechanism. Two signaling pathways are currently associated with Wnt–Fz(Frizzled) interactions. The first one discovered is by far the best understood, and the one to which most Fz receptors appear coupled. It centers on β-catenin protein, the target molecule of a cytosolic complex dedicated to regulating its activity, which is also called canonical pathway.

In the absence of Wnt signaling (Fig 1 B), β-catenin is phosphorylated by the serine/threonine kinases, Casein Kinase [49-51] and GSK-3β [52]. The interaction between these kinases and β-catenin is facilitated by the scaffolding proteins, Axin and APC [53; 30]. Together, these proteins form α-catenin 'degradation complex,' and allow phosphorylated β-catenin to be recognized by β-TrCP, targeted for ubiquitination, and degraded by the proteasome [54-56]. Activation of Wnt signaling inhibits β-catenin phosphorylation and hence its degradation. In the absence of the Wnt signal, Tcf acts as a repressor of Wnt target genes [57], by forming a complex with Groucho [58] (Fig. 1). The repressing effect of Groucho is mediated by interactions with Histone Deacetylases (HDAC), which are thought to make DNA refractive to transcriptional activation [59].

In the presence of Wnt signaling, the Wnt signaling disrupts the β-catenin degradation process by inducing phosphorylation of Dishevelled (Dsh), upstream of the β-catenin complex, thus reducing GSK-3β activity and stabilizing β-catenin, and allowing it to accumulate in cytoplasm and translocate to the nucleus. In the nucleus, β-catenin binds to Tcf homologues and lymphoid-enhancing factor (LEF). Without Wnt activation, Tcf/LEF binds to promoter and enhancer regions of target genes with sequence specific-

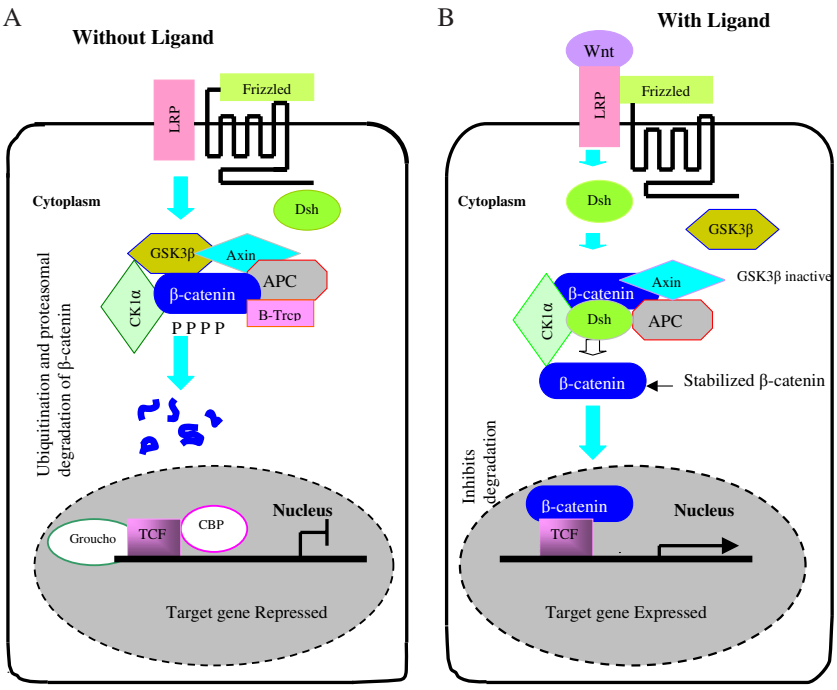

Fig. 1 The canonical Wnt signaling pathway. In absence of Wnt signaling, in cells (Fig. 1A), β-catenin is degraded by cellular proteasomal action (proteolysis) through interactions with Axin, APC, and the protein kinase GSK-3β. In presence of Wnt signal, Wnt proteins (Fig. 1B) bind to the Frizzled/LRP receptor complex at the cell surface. These receptors transduce a signal to Dishevelled (*Dsh*), which induces phosporylation of Dsh and reduces the activity of GSK-3β. As a consequence, the degradation of β-catenin is inhibited, and this protein accumulates in the cytoplasm and nucleus. β-catenin then interacts with TCF to control transcription.

ity and prevents their expression through interaction with the Groucho family of transcriptional repressors. When β-catenin binds to Tcf/LEF, however, it alters the transcriptional machinery to remove this repression and activate the target genes. Nuclear levels of β-catenin are normally moderated by interaction with nuclear APC and Axin-2, which shuttle β-catenin back into the cytoplasm.

The elevation of β-catenin levels leads to its nuclear accumulation and complex formation with LEF/Tcf transcription factors. β-catenin mutant form that lack the phosphorylation sites required for its degradation are Wnt un-responsive and can activate Wnt target genes constitutively [60]. β-catenin, APC and Axin mutations that promote β-catenin stabilization are found in many different cancers [61], indicating that constitutive Wnt signaling is a common feature in many neoplasms [62].

Therapeutic approach by targeting Wnt/β-catenin signaling. There are few approaches that have been developed so far to fight against tumorigenesis. These have been listed in Table 2. The main target of all these strategies is to check the β-catenin translocation to the nucleus/inhibits its expression or enhances its proteasomal degradation process and thus prevents the expression of Wnt target genes.

Dkk proteins as anticancer drugs: Several recent studies have investigated the anti-cancer potential of Dkk proteins, a group of antagonists of the Wnt co-receptors LRP5/6. The Dkk family comprises of four members Dkk-1 to Dkk-4, although their exact biological functions are not known. The Dickkopf (Dkk) genes code for inhibitors that are involved in Wnt signaling [63]. The loss of Dkk expression plays a role in development or progression of malignant melanoma [64].

There have been reports that the expression of Dkk-3 was down-regulated in many human immortalized and tumor-derived cell lines [65] and the expression of Dkk-3 was significantly down-regulated in primary non-small cell lung carcinomas, and expression of exogenous Dkk-3 gene in non-small cell lung carcinoma cells inhibited cell growth [66]. Recently, Dkk-3 has been demonstrated to inhibit invasion and motility of Saos-2 human osteosarcoma cells by modulating the Wnt/β-catenin pathway [67].

In a separate investigation it was reported that exploring Dkk-1 as a sensitizing agent for tumor cells are exposed to genotoxic stresses because Dkk-1 is a transcriptional target of the p53 tumor suppressor [68]. Various chemotherapeutic and other agents, which induce DNA adducts and compromise genome integrity, were shown to significantly increase the expression of Dkk-1. The effect of expression of Dkk-1 to gastrointestinal epithelial proliferation in adult mice was investigated using an adenoviral vector expressing Dkk-1 and it was demonstrated that adenoviral Dkk-1 expression of adult mice repressed the expression of the Wnt target genes within 48 hours and significantly reduced proliferation in both the

small intestine and colon. The result was also accompanied by progressive architectural degeneration of crypts, villi, and glandular structures [69], which is indicative of the efficacy of systemic expression of Dkk-1 as a general therapeutic target for deactivation of Wnt signaling in adult organisms.

Regulation at the protein level: Many of the proteins in Wnt/β-catenin signaling pathway are negative regulators, which function as tumor suppressors. It is well understood that in the development of human colorectal cancers, the β-catenin binding domain of APC is sufficient for tumor suppression and based on the idea, a recombinant adenovirus (Ad-CBR) was constructed to constitutively express the central third of APC, which includes all of the known β-catenin binding repeats [70]. It was observed that the expression in colon cancer cells, Ad-CBR blocked the nuclear translocation of β-catenin and inhibited β-catenin/Tcf-4-mediated transactivation and also substantial growth arrest of tumor cells followed by apoptosis [70], suggesting that the β-catenin binding domain of APC is sufficient for its tumor suppressor activity, and this mini-APC fragment can be used as anti-cancer agent.

Many other negative regulators of Wnt/β-catenin signaling can also be conceptually developed as anti-cancer drugs. These factors include Idax [71], Axam [72], and ICAT [73]. A recent study using a recombinant adenovirus encoding ICAT, reported ICAT inhibited proliferation of colorectal tumor cells mutated in APC or β-catenin and hepatocellular carcinoma cells mutated in Axin [74]. By contrast, ICAT did not inhibit growth of normal or tumor cells containing the wild-type APC, β-catenin, and Axin genes. These results suggest that expression of ICAT or drugs, which can mimic its functions, might be useful in the treatment of human tumors.

Activation of the proteasomal degradation process of β-catenin: Acceleration of the turnover rate of β-catenin through proteasomal degradation process in human tumor cells could be a feasible way to control of many cancers. Stabilization of the β-catenin protein in the cytoplasm is the key to activation of Wnt/β-catenin signaling. The stability of cytoplasmic β-catenin is regulated by the cellular Skp1/Cull1/F box (SCF) ubiquitination machinery [56, 75, 53, 76]. On the basis of this idea, a chimeric protein with the β-catenin binding domain of E-cadherin fused to β-TrCP ubiquitin protein ligase was engineered [77] and thus stable β-catenin mutant was recruited to the cellular SCF ubiquitination machinery for ubiquitination and degradation.

Controlled expression of β-TrCP-E-cadherin in DLD1 colon cancer cells selectively knocked down the cytosolic β-catenin and as a result, cells lost their growth and clonogenic ability *in vitro*, and lost their tumorigenic potential in nude mice [77]. Su *et al.* in the similar fashion engineered a chimeric F box protein (CFP) with multiple copies of the APC 15-amino acid repeat unit and demonstrated that introduction of CFP to colon cancer cells induced targeted ubiquitination and proteolytic degradation of nuclear and cytoplasmic free β-catenin [78] and subsequently it was observed that elimination of pathogenic β-catenin suppressed constitutive Wnt signaling and inhibited *in vitro* and *in vivo* tumor cell growth [78]. Liu *et al.* constructed chimeric F-box fusion proteins by replacing the WD40- repeat of β-TrCP with the β-catenin-binding domains of Tcf-4 and E-cadherin and it was found that the expression of this chimeric F-box fusion protein successfully promoted degradation of β-catenin independent of GSK-3β-mediated phosphorylation [79]. These results show a practical utility of an SCF-based knockout system as a tool in targeting β-catenin protein in human tumors.

Reducing the expression of β-catenin by antisense and RNA interference: Direct targeting β-catenin has attracted a broad range of attentions since the commonality of many human cancers with aberrant Wnt signaling results in increased β-catenin levels. Several approaches have been tested, including antisense, RNA interference, and protein knockdown strategies. The two major strategies, antisense oligonucleotides (Table 1.) and RNA interference (Table 1), yield the same or similar results.

An anti-sense approach has gained popularity in the last decade. With increasing interest in antisense directed therapy, several investigators have begun to examine β-catenin as a potential target. Antisense oligonucleotides are single-stranded DNA or RNA or chimeric DNA/RNA, which are designed to specifically hybridize to a targeted mRNA and subsequently prevent protein synthesis. As early as 1994, antisense strategies were used to decrease expression of β-catenin. These early antisense studies were carried out to ascertain the roles of β-catenin in embryonic development and organogenesis [80–85]. These studies helped to determine where and during which phases of development β-catenin is necessary. Currently, there are over 20 antisense drugs in clinical trials [86]. The track record of safety and efficacy of antisense oligonucleotides makes them an attractive molecule for developing into an anti-β-catenin therapy. Several clinical trials are underway using antisense oligonucleotides against bcl-2, c-Myc, H-ras, and PKCα [87–92]. Utilizing antisense oligonucleotides is an excellent approach to temporarily decreasing or completely blocking the expression of a gene of interest. Still another antisense sequence was used to study Wnt signaling in mouse mammary epithelial cells [93]. The most interesting finding of this group might be that Wnt3 has β-catenin-independent effects. As early as 1994, antisense strategies were used to decrease expression of β-catenin. Haertel-Wiesmann *et al.* reduced β-catenin mRNA levels by 50-80%, resulting in a 90% reduction in Lef transcription activity [Haertel-Wiesmann et al., 2000].

In another two cases the use of antisense oligonucleotides in colon cancer showed that the oligonucleotides reduced the amount of β-catenin mRNA in a dose-dependent manner, as well as decreased protein levels, Tcf transcription, and Cyclin D1 expression [94, 95]. There was also a reduction in cell proliferation, invasiveness and anchorage-independent growth [94]. The same oligonucleotide has been studied also in an *in vivo* xenograft model (SW480 cells) and in five esophageal

Table 1. Some changes in key oncogenes and protooncogenes in Wnt pathway typical for human tumors [22]

Oncogenes/ Protooncogenes	Function of protein	Changes	Tumors
C-MYC	transcription factor, regulates cell cycle and telomerase activity	a) chromosome translocations positioning gene under control of regulatory elements of immunoglobulin genes; b) gene amplification and/or overexpression; mutations stabilizing protein	many forms of neoplasms
Cyclin D1	regulates cell cycle	gene amplification and/or	breast cancer and salivary overexpression
CTNNB1 (β-catenin)	a) Transcription factor,regulates c-MYC and cyclin D1 b) participates in formation of adhesion contacts via binding to cadherin	mutations leading to increase in E-cadherin-unbound β-catenin which functions as transcription factor	Heriditary Adenomatous Polyposis of large intestine; various forms of sporadic tumors.
APC	Binds and destroys Cytoplasmic β-catenin; prevents transcription complexes β-catenin/Tcf	mutations cause prevents of β-catenin degradation	Hereditary adenomatous polyposis and sporadic tumors of large intestine
Axin	binds to the APC/GSK3β/ CKI complex and plays Central role in regulating β-catenin degradation	mutations leading to prevent the degradation of β-catenin	colorectal cancer, esophageal squamous cell carcinoma,medullo--blastoma,hepatocellular carcinoma

carcinoma cell lines. Both cases a decrease in β-catenin protein level, Tcf transcription and tumor cell growth were observed [95, 96]. The, nuclear expression of β-catenin was greatly reduced and more importantly, apoptosis and caspase 3 activity were enhanced, and cell viability and proliferation were reduced approximately 80% [96]. Chung *et al.* examined the effects of β-catenin expression level in leukemia and lymphoma cell lines and found a reduction in tumorigenesis [97].

Similar to antisense oligonucleotides, RNAi has been used successfully in few animal models. These studies have examined the therapeutic potential of RNAi targeting β-catenin. Van de Wetering *et al.* constructed an inducible vector system to express siRNA in LS174T colon cancer cells and demonstrated a decrease in Tcf transcription, increase in G1 cell cycle arrest, and promotion of cell differentiation [98]. In SW480, HCT 116, HeLa Cell lines, the siRNA reduced β-catenin expression, Tcf transcription, Colony formation and decrease of both cell growth for both the *colon cell lines.* The same siRNA while used in SW480 xenograft model, tumor growth was inhibited significantly and cell survival was also increased substantially [99].

Anti-cancer drugs. Various anti-cancer drugs have been reported to be either under trial or have already been approved by Food and Drug Administration (FDA). The most prominent among them are non-steroidal anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase (COX)-2 inhibitors. Recently, studies have reported that human breast carcinomas aberrantly express cyclooxygenase-2 (COX-2), and raised levels of COX-2 have prognostic value in patients with breast cancer [100]. COX-2 overexpression is correlated with cytoplasmic β-catenin expression in many cases [101]. The cancer protective activity of NSAIDs generally has been attributed to direct inhibition of COX-2. Numerous studies from cell lines and animal models lacking expression of COX-2 point to additional mechanisms likely contributing to the anti-neoplastic effect. Experimental and pre-clinical studies suggest, meanwhile, that NSAIDs and several other anti-neoplastic agents also target the Wnt-signaling pathway.

Indomethacin. Indomethacin belongs to a group of carbocyclic acids, which are inhibitor of COX-1 and COX-2 [102]. In *in vitro* studies on different CRC cell lines, the drug was shown to down-regulate [103] aberrant Wnt/β-catenin signaling activity to normal levels when applied at concentrations above 100 μM [104]. The mechanism of action independent of APC/GSK-3β-mediated, phosphorylation-dependent degradation because downregulation was possible in CRC cells with either mutant APC or mutant β-catenin. No reduction of

β-catenin protein level was seen under these conditions. At concentrations above 400 μM, indomethacin-induced G1 arrest and apoptosis of human CRC cells is associated with a dose-dependent decrease in β-catenin protein levels [105]. Moreover, disappearance of nuclear β-catenin was accompanied by its relocation to the plasma membrane in part of the cells. In agreement with the effect on Wnt-β-catenin signaling activity, reduction of β-catenin protein levels by indomethacin was achieved in cells harboring mutant APC or mutant β-catenin.

Sulindac and sulindac metabolites. Sulindac, another NSAID within the group of carbocyclic acids, is a sulfoxide prodrug that is converted to the metabolites sulindac sulfide and sulindac sulfone (exisulind), *in vivo*. Sulindac sulfide, but not sulindac sulfone, inhibits cyclooxygenase (COX) enzyme activities, yet both derivatives have growth inhibitory effects on colon cancer cells [106-108]. Accordingly, at least part of the antineoplastic effect seems to be exerted by COX-independent mechanisms. In different mouse models, in which mice develop multiple intestinal adenomas due to a germline mutation in *Apc*, sulindac causes regression of most preexisting small intestinal tumors within a few days, whereas colonic tumors are largely resistant to the treatment. Just like with indomethacin, β-catenin seems to be a molecular target for sulindac because the distribution of β-catenin before and after sulindac treatment correlates well with tumor behavior [106, 109].

Aspirin and nitric oxide releasing aspirin-derivatives. Similar to indomethacin and sulindac, aspirin (acetyl salicylic acid; ASA) affects cell growth by COX-dependent and COX-independent mechanisms and was shown to down-regulate β-catenin/TCF signaling activity in CRC cells resulting in reduced transcription of target genes [110, 111]. Likewise, the effect is detectable in both, cells with *APC* mutations or with mutationally activated β-catenin. Unlike the other NSAIDs, however, aspirin does not affect β-catenin protein levels, cellular localization or turn-over. Instead, the drug seems to stabilize β-catenin in its transcriptionally inactive, serine/threonine-phosphorylated form, thereby preventing its function as a co-transcription factor.

Glivec/Gleevec. Imatinib mesylate, (formerly called STI571 and marketed under the name Gleevec in the United States and Glivec in Europe), is a small molecule antagonist with activity against protein tyrosine kinases. β-catenin signaling activity is downregulated by Glivec in human CRC cells and in Wnt-1-induced cancer cells as demonstrated by using a β-catenin/ TCF-responsive reporter [112]. Although inhibition of Wnt/β-catenin signaling might not be the primary tumor-inhibiting effect of Glivec, the findings corroborate the hypothesis that tyrosine phosphorylation has an important impact on β catenin signaling as well and downregulation of Wnt/β-catenin target genes by Glivec may be further explored as an adjuvant treatment of human colon cancer.

Bcr. The Wnt signaling pathway can activate transcription of genes such as c-myc through β-catenin. Bcr, which has been described as a negative regulator of this pathway, can form a complex with β-catenin and negatively regulate expression of c-Myc. Expression of Bcr in the human colon carcinoma cell line HCT116, which has a high level of endogenous β-catenin, leads to reduced c-Myc expression. Thus, the results contributed to the understanding of Bcr as a putative tumour suppressor, by negatively regulating the expression of proliferation-promoting genes.

Endostatin. Endostatin is a well-known antiangiogenic protein that has been found to be a potential inhibitor of this signaling pathway [113]. It was demonstrated that endostatin directly induces apoptosis and inhibits the Wnt signaling pathway in colorectal cancer cell lines bearing mutations on the APC gene as a model of FAP-related malignant cells [113]. Endostatin gene therapy may be represented an attractive new therapeutic approach and can also be used to examine the effect of other cancer diseases. In many tumors, such as those of the pancreas, prostate, skin or lung, ectopic activation of GLI proteins, transcription factors constitute the final effec-

Table 2. Few Approaches to Targeting Wnt/β-Catenin Signaling Pathway for Cancer Therapy [1]

Type of Drug	Target molecule	Tumor targeted
Antisense Monoclonal antibodies	Wnts	Breast, NSCLC, Melanoma, Mesothelioma, SS Sarcoma
sFRPs overexpression	Fzds	Colon, Mesothelioma
wt-Dkk overexpression Chemosensitization	Dkks	Colon, Mesotheliomma
wt-APC overexpression wt-Axin overexpression	APC Axin	Colon Esophageal SCC
Antisense oligos RNA interference Protein knockdown	β-Catenin	breast, colon, Esophageal SCC, Colon
Inhibition of COX-2	COX-1	Breast, Colon, NSCLC
Apoptosis/Suicide HSV-TK/ganciclovir Oncolytic virus	LEF/Tcfs	Colon, NSCLC
Inhibition of CBP Antisense oligos Chemosensitization	CBP c-Myc	Colon Prostate, Melanoma, Colon
Cdk inhibitor	cyclin D1	Colon, RCC, NSCLC,Prostate, SS Sarcoma, Breast, SCC, Uterine, Lymphoma,Melanoma, Glioblastoma, Leukemia

Abbreviations: NSCLS: non-small cell lung cancer; SS: soft tissue; SCC: squamous cell carvinoma; RCC: renal cell carcinoma; DFSP: dermatofibrosarcoma protuberans; CML: chronic myeloid leukemia; GIST: gastrointestinal stromal tumor; ALL: acute lymphoblastic leukemia.

tors of the Hedgehog (HH) signaling pathway, has been linked to tumorigenesis [114]. In a separate case study it has been suggested that Gli1 plays an inhibitory role in the development of colorectal cancer involving Wnt signaling, even in cases with the stabilizing mutation of β-catenin [115].

GnRH-based chimeric proteins. One of the approaches developed in recent years for targeted cancer therapy is the construction and use of chimeric proteins. Chimeric cytotoxins are a class of targeted molecules designed to recognize and specifically destroy cells over-expressing specific receptors. These molecules, designed and constructed by gene fusion techniques, comprise both the cell-targeting and the cell-killing moieties. A number of chimeric proteins have already been developed based on an analog of Gonadotropin Releasing Hormone (GnRH) as their targeting moiety [116]. These chimeras recognize a GnRH-binding site that was overexpressed on a surprisingly wide variety of cancers, all confined to the adenocarcinoma type. All GnRH-based chimeric proteins selectively killed adenocarcinoma cells both *in vitro* and *in vivo*. Utilizing GnRH-based chimeric proteins for targeted therapy could open up new vistas in the fight against adenocarcinomas in humans.

Dab2. Overexpression of a new molecule, Disabled-2 (Dab2), was demonstrated to link cell surface receptors to downstream signaling pathways, was shown to inhibit Wnt-3A-stimulated accumulation of β-catenin, leading to decreased canonical Wnt/β-catenin-mediated gene induction, while simultaneously potentiating non-canonical Wnt-5A-stimulated JNK activation in NIH-3T3 cells [117]. Ablation of the Dab2 protein was found to lead to increased nuclear β-catenin levels and elevated β-catenin/Tcf/LEF-1-dependent gene induction [117]. These results thus suggest that Dab2 plays an important, non-redundant, negative regulatory role in the canonical Wnt/β-catenin signaling pathway.

CHOP. A recent finding was reported about a molecule, CHOP (GADD153) that is a protein of the C/EBP family of transcriptional regulators, which dimerizes with other C/EBP members and changes their DNA-binding and transactivation properties [118]. It induces growth arrest and apoptosis after endoplasmatic reticulum stress or DNA damage. CHOP is also expressed during early embryogenesis and upregulated in tumor tissues with defective Wnt signals. It has also been demonstrated that CHOP functions as a specific inhibitor of Wnt/Tcf signaling [118]. CHOP inhibits Tcf-dependent transcription in human embryonic and colon cancer cell lines. CHOP binds to TCF factors, thereby preventing the binding of TCF to its DNA recognition site. This finding demonstrated a novel function of CHOP as a Wnt repressor.

DMC. 2,5-dimethyl-celecoxib (DMC) is a derivative of celecoxib, a COX-2 inhibitor with anticancer activity in both preclinical studies and clinical practice, and lacks COX-2-inhibitory activity [119]. Several preclinical studies have demonstrated that DMC has better apoptosis-inducing activity than celecoxib, albeit with undefined mechanisms, and exhibits anticancer activity in animal models.

Conclusion

Although the understanding of the Wnt pathway continues to expand, there are a number of important questions that remain unanswered. The details of how signaling is initiated upon Wnt binding to Fz and LRP need to be explored further, as a whole the mechanism by which the β-catenin destruction complex is regulated, should be investigated in details. More important probably is the question of how specificity is achieved in the nuclear activity of β-catenin and its regulation of target genes. Manipulating the specificity using small molecules that target the proteins involved could hold promise in treating specific disease processes.

Conventional chemotherapeutic drugs used for the treatment of cancer patients in advanced stages have yielded only limited benefit, regarding survival time but not to mention cure of the patients. To improve the clinical outcome of cancer, agents aimed at novel molecular targets are required. Hence, accordingly, disruption of this signaling pathway holds promise for the development of new anti-cancer drugs. Although classic anticancer agents that target DNA have led to cures in a few solid tumors, the prognosis for most patients with neoplastic disease is still very poor.

Although many biological agent-based anti cancer drugs, have been discovered, in many cases, the targets themselves are inefficient and in many cases leads to drugs having poor efficacy and undesirable side effects. Indeed, some rationally designed drugs (e.g., inhibitors of receptor tyrosine kinases, tumor necrosis factor (TNF), COX-2, vascular endothelial growth factor (VEGF), bcr-abl, and proteasomes) are ineffective against cancers and other inflammatory conditions and produce serious side effects [120]. Anti-Wnt monoclonal antibodies and proteins targeting extracellularly and that mimic the effects of Dkks or sFRPs have a common advantage of a simple and specific delivery route, but a common disadvantage is their ineffectiveness to treat those tumors containing mutational effect. Although, viral or DNA/RNA based therapies targeting Tcf/LEF, CBP, and c-Myc would inhibit the signaling pathway in the nucleus. But the big challenge in this case is to develop an efficient delivery system that is specific to cancer cells. Viral vectors, on the other hand, can efficiently deliver the therapeutic agents, but may be nonspecific to the cancer cells, and have a potential to elicit host immune responses. COX-2 inhibitors have been on the market for over five years and have a simple delivery route, although they may not be tumor-specific and cause adverse effects.

Therefore, finding a suitable and effective anti cancer drug is still a problem. Thus, it has become necessary to rethink drug development strategies. Only a detail understanding of this enormously complex family of signaling proteins can lead us to the answer. The next few years are likely to see novel therapeutic agents aimed at controlling Wnt signaling in order to alleviate these conditions.

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