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β-Catenin as a valid molecular target for the development of therapeutic inhibitors

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ABSTRACT

The Wnt/β-catenin signaling pathway plays important roles in the development, possibly by maintaining the stem cells. Activation of Wnt/β-catenin signaling is also a critical initiating event in adenocarcinoma of the colon and the formation of various human cancers. β-Catenin, a key regulator of Wnt signaling pathway, interacts with the TCF transcription factors and activates the transcription of oncogenic target genes during tumorigenesis. Since the mutations in β-catenin have been found to be responsible for the human tumorigenesis, it is tempting to speculate that β-catenin is a molecular target for the effective anticancer therapy. Here we describe the structure and function of β-catenin as a central player in the cellular signaling network and discuss its potential roles in cancer cell development. Evidences of the involvement of β-catenin in the Alzheimer's disease are also presented. We also listed various pharmacological inhibitors which can modulate β-catenin signaling and reverse the tumorigenesis. These data provides the proof-of-principle for β-catenin as a valid molecular target for the effective anticancer therapy.

Key words : β-catenin, TCF, tumorigenesis, colon cancer, anti-cancer therapy

Introduction

The Wnt/β-catenin signaling pathway have attracted wide attentions from cancer researchers and phamacologists in recent years. Activation of Wnt signaling pathway is considered to be the initiating event in the transformation of intestinal epithelial cells and β-catenin is a key regulator protein in this process. According to the most widely accepted current model, CK-Iα (Casein Kinase Iα) and GSK-3β (Glycogen synthase Kinase-3β) phosphorylate β-catenin in the APC/Axin complex. Phosphorylated β-catenin is ubiquitinated, degraded by the proteasome; therefore, the level of cytoplasmic β-catenin protein is quite low in inactivated normal cells. When Wnt acts on Frizzled receptor, Dvl (Dishevelled) antagonizes the action of GSK-3β, leading to accumulate cytoplasmic β-catenin by dissociating β-catenin from the APC/Axin complex and translocates to the nucleus. In the nucleus, it binds to the transcription factor TCF/LEF and thereby stimulates the expression of various genes.

Among various molecules involved in this pathway, alterations in the β-catenin, APC and Axin genes have been frequently found in several human cancers. β-Catenin is abnormally accumulated in the cytoplasm and nucleus of these cancer cells and TCF-mediated gene expression is increased. Since APC and Axin induce the degradation of β-catenin, it is conceivable that β-catenin functions as an oncogene product, while APC and Axin act as tumor suppressor gene products. Additionally, β-catenin is also shown to be involved in the neurodegenerative disease, especially in Alzheimer's disease

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by interacting with presenlin 1 (PS1). Recent studies have shown that PS1-β-catenin complex increases β-catenin stability, and that FAD (Familial Autosomal Dominant)-linked PS1 mutations causes β-catenin destabilization and mistrafficking, which eventually induces neuronal apoptosis.

During last decade, various anti-cancer agents have been introduced to interfere with tumor growth by blocking the unlimited activation of the Wnt/β-catenin signaling. The most prominent among these are non-steroidal anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase (COX-2) inhibitors. Despite the powerful NSAIDs based cancer therapy, considerable disadvantages still remained. Therefore, mechanism- and structure-based specific inhibitors must be developed to selectively modulate the β-catenin activation in the tumorigenesis as well as in the neurological apoptosis.

Structure of β-catenin

β-Catenin protein forms a dumb-bell shape structure with armadillo repeats in the middle and globular domains at both ends. Main body of β-catenin protein is composed of twelve imperfectly homologous armadillo sequences of ~42 amino acids as tandem repeats (called arm repeats), which are also found in many other cytoplasmic proteins (Fig. 1A). Each arm repeat is made up of three helices, H1, H2 and H3. Arm repeats are flanked by an N-terminal head domain of ~130 amino acids and a C-terminal region of ~100 amino acids (1) (Fig. 1B). The three regions have distinctive charge distributions; the head and tail regions are acidic and the arm repeat region is highly basic. The terminal domains have thus far resisted high-resolution structural analysis, but the structure of the core arm repeat region, which is implicated in most binding interactions, has been determined at 2.1 Å resolutions (2-3). The 12 arm repeats form a single domain with a continuous hydrophobic core contributed by interactions between adjacent repeats.

Multiple functions of β-catenin

β-Catenin plays multiple roles in diverse locations of the cells. In the plasma membrane, β-catenin associates with the

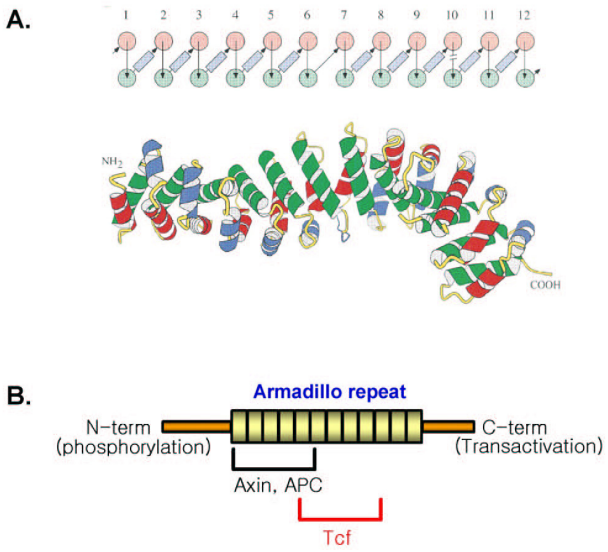

Fig. 1. Structure of β-catenin protein. A. Armadillo domain of β-catenin is depicted with highly conserved armadillo repeats (12 repeated boxed). B. N-terminal phosphorylation sites are required for proteasome-mediated degradation. C-terminal domain is required for β-catenin-mediated transcriptional activation. Protein-protein interaction domain for Axin, APC and TCF protein are shown.

cytoplasmic domain of E-cadherin and forms the cell adhesion complex. Through a distinct binding site, β-catenin also binds to the unrelated protein α-catenin, which in turn binds to actin protein. β-Catenin therefore provides the physical linkage between transmembrane adhesion proteins and the cytoskeleton proteins. This linkage to the cytoskeleton is crucial to the cell adhesion function of cadherins because cadherins cannot mediate adhesion in cells that lack either α- or β-catenin (4-5). There is a dynamic element to this complex that allows cell movement during development and wound healing. Under these conditions, epithelial migration is accompanied by tyrosine phosphorylation of β-catenin, separation of the cadherin-catenin complex and an increase in the free cytoplasmic pool of β-catenin. Indeed, inhibition of tyrosine phosphorylation of β-catenin has been shown to inhibitepithelial cell migration.

β-Catenin can also be found in the nucleus and plays critical roles in signal transduction. In signal transduction role, β-catenin is a central component of the developmentally important Wnt pathway (Fig. 2). The component and function

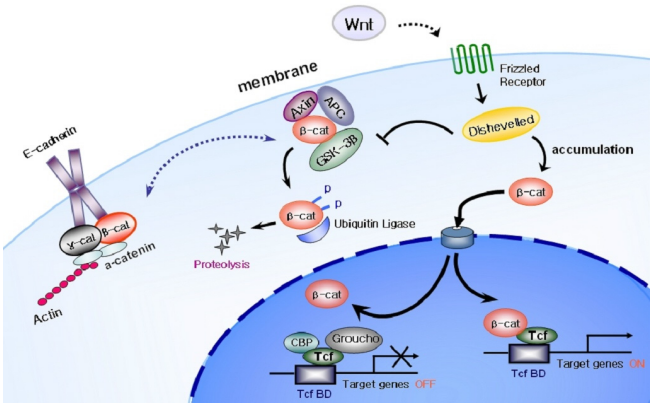

Fig. 2. Overview of Wnt signaling pathway. In the plasma membrane, β-catenin forms the cell adhesion complex with E-cadherin, a-catenin and actin. Binding of Wnt protein to frizzled receptors activates Dishevelled which blocks the function of the complex assembled over the scaffold proteins Axin. Note that Axin can form similar complexes with APC, GSK3β and β-catenin. In the absence of Wnt, the axin/conductin complexes promote phosphorylation of β-catenin by GSK3β. Phosphorylated β-catenin becomes multi-ubiquitinated (Ub) and subsequently degraded in proteasomes. In the presence of Wnt or by the mutations of APC or β-catenin, phosphorylation and degradation of β-catenin is blocked which eventually leads to the association of β-catenin with TCF transcription factors. The β-catenin/TCF complexes bind to DNA and activate Wnt target genes together with various transcriptional activators.

of Wnt pathway are highly conserved in evolution from invertebrate to human. Wnt protein is a secreted glycoprotein and has 20 isotypes in human, regulating cell growth and differentiation during embryonic development or tumorigenesis (6-7). In the absence of Wnt, in differentiated epithelial cells, most of β-catenin is attached to the plasma membrane, where it associates with E-cadherin in adherence junctions. Cytosolic β-catenin is located in a multi-protein-complex, consisting of the adenomatous polyposis coli (APC) protein, axin/conductin, casein kinases (CK) 1α or 1ε and glycogen synthase kinase-3β (GSK-3β). CK1 induces the ser/thr-phosphorylation of β-catenin at residue 45 followed by phosphorylation through GSK-3βat residues 41, 37 and 33. This process targets β-catenin for ubiquitination by the F-box-ligase β-TrCP (beta-transducin repeat-containing protein) and subsequent degradation by the proteasome. Mutations in APC, also in Axin and Conductin (Axin 2) proteins reduce β-catenin degradation thereby constitutively activates the β-catenin/TCF complex in the nucleus. Similarly, mutations in one of the ser/thr-phosphorylation sites of β-catenin lead to its stabilization and transcription of target genes even in the absence of external Wnt signals (8-12)

Central role of β-catenin in cellular signaling network

Extracellular Wnt ligand can interact with a Frizzled (Fz) receptor. Binding of Wnt to Fz receptor leads to activation of the phospho-protein Dishevelled (Dsh). Activated Dsh probably recruits Axin at plasma membrane, which eventually decreases β-catenin degradation. Activation of Dsh also leads to the inhibition of GSK-3β, which further reduces the phosphorylation and degradation of β-catenin. As the protein level of β-catenin rises through Wnt-dependent inhibition of GSK-3β, it accumulates in cytoplasm and translocates to the nucleus, where it interacts with DNA bound TCF and LEF family members to activate the transcription of target genes, such as cyclin D1 and c-myc (13-17). In the absence of β-catenin, TCF is associated with members of the Groucho proteins that act as a transcriptional repressor of Wnt target genes (18). Therefore, the function of TCF could be balanced by the selective binding either to transcriptional activator β-catenin or to transcriptional repressor Groucho. In Drosophila, CREB-binding protein (dCBP) has also been shown to bind to dTCF and keep dTCF inactive in the absence of Wingless (Wnt) signaling. dCBP binds to the HMG domain of dTCF and acetylates a conserved lysine in the Armadillo-binding domain of dTCF, and lowers the Armadillo binding to dTCF. Consistent with these observations, dCBP mutants show mild Wingless over reactivation phenotypes in various tissues. β-catenin/TCF-mediated transcription is also regulated by phosphorylation of TCF/LEF. MAP kinase-related NEMO-like kinase (NLK) stimulated by TAK1 (a kinase activated by transforming growth factor-β) mitogen activated protein kinase-kinase kinase phosphorylates TCF/LEF and inhibits the interaction of the β-catenin/TCF complex with DNA. Thus,

TAK1 and NLK downregulate transcriptional activation mediated by the β-catenin/TCF complex and negatively regulates Wnt signaling both in mammalian cells and C. elegans (19-20).

Colorectal carcinoma cells in vivo have been shown to express several growth factors, including EGF and platelet-derived growth factor, and to express receptors for these and other growth factors, for example, EGF-R, erbB-2, and c-met (receptor for hepatocyte growth factor). Further, it is well known that EGF and hepatocyte growth factor treatment leads to transformation of colonic epithelial cells into a more malignant phenotype. This change has been attributed to the ability of the growth factors to promote tyrosine phosphorylation of β-catenin and to disrupt the interaction between E-cadherin and β-catenin complex (22-23). Mutations of the ras oncogenes, which lead to overexpression of their gene products, are found in at least 50% of sporadic CRC (Colorectal cancers). Cytoplasmic expression of the trefoil peptide TFF-3, which has mitogenic and prokinetic properties, is significantly higher in CRC tissue compared with normal colonic mucosa (24-25). Inducing overexpression of ras oncogenes in cell lines or treatment with TFF-3 have a similar effect in promoting tyrosine phosphorylation of β-catenin. Release of β-catenin from its membrane-bound pool by tyrosine phosphorylation might be expected to increase nuclear translocation. However, in the case of TFF-3 treatment, increased tyrosine phosphorylation of β-catenin was seen without demonstrable nuclear accumulation of the protein. It is possible that additional factors, GSK-3 inhibition or loss of functioning APC protein, are needed to transfer membrane-released β-catenin into the nucleus.

Critical roles of β-catenin in cancers

The Wnt/β-catenin pathway contains many repressors, indicating that it is important for this pathway to be tightly regulated. Supporting this idea, any mutations that strongly and constitutively activate the β-catenin pathway are probably involved in the initiation and progression of cancer. The involvement of β-catenin signaling in oncogenesis conforms to the role of this pathway as a master switch that controls proliferation versus differentiation. Sporadic mutations in the tumor suppressor APC may account for up to 80% of colorectal cancer (CRC). Mutations in the β-catenin gene have been found in almost 50% of CRC without APC mutation (18-21). Inactivation of the second APC allele or truncations of APC protein were probably found in familial adenomatous polyposis (FAP) patients (Fig. 3). Mutated APC protein can bind but not down-regulate β-catenin, resulting in high level of free cytoplasmic β-catenin. Similarly, gain-of-function mutations in the amino terminal phosphorylation sites of β-catenin, and loss-of-function mutations in Axin, also activate β-catenin signaling and links to CRC development. Human sporadic CRC including 24 primary human adenocarcinoma, 16 adenomas and 33 cell lines derived from adenocarcinoma were investigated to clarify the relationship among mutations in APC, β-catenin, GSK-3β and TCF-4 genes. None of 46 APC deficient tumors contains

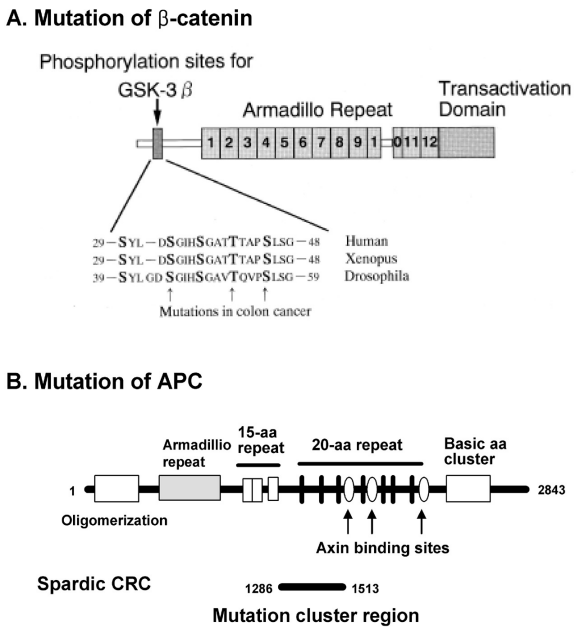

Fig. 3. Mutations in β-catenin and APC in colorectal cancer. A. Mutations of GSK3β dependent phosphorylation sites are frequently found in β-catenin. Phosphorylation sites are located in the N-terminal parts of β-catenin. B. Mutations of APC protein. The β-catenin binding sites are a series of three repeat of 15 residues (15-aa) and seven repeat of 20 residues (20-aa), located near the center of the protein. Truncation in the mutation cluster region account for 〉 60% of oncogenic mutation in APC.

a mutation within β-catenin gene, but approximately half of the 27 tumors with intact APC shows mutation in β-catenin. Mutation in APC or β-catenin are mutually exclusive, and no tumor lacking mutations in the GSK-3β or Tcf-4 genes, suggesting that β-catenin is unique in its ability to substitute for APC mutations.

Regulation of apoptosis plays an important role in CRCs. Nuclear β-catenin signaling seems to have an anti-apoptotic effect in which reduces the activation of APC or Axin. The inter-relationship between p53 and β-catenin has also been reported. Overexpression of nuclear β-catenin in lung adenocarcinoma cells led to accumulation of transcriptionally active p53 probably through inhibition of its degradation. These findings seem to contradict the pre-mentioned anti-apoptotic effect of nuclear β-catenin signaling (26). There have been more recent data suggesting that p53 may in turn reduce expression of β-catenin by inducing Siah-1-mediated degradation of β-catenin. These exciting findings provide the clues for why mutational loss of p53 function may promote colorectal carcinogenesis. Low levels of Siah-1 have been observed in SW480, a CRC cell line known to express mutant p53. However, future studies will be needed to quantify the exact contribution of loss of p53 and Siah-1 functions to β-catenin signaling in CRCs in vivo (27-28).

Recent attention has been focused to the role of prostaglandin and COX-2 (Cyclooxygenase-2) in tumorigenesis as well as in inflammation. Since the expression of COX-2 is higher in human CRCs than that in normal colonic mucosa, aspirin or specific COX-2 inhibitors reduce the risk of developing CRC. The first observation of an interaction between COX-2 and β-catenin pathways came from studies on mutant APC mice; intestinal polyps from the mutant APC mice showed elevated levels of COX-2, and introduction of a COX-2 null mutation gene to the animals reduces the number and size of intestinal tumors (29). Furthermore, addition of wild-type APC to mutant APC colorectal cell lines reduces COX-2 protein expression. However, the overexpression of β-catenin in murine mammary cell lines increases basal COX-2 protein and mRNA (30). Although these data suggest β-catenin/TCF transcriptional activity may directly induce COX-2 expression, a precise mechanism needs to be investigated (31). Whatever the mechanism, various pharmacological agents were shown to be effective in blocking COX-2 expression in CRCs.

Possible role of β-catenin in Alzheimer's diseases

Even though the aberrant activation of β-catenin is responsible for the development of CRC, reduction of β-catenin signaling is likely to be involved in the neurodegenerative diseases. Alzheimer's disease (AD) is the most common neurodegenerative disease that affects the elderly population and is characterized by three main pathological hallmarks: extracellular deposits of the amyloid β-peptide (Aβ), intracellular deposits of hyperphosphorylated tau and selective death of the cholinergic system of neurotransmission. A group of serine–threonine kinases, including GSK3β can regulate tau hyperphosphorylation, indicating the possibility that Wnt/β-catenin signaling pathway might involve in the development of AD. Indeed, it is generally accepted that GSK3β has an important role in the stability of tau and other microtubule-associated proteins. It is also likely that the inhibition of GSK3β prevents tau hyperphosphorylation, the breakdown of neuronal microtubules and the initiation of cell-death processes of AD. The study on the early-onset familial autosomal-dominant forms of AD led to the identi-

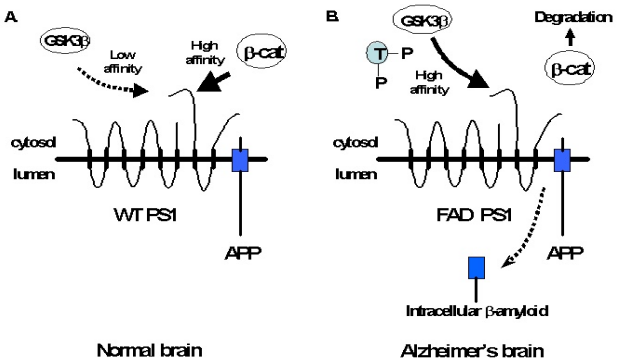

Fig. 4. Interaction of β-catenin to presenilin 1 (PS1). A. In normal brain, the cytoplasmic loop of PS1 between the sixth and seventh transmembrane domains binds to β-catenin. PS1-β-catenin complex increases β-catenin stability B. In Alzheimer's patient brain, GSK3β and FAD-linked mutations in microtubule associated protein (tau) decrease the binding affinity of PS1 to β-catenin (leading to β-catenin degradation), while increasing the binding affinity to GSK3β and tau.

fication of mutations in the amyloid precursor protein (APP) or presenilins (PS1 and PS2). Most of these mutations affect the processing of APP to yield the most pathogenic form of Aβ peptide. A wealth of data were accumulated to demonstrate that β-catenin and GSK3β are components of PS1 multiprotein complexes, in which PS1 helps to regulate the pool of β-catenin that is available for signaling (32-33) (Fig. 4). Other studies have been proposed that the loss of Wnt/β-catenin function underlies the onset and development of AD (34). Interestingly, recent data indicate that APP processing or Aβ neurotoxicity might be modulated by Wnt signaling components or compounds that mimic its activity, such as the GSK3 inhibitor, lithium (35). However, several issues need to be confirmed to relate Wnt signaling to the death of the cholinergic neurotransmitter system in AD.

Therapeutic modulation of β-catenin

Activation of an abnormal β-catenin/TCF signaling pathway through changes in β-catenin homeostasis are an initiating factors in the development of CRCs. Understanding these complex molecular pathways may identify new target protein for the battle against colorectal cancer. At a genetic level, gene therapy with wild-type APC transfected into CRC cell lines containing mutant APC has been shown to cause a pronounced reduction in total β-catenin levels and increased tumor cell death through apoptosis (36-37). Antisense oligonucleotides against β-catenin itself have been shown to suppress neoplastic growth in APC mutant CRC cells.

Various drugs that may modulate the TCF/β-catenin signaling pathway, non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to have anti-neoplastic effects in the colon (Table 1). Indomethacin, which inhibits COX-1 and COX-2 activities, was shown to down-regulate aberrant Wnt/β-catenin signaling activity to normal levels when treated at concentration above 100 uM. It induces G1 arrest in cellular proliferation and apoptosis of human CRCs and also reduces the localization of nuclear β-catenin. Consequently, indomethacin represses the β-catenin/TCF mediated transcriptional activity (38-39). Sulindac, another NSAID within the group of carbocyclic acids, seems to have an anti-neoplastic effect by COX independent mechanism. β-catenin was recently shown to be down-regulated by sulindac in human colorectal adenomas. In addition, sulindac abrogates β-catenin/TCF mediated transcription in CRC cell lines, and decreases the level of nonphosphorylated β-catenin. Sulindac has also been shown to increase the expression of APC mRNA in malignant colonic epithelial cells in vitro and de-

Table 1. Inhibitors of Wnt signaling as potential anti-cancer drugs

Agent	Type	Mechanism of Action	Developmental stages for cancer treatment
Indomethacin	ASAID	Repression of β-catenin expression Decreased expression of Wnt/β-catenin target genes	Phase Ⅱ/Ⅲ
Sulindac	NSAID	Induction of β-catenin degradation by proteasome	Phase Ⅱ
Aspirin	NSAID	Induction of β-catenin phosphorylation	Phase Ⅱ (clinical trial)
NO-Aspirin	NSAID	Disruption of β-catenin/TCF interaction	Phase Ⅰ (toxicity test)
Celecoxib/Roecoxib	COX-2 inhibitor	Relocalization of β-catenin to plasma membrane	Phase Ⅲ(clinical trial)
Gilivec	Tyrosine kinase inhibitor	Relocalization of β-catenin to plasma membrane	clinical trial
PFK 115-584 (fungal derivative)	Small molecule	Disruption of β-catenin/TCF interaction	In vitro bioassay
CGP049090 (fungal derivative)	Small molecule	Disruption of β-catenin/TCF interaction	In vitro bioassay

crease polyp burden in animal models of CRC (40-41). It is found to induce the caspase- and proteasome-dependent degradation. Aspirin (Acetyl Salicylic Acid; ASA), the prototype of anti-inflammatory and analgesic agent, was the first NSAID shown to have cancer preventive properties in CRCs (42). Similar to indomethacin and sulindac, aspirin arrests cell growth by repressing β-catenin/TCF signaling activity in CRC with APC mutation or β-catenin mutation. Unlike the other NSAIDs, however, aspirin dose not affect β-catenin protein level, cellular localization or turn-over, but mostly prevent β-catenin function as a transcriptional co-activator (43). To improve the efficiency of NSAIDs in cancer cells, nitric-oxide (NO) releasing NSAIDs have been developed. NO-ASA is the most popular among them. In vitro, NO-ASA was found to be 2,500-5,000 fold more potent than traditional aspirin in inhibiting the growth of colon cancer cells (44-45). Despite the powerful NSAIDs based cancer therapy, there are some disadvantages still remained. First, there is bio-safety concern with the prospect of toxic long-term uses of the drugs. Second, application of these NSAID based molecules is highly time-consuming and cost-intensive. Therefore, therapeutic drugs must be developed for the specific modulation of β-catenin signaling as an effective anti-cancer therapy.

Conclusion

The Wnt/β-catenin signaling pathway plays an important roles in development and tumorigenesis. Ongoing studies in this exciting field are now revealing additional components and critical relations to cellular signaling pathways. These findings will expand the view of Wnt/β-catenin signaling pathway and of its mechanism of action. It may imply that β-catenin regulates not just a signaling pathway but also a signaling network. This concept will undoubtedly accelerate a step forward to develop anti-cancer drugs.

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