Atlas of Genetics and Cytogenetics in Oncology and Haematology

The WNT Signaling Pathway and Its Role in Human Solid Tumors

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The current knowledge of the canonical Wingless-type MMTV integration site family (Wnt) signaling pathway emerges from studies of the Wingless (Wg) pathway in Drosophila melanogaster and the Wnt pathway in Xenopus laevis, Caenorhabditis elegans (C. elegans) as well as in mammalians. The Wnt signaling pathway is evolutionary conserved and controls many events during the embryogenesis. At the cellular level this pathway regulates morphology, proliferation, motility and cell fate. Also during tumorigenesis the Wnt signaling pathway has a central role and inappropriate activation of this pathway are observed in several human cancers (Spink et al., 2000).

In the first part of this review, central Wnt pathway proteins and their binding partners will be described, whereas the second part will focus on genetic and epigenetic alterations of WNT components in human solid tumors.

List of abbreviations:

Ala	Alanine
APC	Adenomatous polyposis coli
Arm	Armadillo
Asef r	APC-stimulated guanine nucleotide exchange factor

-TrCP	b-transducin repeat-containing protein
CBP	CREB binding protein
CK1, 2	Casein kinase 1 and 2
CtBP	C-terminal binding protein
DIX	Dishevelled homologous
Dkk	Dickkopf
DLG	Disc large tumor suppressor protein
Dsh/Dvl	Dishevelled
FAP	Familial adenomatous polyposis coli
FRAT	Frequently rearranged in advanced T-cell lymphomas
Fz	Frizzled
GBP	GSK-3 binding protein
GSK-3	Glycogen synthase kinase-3
HMG	High mobility group
HCC	Hepatocellular carcinoma
ICAT	Inhibitor of b-catenin and TCF-4
ILK	Integrin-linked kinase
LEF	Lymphoid enhancing factor
Lgs	Legless
LRP	Low density lipoprotein receptor related protein
Met	Methionine
MMTV	Mouse mammary tumor virus
MSI	Microsatellite instability
NES	Nuclear export signal
NLK	Nemo-like kinase
NLS	Nuclear localization signal
PHD	Plant homology domain
Pin-1	Peptidyl-propyl cis-trans isomerase-1
PI-3K	Phosphatidylinositol-3 kinase
PP2A	Protein phosphatase 2A
Pro	Proline
Pygo	Pygopus
RGS	Regulators of G-protein signaling
SAMP	Ser-Ala-Met-Pro
SARP	Secreted apoptosis-related protein
Ser	Serine
sFRP	secreted frizzled-related protein
Tak	TGFb-activated kinase
TCF	T-cell factor
TGF	Transforming growth factor
Thr	Threonine
TLE	Transducin-like enhancer of split
Wg	Wingless
WIF-1	Wnt-inhibitory factor-1
Wnt	Wingless-type MMTV integration site family member

The Wnt signaling pathway

The Wnt signaling pathway is essential in many biological processes and numerous studies of this pathway over the last years have lead to the identification of several novel components. Nevertheless, many of the mechanisms involved in activation or inactivation of this particular pathway still remains to be elucidated. The pathway with or without a Wnt signal is schematically presented in Figure 1.

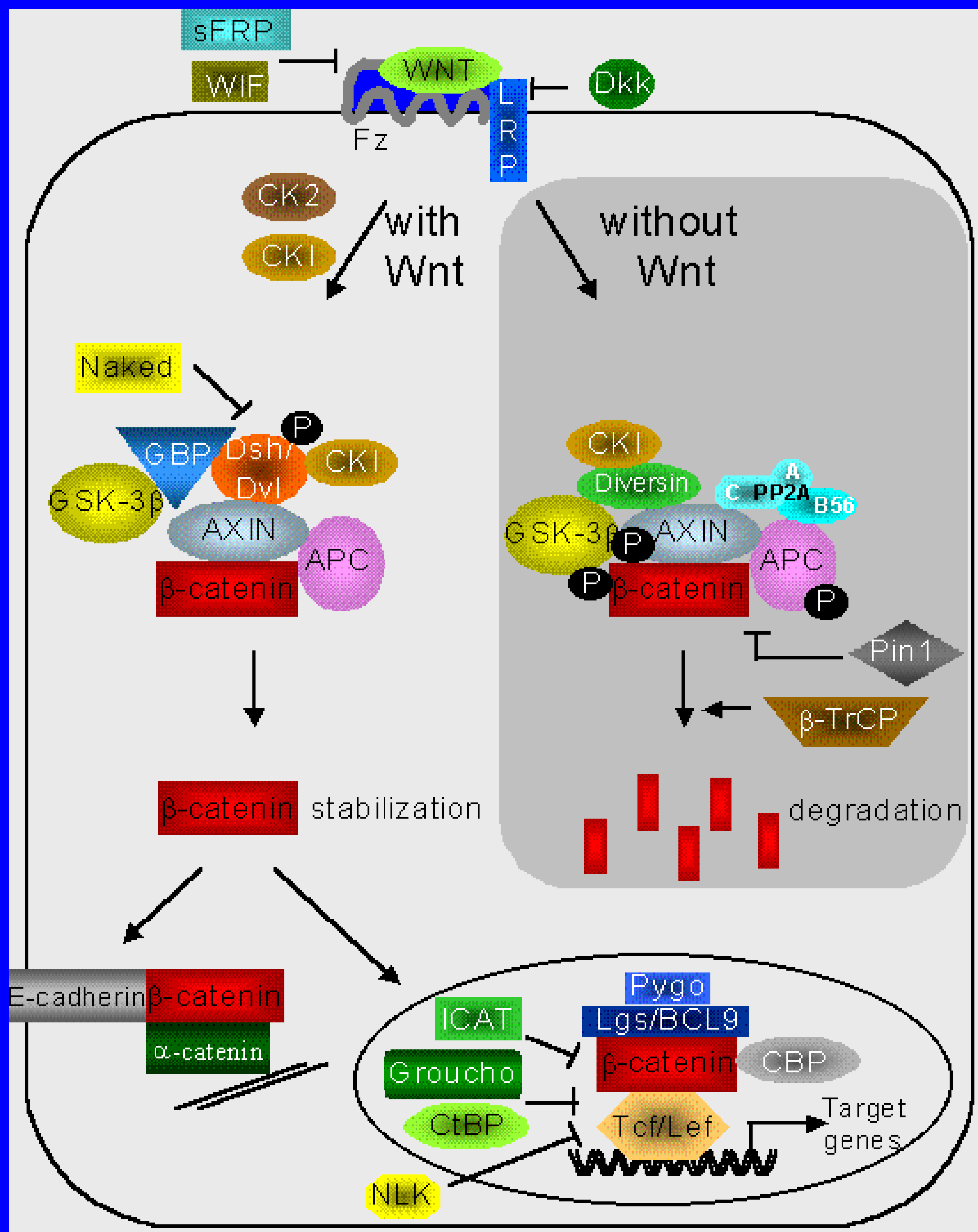

Figure 1. Schematic presentation of the Wnt pathway

In the presence of a Wnt ligand, if not inhibited by secreted antagonists, the Wnt ligand binds a frizzled (Fz)/low density lipoprotein receptor related protein (LRP) complex, activating the cytoplasmic protein dishevelled (Dsh in Drosophila and Dvl in vertebrates). Precisely how Dsh/Dvl is activated is not fully understood, but phosphorylation by casein kinase 1 (CK1) and casein kinase 2 (CK2) have been suggested to be partly responsible (Willert et al., 1997; Sakanaka et al., 1999; Amit et al., 2002).
Dsh/Dvl then inhibits the activity of the multiprotein complex (b-catenin-Axin-adenomatous polyposis coli (APC)-glycogen synthase kinase (GSK)-3), which targets -catenin by phosphorylation for degradation by the proteasome. Dsh/Dvl is suggested to bind CK1 and thereby inhibiting priming of -catenin and indirectly preventing GSK-3 phosphorylation of -catenin (Amit et al., 2002). Upon Wnt stimulation, Dvl has also been shown to recruit GSK-3 binding protein (GBP) to the multiprotein complex. GBP might titrate GSK-3 from Axin and in this way inhibits phosphorylation of -catenin. Finally, sequestration of Axin at the cell membrane by LRP has been described (Mao et al., 2001b). The overall result is accumulation of cytosolic -catenin.
Stabilized -catenin will then translocate into the nucleus and bind to members of the T-cell factor (Tcf)/Lymphoid enhancing factor (Lef) family of DNA binding proteins leading to transcription of Wnt target genes.

In the absence of a Wnt ligand, Axin recruits CK1 to the multiprotein complex causing priming of -catenin and initiation of the -catenin phosphorylation cascade performed by GSK-3 . Phosphorylated -catenin is then recognized by -transducin repeat-containing protein (-TrCP) and degraded by the proteaosome, reducing the level of cytosolic -catenin.

1. Extracellular inhibitors

At least three classes of Wnt antagonists are reported in *Xenopus*, all with human homologues, however, none of them have been identified in *Drosophila* or *C. elegans*.
The first class, ***secreted frizzled-related proteins (sFRPs)***, are also called secreted apoptosis-related proteins (SARPs) due to their effect on cell sensitivity to pro-apoptotic stimuli (Melkonyan et al., 1997). They contain a cysteine-rich domain with similarity to the ligand-binding domain of the Fz transmembrane protein family, but lack the 7-transmembrane part that anchors Fz proteins to the plasma membrane (Rattner et al., 1997). The sFRPs thus compete with the Fz proteins for binding to secreted Wnt ligands and antagonize the Wnt function. However, a contradictory effect of the sFRPs has been described, in which the sFRPs enhance the Wnt activity by facilitating the presentation of the ligand to the Fz receptors (Uthoff et al., 2001). Three human homologues are identified, SARP1-3, but they show distinct expression pattern (Melkonyan et al., 1997).
Wnt-inhibitory factor-1 (WIF-1) represents the second class of secreted Wnt antagonists, and in Xenopus WIF-1 binds to Wnt proteins and inhibits their activities by preventing access to cell surface receptors (Hsieh et al., 1999). A human homologue, located at chromosome 12, is identified (Hsieh et al., 1999).

The third type of secreted antagonists, ***Dickkopf (Dkk)***, includes four known human proteins, DKK1-4 (Krupnik et al., 1999). In Xenopus, Dkk1 does not inhibit Wnt ligands directly, but interacts with the Wnt co-receptor, LRP, and prevents formation of an active Wnt-Fz-LRP receptor complex (Mao et al., 2001a). Recently, it was found that Kremen1 and Kremen2 worked as Dkk receptors (Mao et al., 2002) and a ternary complex between Kremen2, Dkk1 and LRP6lead to endocytosis and thus removal of LRP6 from the plasma membrane (Mao et al., 2002). Surprisingly, Dkk2 was reported to induce Wnt signaling by working synergistically with the Fz family rather than inhibiting Wnt stimulation(Wu et al., 2000).

2. Ligands and receptors

Wnt ligands belong to a family of proto-oncogenes expressed in several species ranging from the fruit fly to man. This large family of secreted glycoproteins is considered one of the major families of signaling molecules. The first Wnt gene, mouse Int-1, was identified by its ability to form mammary tumors in mice when activated by integration of the mouse mammary tumor virus (MMTV)(Nusse and Varmus, 1982). Int-1 was later renamed Wnt-1 due to the relationship between this gene and the Wg gene in Drosophila (Nusse et al., 1991). At present, 19 human WNT genes are characterized (http://www.stanford.edu/~rnusse/wntwindow.html). Although the individual members of this family are structurally related, they are not functionally equivalent and each may have distinct biological properties (Dimitriadis et al., 2001).

In *Drosophila*, the *Fz* genes play an essential role in development of tissue polarity. The Fz genes code for seven-transmembrane proteins and lines of evidence showing that Fz proteins work as receptors for Wg in *Drosophila* exist (Bhanot et al., 1996). Several mammalian homologues have been identified (http://www.stanford.edu/~rnusse/wntwindow.html). Both the extracellular cysteine rich domain and the transmembrane segment are strongly conserved, but nevertheless, the Fz proteins differ in both function and ligand specificity (Wang et al., 1996). Although it is known that the Wnts interact with the Fz receptor, the mechanism of Fz signaling is not fully understood (Uthoff et al., 2001).
In *Drosophila, Xenopus*, and mouse the Arrow (*Drosophila*)/LRP (in vertebrates) is required during Wnt signaling, possibly by acting as a co-receptor for Wnt (Pinson et al., 2000; Tamai et al., 2000; Wehrli et al., 2000). The LRP gene encodes a long single-pass transmembrane protein, and the extracellular domain binds Wnt directly making a ternary complex with the Fz receptor (Tamai et al., 2000). Recently it was observed that the intracellular part of LRP binds Axin (Mao et al., 2001b). The authors hypothesized that Fz-Wnt-LRP complexes and subsequently LRP recruits Axin to the complex, thereby inactivating Axin leading to release of -catenin from the multiprotein complex (see later) and consequently transcription of downstream Wnt target genes.

Two mammalian homologues have been described, LRP5 (Hey et al., 1998) and LRP6 (Brown et al., 1998).

3. Downstream of the receptor complex

Dsh in *Drosophila* and Dvl in vertebrates encode a cytoplasmic phosphoprotein and is a positive mediator of Wnt signaling (Klingensmith et al., 1994). In the human genome, three homologues have been described, DVL1-3 (Pizzuti et al., 1996; Bui et al., 1997). Dsh/Dvl works downstream of Fz receptor, but upstream of -catenin (Uthoff et al., 2001). However, its exact mechanism of action remains unknown, but several binding partners have been detected (Figure 2).

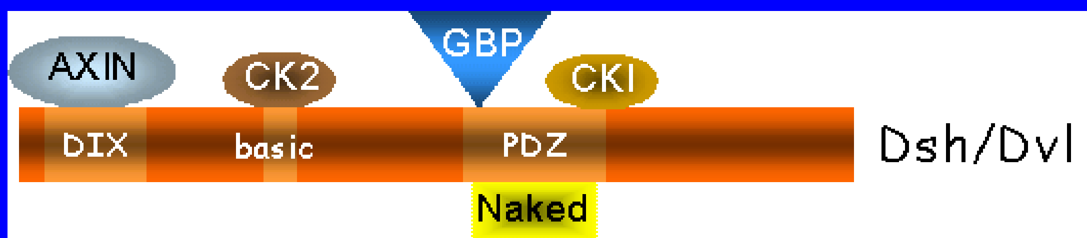

Figure 2. Dsh/Dvl and binding partners

The dishevelled homologous (DIX) domain of Dvl binds **Axin**. This binding inhibits Axin promoted GSK-3 dependent phosphorylation of -catenin (Kishida et al., 1999).

In *Drosophila*, **CK2** works as a positive mediator of Wg signaling by interacting with the basic domain of Dsh and subsequently activates it (by phosphorylation)(Willert et al., 1997).

Upon Wnt stimulation, Dsh/Dvl binds **CK1**. This binding probably inhibits phosphorylation priming of the Serine (Ser) 45 site in -catenin causing stabilization of -catenin and activation of the Wnt pathway (Sakanaka et al., 1999; Amit et al., 2002). However, the exact mechanism of this event is yet unknown. In the absence of a Wnt signal, CK1 associates and cooperates with Axin, probably through diversin (see below). This drives the phosphorylation and degradation cascade of -catenin, and subsequently inhibits the Wnt signaling pathway (Amit et al., 2002).

During Wnt stimulation in *Xenopus*, **GBP** interacts with the PDZ domain of Dvl (Li et al., 1999). The name PDZ derives from three proteins that contain repeats of the same type as found in this domain: mammalian postsynaptic density protein, PSD-95, *Drosophila* discs-large tumor suppressor, Dlg, and the mammalian tight junction (zonula occludens) protein, ZO-1. As mentioned, Dvl also interacts with Axin. Both GBP and Axin bind GSK-3 , however these two components share overlapping binding sites on GSK-3 and thus compete in binding to this protein. One theory suggests that in the presence of a Wnt signal, Dvl recruits GBP to the multiprotein complex. GBP then titrates GSK-3 from Axin leading to accumulation of -catenin in the cytoplasm (Fraser et al., 2002). The human homologue of GBP is frequently

rearranged in advanced T-cell lymphomas (FRAT). Newly, FRAT/GBP was shown to contain a nuclear export sequence, leading to nuclear export of itself as well as the bound GSK-3 . Thus, FRAT/GBP is involved in regulating the accessibility of cytoplasmic GSK-3 (Franca-Koh et al., 2002). Two human family members, FRAT-1 and FRAT-2, are identified (Jonkers et al., 1997; Saitoh et al., 2001), but none is described in *Drosophila*.

In the fly, the **naked** protein binds Dsh and downregulates its activity. The expression of Naked is induced by Wg, indicating a negative feedback mechanism (Zeng et al., 2000).

4. The multiprotein complex

The stability of -catenin (encoded by the gene *CTNNB1*) is regulated by a multiprotein complex consisting of -catenin, Axin/Conductin, APC, and GSK-3 (Schwarz-Romond et al., 2002). In this scaffolding complex, GSK-3 phosphorylates primed -catenin, thus marking -catenin for ubiquitylation and subsequent proteasome degradation. During the last years, several novel players that interact with the components of the multiprotein complex have emerged. Still, the exact mechanisms of action of the multiprotein complex need further clarification.

-catenin

-catenin was first described in humans as a protein which interacts with the cytoplasmic domain of E-cadherin and with -catenin, anchoring the cadherin complex to the actin cytoskeleton (Kemler and Ozawa, 1989). Then, the homology between -catenin and the Armadillo (Arm) of *Drosophila* and -catenin in *Xenopus* lead to the discovery of an additional role for mammalian -catenin, namely as the key mediator of Wnt signaling (McCrea et al., 1991; Gumbiner, 1995).
The primary structure of -catenin comprises an amino-terminal domain of approximately 130 amino acids, a central region of 12 imperfect repeats of 42 amino acids known as arm repeats (since they show homology with repeats found in the Arm protein of *Drosophila*), and a carboxy-terminal domain of 110 amino acids. The amino-terminus of -catenin is important for regulating of its stability, whereas the carboxyl terminus works as a transcriptional activator domain (Willert and Nusse, 1998).
Interestingly, plakoglobin, also called -catenin, shares overall 70% amino acid identity with -catenin and as much as 80% within the arm repeat domain (Huber and Weis, 2001). Plakoglobin binds E-cadherin, -catenin, APC, Axin and Tcf/Lef transcription factors, and is involved in cell adhesion as well as Wnt signaling. However, differences between -catenin and plakoglobin in these processes exist (Kolligs et al., 2000).
-catenin activity is controlled by a large number of binding partners that affect the stability and localization of -catenin (Figure 3).

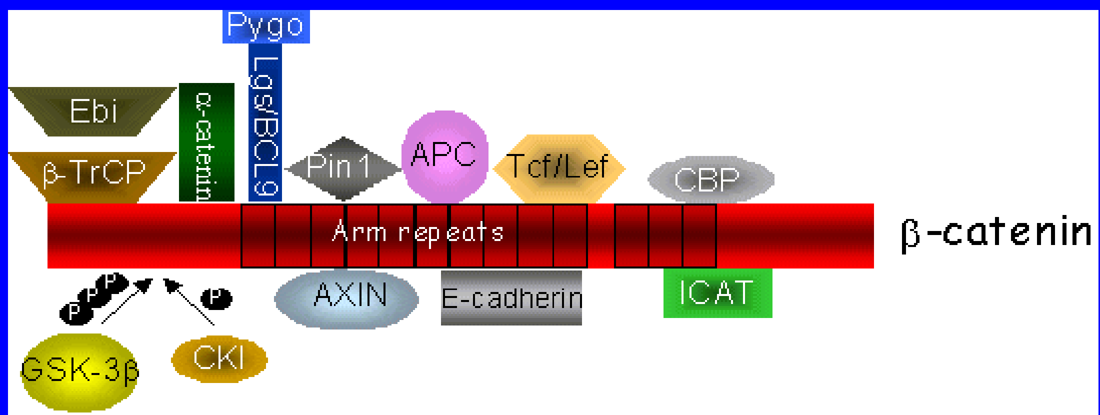

Figure 3. -catenin and binding partners

Two ubiquitin-mediated degradation systems are involved in the catabolism of -catenin. Both F-box proteins, **-TrCP** and **Ebi**, recognize and bind to the same sites on the N-terminal domain of -catenin (Polakis, 2001). However, unlike -TrCP, Ebi probably does not require phosphorylation of -catenin for recognition. Ebi works in complex with SIAH-1, a TP53 induced protein, linking activation of TP53 to the degradation of -catenin (Liu et al., 2001; Matsuzawa and Reed, 2001). Both degradation systems require an intact APC protein (Polakis, 2001).

GSK-3 sequentially phosphorylates threonine (Thr) 41, Ser 35, and Ser 33 of -catenin after -catenin has been primed (phosphorylated at Ser 45) by **CK1** (Amit et al., 2002; Schwarz-Romond et al., 2002; Liu et al., 2002).

Binding of **-catenin** to the N-terminal region of -catenin (Nagafuchi et al., 1994) and E-cadherin to the arm repeat (Huber and Weis, 2001) connects -catenin to cell adhesion.

The arm repeat domain of -catenin mediates binding of **cadherins** (Hulsken et al., 1994; Pai et al., 1996), **APC** (Hulsken et al., 1994; Rubinfeld et al., 1995), **Axin** (Behrens et al., 1998; Ikeda et al., 1998), and **Tcf/Lef** family of transcription factors (Behrens et al., 1996; van de et al., 1997). E-cadherin, APC and Tcf/Lef interact with this domain of -catenin in an overlapping and mutually exclusive manner (Willert and Nusse, 1998).

In Drosophila, Legless (**Lgs**) and Pygopus (**Pygo**) have recently been shown to be required for Arm to function as a transcriptional co-activator in the Wg signaling pathway (Kramps et al., 2002). Lgs encodes the homologue of human , whereas Pygo codes for a PHD (plant homology domain) finger protein and two human homologues have been identified, hPYGO1 and hPYGO2 (Kramps et al., 2002). Lgs/BCL-9 is shown to bind the arm repeats of Arm/ -catenin and work as a linker molecule between Pygo and the Arm/ -catenin - Pan/Tcf complex in the nucleus leading to transcription of Wg/Wnt target genes (Kramps et al., 2002). The exact mechanism of action of Pygo remains unknown.

Peptidyl-propyl cis-trans isomerase 1 (**Pin1**) binds a phosphorylated Ser-

Proline (Pro) motif next to the APC binding site in -catenin and inhibits interaction between APC and -catenin, consequently acting as a positive regulator of Wnt signaling (Ryo et al., 2001).

In Xenopus, the transactivating domain of -catenin interacts with CREB binding protein (**CBP**) and these synergistically stimulate transcription of Wnt target genes (Takemaru and Moon, 2000).

In mouse studies, inhibitor of -catenin and TCF-4 (**ICAT**) binds the C-terminal domain of -catenin and inhibits its interaction with TCF-4. -catenin-TCF-4 mediated transactivation of Wnt target genes is then repressed (Tago et al., 2000).

APC

The first hint of the mode of action of APC came from studies showing that APC binds -catenin (Rubinfeld et al., 1993; Su et al., 1993). Later it has been demonstrated that APC plays a central role in regulating the -catenin level in the Wnt signaling pathway in addition to be involved in cell migration, cytoskeleton regulation, and chromosome segregation (Fodde, 2003), functions of APC that will not be dealt with in this paper. APC encodes a large protein consisting of several distinct conserved domains (Groden et al., 1991), interacting with a number of different proteins (Figure 4).

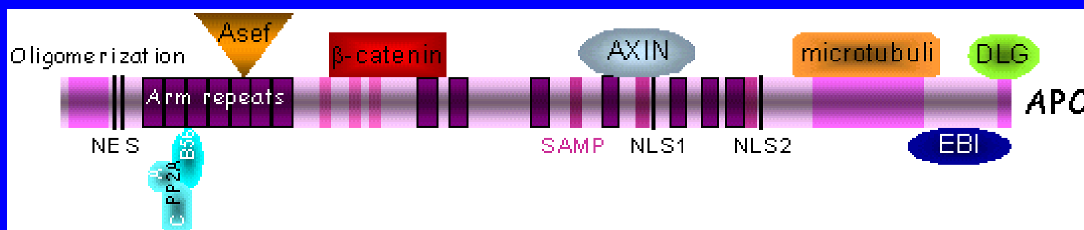

Figure 4. APC and binding partners

The amino-terminal end of APC contains heptad repeats involved in oligomerization of APC (Joslyn et al., 1993).
The holoenzyme, protein phosphatase 2A (PP2A), comprises three subunits, the structural- (A), the regulator- (B), and catalytic- (C) subunit. APC, like -catenin, harbors arm repeats to which the regulatory subunit of PP2A, **B56**, binds (Seeling et al., 1999). In Xenopus, the PP2A holoenzyme (containing B56) is present in the -catenin degradation complex, and PP2A is suggested to dephosphorylate and activate GSK-3 , leading to degradation of -catenin and inhibition of Wnt signaling (Li et al., 2001a).
The arm repeats of APC also bind to APC-stimulated guanine nucleotide exchange factor (**Asef**) and enhances the interaction between Asef and , a member of the Rho family of small GTPases. This further modulates the actin cytoskeleton and influence cell adhesion and cell motility (Kawasaki et al., 2000).
Three 15-amino acid repeats and seven 20-amino acid repeats within APC are known to bind **-catenin** (Rubinfeld et al., 1993; Su et al., 1993).

Phosphorylation of APC by GSK-3 increases the negative charge on APC and strengthens the interaction between APC and the positively charged arm repeat domain of -catenin (Oving and Clevers, 2002). Down regulation of -catenin requires at least three of the seven 20 amino acid repeats to be intact (Rubinfeld et al., 1997).
Three Ser-Alanine (Ala)-Methionine (Met)-Pro (SAMP) motifs are located within the 20 amino acid repeats of APC and these mediate binding to **Axin** (Behrens et al., 1998).
APC also contains two intrinsic nuclear localization signals (**NLSs**) located in the middle and C-terminal region of APC (Zhang et al., 2000) and at least two intrinsic nuclear export signals (**NESs**), located near the amino terminus (Neufeld et al., 2000a; Henderson and Fagotto, 2002). Recently, it was shown that APC binds nuclear -catenin and stimulates its nuclear export and subsequently its cytoplasmic degradation (Neufeld et al., 2000b).
Phosphorylation sites near the NLS2 site were shown to be critical for regulation of APC's nuclear distribution (Zhang et al., 2001).
A basic domain in the C-terminal region of APC binds **microtubules** directly, inducing stabilization of their ends (Zumbrunn et al., 2001). APC also contains a binding site for , another microtubuli binding protein, which is required for APC-mediated attachment of microtubules to the chromosomes' kinetochores, ensuring proper chromosome segregation during mitosis (Su et al., 1995; Kaplan et al., 2001; Fodde et al., 2001). The interaction of APC with microtubules is decreased by phosphorylation of APC by GSK-3 (Zumbrunn et al., 2001).
The C-terminus of APC also interacts with the human homologue of the Drosophila discs-large tumor suppressor protein (**DLG**) (Matsumine et al., 1996). However the effect of this interaction is not yet fully understood.

Axin

In 1997, Axin, the product of mouse fused locus, was introduced as a novel component in the Wnt signaling pathway (Zeng et al., 1997). Axin works as a scaffold protein involved in forming the multiprotein complex leading to phosphorylation and degradation of -catenin and thereby acts as a negative regulator of Wnt signaling. Later, a homologue of Axin in mouse, Conductin, and in rat, Axil, were identified (Behrens et al., 1998; Yamamoto et al., 1998). Two human homologues also exist, AXIN1(the Axin homologue)(Zeng et al., 1997) and AXIN2(the Conductin/Axil homologue)(Mai et al., 1999). Axin and Conductin share 45% amino acid identity. Interestingly, Axin is homogeneously distributed, whereas Conductin is more selectively expressed in specific tissues (Lustig et al., 2002). Recently, it was shown that Conductin is a downstream target gene of the Wnt pathway and might work in a negative feedback loop controlling Wnt signaling activity (Lustig et al., 2002). TCF binding sites has also been identified in the human homologue, AXIN2 (Leung et al., 2002).
Several components of the Wnt signaling pathway interact with Axin (Figure 5).

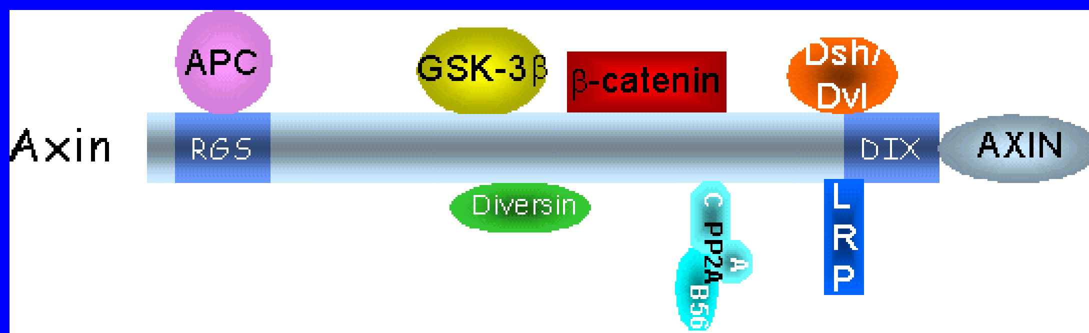

Figure 5. Axin and binding partners

APC binds to Axin at a region with significant homology to the regulators of G-protein signaling (RGS) family (Spink et al., 2000).

GSK-3 is recruited to the multiprotein complex by Axin (Behrens et al., 1998) and then phosphorylates Axin and APC and increases their interaction with -catenin (Peifer and Polakis, 2000). Subsequently, primed **-catenin** bound to Axin and APC (Behrens et al., 1998), is phosphorylated by GSK-3 , marking -catenin for proteasome degradation.

In *Xenopus* and cell cultures, **diversin** (an ankyrin repeat protein) also binds to Axin and recruits CK1 to the multiprotein complex (Schwarz-Romond et al., 2002). Diversin/CK1 and GSK-3 cooperate in -catenin degradation, however diversin and GSK-3 use identical binding sites on Axin suggesting that a homodimeric Axin complex is present to perform phosphorylation and degradation of -catenin. One of the Axin molecules binds diversin that recruits CK1, leading to priming of -catenin, whereas the other Axin molecule binds GSK-3 causing phosphorylation of primed -catenin. A closely related diversin gene is identified in humans, ANKRD6 (Schwarz-Romond et al., 2002).

The **PP2A** catalytic subunit binds Axin (Hsu et al., 1999), and the PP2A holoenzyme works as a negative regulator of Wnt signaling (Li et al., 2001a).

The DIX domain of Axin binds **Dsh/Dvl** (Kishida et al., 1999) and **LRP5**, the co-receptor for the Wnt ligand (Mao et al., 2001b). In addition, this domain is necessary for oligomerization of Axin (Kishida et al., 1999). The putative effects of these interactions have been described previously in the text. The DIX domain is essential for degradation of -catenin (Kikuchi, 1999).

GSK-3

GSK-3 , zw3 or shaggy in *Drosophila*, is a member of the Ser/Thr family of protein kinases. This protein is a key enzyme in the Wnt signaling pathway. As outlined above, GSK-3 phosphorylates primed -catenin prior to proteasome degradation, and it phosphorylates Axin and APC and enhances their interaction with -catenin.
Unlike most protein kinases, GSK-3 is constitutively active and phosphorylation of GSK-3 leads to inhibition of its activity (Manoukian and Woodgett, 2002). Two highly related human homologues are identified, GSK-3 and GSK-3 and these two isoforms are more than

95% identical in the protein kinase catalytic domain (Woodgett, 1990). Consequently, GSK-3 can substitute for many, but not all of the functions of GSK-3 in the Wnt signaling pathway (Manoukian and Woodgett, 2002).

5. Nuclear components

The stabilized cytosolic -catenin will be translocated into the nucleus, but how -catenin enters the nucleus is not yet fully understood. Nuclear -catenin associates with the family of Tcf/Lef transcription factors, and is therefore a key factor for expression of Wnt downstream genes.

Tcf/Lef

The Tcf/Lef proteins are a class of related high mobility group (HMG)-box of transcription factors. Four human homologues of Tcf/Lef have been identified, LEF1, TCF1, TCF3 and TCF4. They all recognize the same DNA sequences, however they display tissue specific expression patterns. With Wnt signal, Tcf/Lef acts in a complex with -catenin, BCL-9, Pygo and CBP(see Figure 1) and target genes like c-MYC, cyclin D1, WNT inducible signaling pathway protein (WISP)-3, and matrix metalloproteinase (MMP)-7, are expressed. Without the presence of Wnt stimulation, the Tcf/Lef proteins repress transcription of the Wnt target genes by binding to co-repressors like Groucho and C-terminal binding protein (CtBP).
In Figure 6 the cooperation of Tcf/Lef family members with various proteins are shown.

Figure 6. Tcf/Lef and binding partners

Nuclear **-catenin** makes a heterodimeric complex with the N-terminal region of Tcf/Lef supplying the complex with a transactivating domain, whereas Tcf/Lef contributes with a DNA-binding domain. Potential co-activators bind to -catenin (Figure 3).

In *Xenopus*, , an essential component of the transforming growth factor (TGF) - signaling pathway, interacts with Lef1, making a -catenin-Lef1-Smad4 complex harboring dual DNA recognition specificity. Only target genes containing sites recognized by both the DNA-binding proteins will be

transcribed.

At least two different co-repressors bind and inhibit the effect of Tcf/Lef in the absence of nuclear -catenin. One of these is the **Groucho** (found in *Drosophila*), which interacts with a histone deacetylase resulting in a "closed" chromatin structure that does not allow transcription. Three human homologues of Groucho are described, transducin-like enhancer of split 1-3 (TLE 1-3). In *Xenopus*, the second known co-repressor, **CtBP**, is shown to bind and repress Tcf3 and Tcf4.

TGF -activated kinase (Tak)-1acts upstream of nemo-like kinase (**NLK**). In vertebrates, NLK co-localizes and phosphorylates Tcfs. This reduces the DNA binding capacity of Tcfs and thereby removes -catenin-Tcf complexes from the promoter region of Wnt target genes.

6. Wnt target genes

At present more than 50 Wnt target genes have been described in *Drosophila* and vertebrates. Most of them are listed at: http://www.stanford.edu/~rnusse/wntwindow.html. These are involved in numerous processes, including development, cell proliferation, cell-cell interactions and cell-matrix interactions. The majority of these genes contain Tcf/Lef binding sites in their promoter, however other mechanisms of activation have also been reported. In this review, the target genes will not be described in detail.

Alteration of the Wnt signaling pathway in human solid tumors

Chronic activation of the Wnt signaling pathway has been implicated in the development of a variety of human malignancies, including colorectal carcinomas, hepatocellular carcinomas (HCCs), melanomas and uterine and ovarian carcinomas. Mutations in the regulator genes, *CTNNB1*, *APC* and *AXIN*, as well as in other components of this pathway have been reported. The effect of the various mutations is an increase in the cellular level of -catenin and subsequent transcription of Wnt target genes like c-MYC, cyclin D1 and WISP-1. However, alterations of genes encoding proteins working up-stream of the multiprotein complex have not yet been described in human tumorigenesis, but altered expression has been observed for some of these components. Neither has *GSK-3* been reported mutated in human cancers. This might be explained by the central role of *GSK-3* also in pathways other than the WNT pathway and mutation in this gene may be incompatible with cell viability. Alternatively the closely related gene, *GSK-3* may substitute for loss of *GSK-3* and inactivation of both genes is unlikely in tumor development.

An overview of the different WNT components found mutated in human tumors is presented in Figure 7, followed by a detailed discussion.

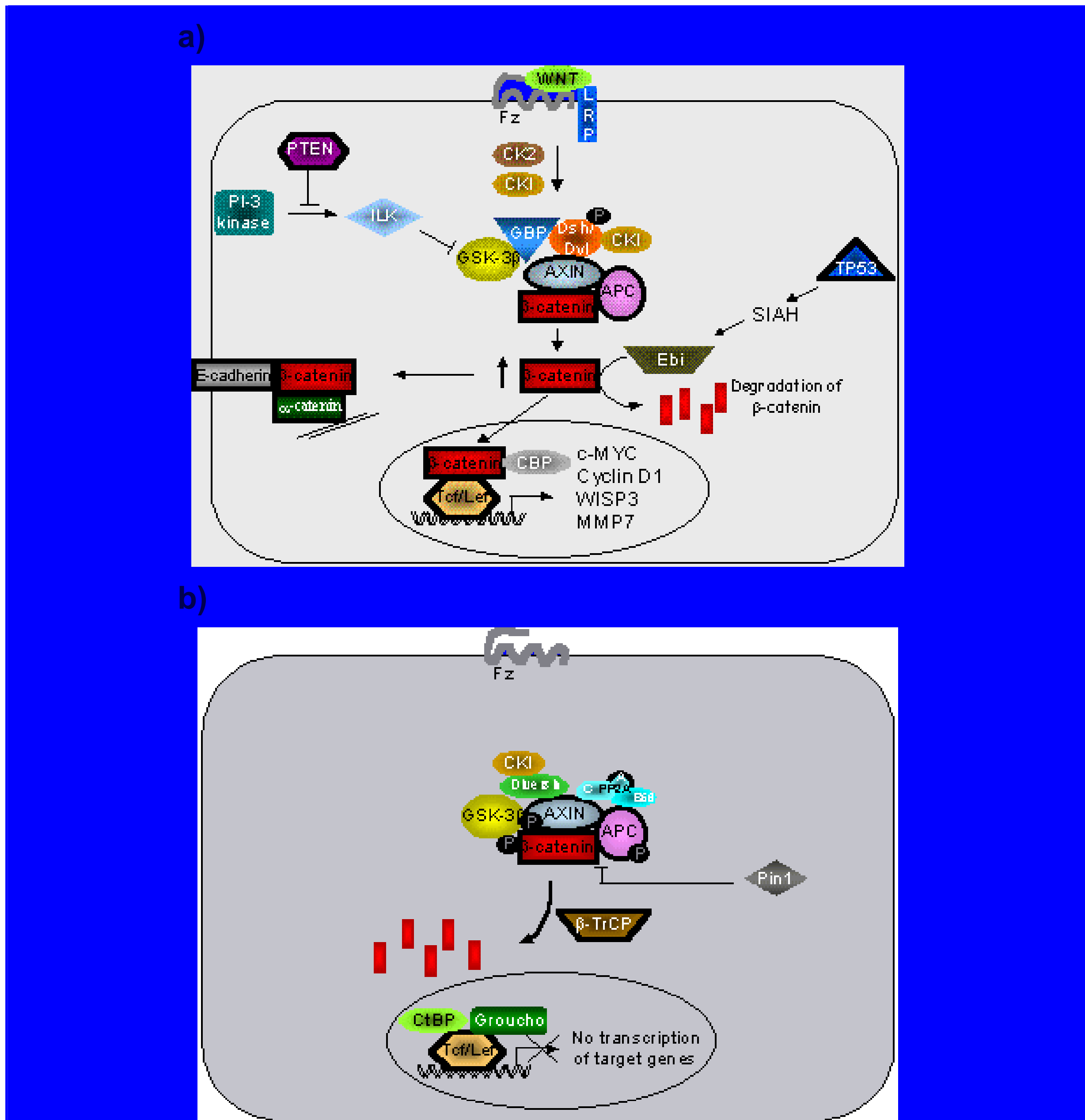

Figure 7. Wnt genes mutated in human solid tumors
a) Wnt is present, b) No Wnt stimulation. Genes marked with a black circle are found mutated in human solid tumors (for details see the text).

-catenin

The *CTNNB1* gene encodes -catenin. Exon 3 of this gene is hot spot for mutation in human tumors. This exon encodes the critical Ser/Thr residues, which are sites for priming by CK1 (Ser 45) and phosphorylation by GSK-3 (Ser 33, 37 and Thr 41) and thus the

recognition site of -TrCP marking -catenin for degradation. Therefore mutations within this exon increase the stability of the -catenin protein. Indeed, somatic mutations in exon 3 have been described in a wide variety of human tumors, including colorectal carcinoma, desmoid tumor, endometrial carcinoma, HCC, hepatoblastoma, intestinal carcinoma gastric, medulloblastoma, melanoma, ovarian carcinoma, pancreatic carcinoma, pilomatricoma, prostate carcinoma, squamous cell carcinoma of the head and neck, thyroid carcinoma, and Wilms' tumor (http://www.stanford.edu/~rnusse/wntwindow.html). In colorectal carcinomas, desmoid tumors and hepatoblastomas an inverse correlation between *CTNNB1* mutations (exon 3) and *APC* mutations are observed. Only two reports, both on colorectal tumors, have examined other exons of *CTNNB1* than exon 3. The consequence of mutations outside exon 3 is presently unknown, however the majority of the tumors and cell lines with mutation outside exon 3 also showed mutation in *APC*. A tendency towards more *CTNNB1* mutations in colorectal tumors with microsatellite instability (MSI) than in those without have been reported, however this correlation is not appearent in all studies and are not found in endometrial carcinomas.

Plakoglobin/ -catenin

Plakoglobin is encoded by , which has so far not been found mutated in human primary tumors. Only one gastric carcinoma cell line and one squamous-cell lung carcinoma cell line have been reported with mutations in this gene. Nevertheless, and in contrast to -catenin, plakoglobin induces neoplastic transfomation of rat epithelial cells in the absence of stabilizing mutations. The cellular transformation performed by plakoglobin is also distinct from -catenin in that activation of the proto-oncogene c-MYC is required. Increased nuclear expression of plakoglobin is seen in several human tumors like colorectal carcinomas, endometrial carcinomas, esophageal carcinomas and testicular germ cell tumors. The C-terminal domain, which harbors the transactivating domain, is very different in plakoglobin and -catenin and these two proteins might therefore activate different target genes by recruiting different transcription co-factors to the plakoglobin/TCF and -catenin/TCF complexes.

APC

Patients with familial adenomatous polyposis coli (FAP) harbor a germline mutation in the tumor suppressor gene *APC*. Germline mutations within different regions of the gene are associated with different disease phenotypes, as for instance mutations in codon 1403 to 1578 are associated with extracolonic manifestations, whereas mutations in codon 78 to 167 and codon 1581 to 2843 are seen in attenuated adenomatous polyposis coli. Although more than 90% of the somatic mutations reported in *APC* are observed in colorectal carcinomas, mutations have also been described in breast carcinomas, desmoid carcinomas, hepatoblastomas, HCCs, intestinal type of gastric

carcinomas, medulloblastomas, ovarian carcinomas, pancreatic carcinomas and thyroid carcinomas (http://archive.uwcm.ac.uk./uwcm/mg/search/119682.html). Approximately 80% of sporadic colorectal carcinomas contain mutations in *APC*. The mutation cluster region, codons 1286-1513, accounts for 10% of the coding region, but harbors 80-90% of all *APC* mutations. The majority of the mutations lead to a truncated protein, missing some or all of the -catenin binding and down regulation sites, in addition to the AXIN binding sites and thus making APC disable to regulate the -catenin level in the cell. Minimum three of seven 20 amino acid repeats have to be intact for proper degradation of -catenin. However, for nuclear export of -catenin only one of seven 20 amino acid repeats in APC is required. In addition to genetic alterations of *APC*, inactivation through promoter hypermethylation has been found in a subset of several human malignancies.

AXIN

It has been suggested that AXIN1, which is constitutively expressed, is important for the regulation of the basal activity of the WNT signaling pathway, whereas AXIN2, which is induced in response to increased -catenin levels, rather regulates the duration and intensity of a WNT/ -catenin signal. Biallelic inactivation (mutation and deletion) of the *AXIN1* gene has been reported in HCC, implying that *AXIN1* acts like a tumor suppressor gene. *AXIN1* mutations have also been detected in some endometrioid ovarian carcinomas, medulloblastomas and microsatellite instable colorectal carcinomas. Exon seven of *AXIN2* contains four repetitive sequences and these are found mutated in about one fourth of colorectal carcinomas with MSI. An inverse correlation between mutations in *AXIN1/AXIN2* and *APC* or *CTNNB1* has been suggested, however some HCCs contain mutations in both *AXIN1/AXIN2* and *CTNNB1* and a few microsatellite instable colorectal carcinomas have mutations in both *AXIN2* and *APC*. As previously described, the DIX domain of AXIN is essential for the inhibitory effect of this protein on the WNT signaling pathway. The majority of the mutations described so far (both in *AXIN1* and *AXIN2*) are predicted to truncate the protein and probably give rise to a protein lacking a part of or the whole DIX domain.

TCF/LEF

TCF/LEF mRNAs undergo extensive alternative splicing. *TCF1* and *LEF1* also exhibit alternative promoter usage generating protein isoforms that either carry or lack binding sites for -catenin. LEF1 has been suggested as a positive feedback regulator of the Wnt signaling pathway in colorectal carcinogenesis. In these tumors the -catenin/TCF complexes selectively activate one of the promoters in *LEF1* leading to expression of a full-length isoform that binds -catenin. It has further been suggested that APC and LEF1 compete for nuclear -catenin and that LEF1 might anchor -catenin in the nucleus by blocking APC mediated nuclear export. On the other hand, up-

regulation of a dominant negative isoform of TCF1, that do not bind -catenin, has been observed in human colon cancer cell lines with an activated WNT signaling pathway. However, so far *TCF4* is the only TCF/LEF family member that has been found mutated in human cancers. *TCF4* contains an (A)9 repeat in exon 17 and this repeat is mutated in a subset of colorectal and gastric -carcinomas with MSI. This mutation decreases the proportion of the long TCF4 isoform, which contain two binding domains for the transcriptional co-repressor CtBP and might therefore constitutively activate transcription of WNT target genes. Interestingly, mutation in either *APC*, *CTNNB1* or *AXIN1* is observed in the tumors harboring a *TCF4* mutation.

b-TrCP

-TrCP is the F-box protein that control degradation of phosphorylated -catenin. Recently, this gene was found mutated in a human prostate cancer cell line and a prostate xenograft. Both alterations were heterozygous, but *in vitro* studies showed that they rendered the -TrCP protein deficient in -catenin binding and accumulation of nuclear -catenin was observed. Wild type *APC* and *CTNNB1* were seen in both cases suggesting that *-TrCP* might substitute for *APC* and *CTNNB1* mutations in prostate cancer. Interestingly, increased expression of -TrCP is detected in cells with an activated WNT signaling pathway, indicating that -TrCP is involved in a negative feedback regulation mechanism.

TP53

Cellular responses to TP53 activation include cell-cycle arrest, apoptosis, DNA repair, senescence and differentiation. Approximately 50% of all human cancers show mutation in *TP53* (http://www.iarc.fr/p53/Index.html). Newly, a functional cross-talk between TP53 and the WNT signaling pathway was observed. TP53 transactivates SIAH-1 leading to ubiquitin-mediated proteasome degradation of oncogenic (unphosphorylated) -catenin. Presently, it is unknown if *TP53* mutations substitute for oncogenic activation of *CTNNB1* during tumor development. However, in HCC *CTNNB1* mutations and *TP53* mutations are mutually exclusive.

PP2A

The *PP2R1B* gene, which encodes the b isoform of the A subunit of PP2A is mutated in 15% of human primary colon tumors. Additionally, some lung cancer cell lines show sequence alterations within this gene. These mutations might destabilize the holoenzyme complex and thus abolish its effect on the WNT signaling pathway.

E-cadherin

The gene encoding E-cadherin, *CDH1*, is altered in human tumorigenesis by different mechanisms. Germline mutations in *CDH1*

predispose to hereditary diffuse-type gastric cancer, whereas somatic mutations in *CDH1* are demonstrated in several human carcinomas, like diffuse type gastric carcinomas, lobular breast carcinomas, endometrial carcinomas, ovarian carcinomas and signet-ring cell carcinomas of the stomach. Certain tumors that display mutation in one allele of *CDH1* also acquire deletion in the other allele, which is consistent with a two-hit mechanism for inactivation. Hypermethylation of the *CDH1* promoter has been observed in some primary tumors without identified *CDH1* mutations, including human breast, colorectal, gastric, HCC, prostate and thyroid carcinomas. Transcriptional silencing of E-cadherin may also result from aberrant expression of transcription factors that repress its promoter. Examples of such transcription repressors are , SLUG, SIP1 and E12/E47. Interestingly, *SNAIL* is located to chromosome band 20q13.1, a region frequently amplified in human cancer. In HCC, breast carcinomas, melanoma and oral squamous cell carcinomas, an inverse correlation between SNAIL and E-cadherin expression is observed. Nevertheless, inactivation of E-cadherin does not appear to significantly increase the level of free cytosolic -catenin, probably because the excess of cytoplasmic -catenin rapidly is removed by an intact degradation system. It has been shown that introduction of *CDH1* into a cell line lacking E-cadherin and demonstrating constitutively transcription of WNT target genes, help sequester -catenin and thus reduce the transcription of WNT target genes. However, the converse has never been proven. Loss of expression of E-cadherin did not result in constitute -catenin/TCF transcriptional activation.

-catenin

The *CTNNA1*gene encodes -catenin, a protein involved in cell adhesion by anchoring the -catenin-E-cadherin complex to the actin cytoskeleton. *CTNNA1* has so far only been found mutated in some lung, prostate, ovarian, and colon cancer cell lines. 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 -catenin inactivation on WNT signaling has not been reported.

PTEN

PTEN is a phosphatase and tensin homologue that by dephosphorylation inhibits the activities of phosphatidylinositol-3 kinase (PI-3K). In *PTEN* null prostate cancer cell lines, PI-3K activates integrin-linked kinase (ILK), which further phosphorylates and inhibits the activity of GSK-3 . Subsequently, -catenin accumulates in the nucleus and increased expression of the WNT target gene, cyclin D1, is observed. Upon reexpression of wild-type PTEN, GSK-3 activity is elevated, leading to an increase in -catenin phosphorylation and subsequent degradation of -catenin. This might present a key mechanism by which PTEN works as a tumor suppressor protein.

Germline mutations in *PTEN* are associated with Cowden disease. *PTEN* is also frequently mutated or deleted in a variety of sporadic human malignancies, such as glioblastoma and carcinomas of the prostate, kidney, and breast. In addition, *PTEN* contains two (A)6 repeats within its coding region and these are found mutated endometrial, colorectal and gastric carcinomas with MSI. Finally, promoter methylation of *PTEN* has been observed in some solid tumors.

Several novel molecular data have during the last few years contributed to the understanding of the complexity of the WNT signaling pathway. However, many of the underlying mechanisms still remain unknown. Both genetic, epigenetic and expression alterations of molecules in the WNT signaling pathway are characteristic in human solid tumors. In the colorectal adenoma-carcinoma sequence, the majority of the tumors show accumulation of nuclear -catenin and this change is even apparent in aberrant crypt foci. A future perspective, when it comes to anti-cancer therapeutics, would be to block the -catenin-TCF complex and thereby transcription of WNT target genes.

References

1. Amit S, Hatzubai A, Birman Y, et al. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev. 16:1066-1076, 2002.
2. Barker N, Clevers H. Catenins, Wnt signaling and cancer. Bioessays 22:961-965, 2000.
3. Behrens J, Jerchow BA, Wurtele M, et al. Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. Science 280:596-599, 1998.
4. Behrens J, von Kries JP, Kuhl M, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature 382:638-642, 1996.
5. Bhanot P, Brink M, Samos CH, et al. A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature 382:225-230, 1996.
6. Blanco MJ, Moreno-Bueno G, Sarrio D, et al. Correlation of Snail expression with histological grade and lymph node status in breast carcinomas. Oncogene 21:3241-3246, 2002.
7. Brabender J, Usadel H, Danenberg KD, et al. Adenomatous polyposis coli gene promoter hypermethylation in non-small cell lung cancer is associated with survival. Oncogene 20:3528-3532, 2001.

8. Brabletz T, Jung A, Dag S, Hlubek F, Kirchner T. beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol 155:1033-1038, 1999.

9. Brannon M, Brown JD, Bates R, Kimelman D, Moon RT. XCtBP is a XTcf-3 co-repressor with roles throughout Xenopus development. Development 126:3159-3170, 1999.

10. Brown SD, Twells RC, Hey PJ, et al. Isolation and characterization of LRP6, a novel member of the low density lipoprotein receptor gene family. Biochem.Biophys.Res.Commun. 248:879-888, 1998.

11. Buda A, Pignatelli M. Genetics--cellular basis. Br.Med.Bull. 64:45-58.:45-58, 2002.

12. Bui TD, Beier DR, Jonssen M, et al. cDNA cloning of a human dishevelled DVL-3 gene, mapping to 3q27, and expression in human breast and colon carcinomas. Biochem.Biophys.Res.Commun. 20;239:510-516, 1997.

13. Caca K, Kolligs FT, Ji X, et al. Beta- and gamma-catenin mutations, but not E-cadherin inactivation, underlie T-cell factor/lymphoid enhancer factor transcriptional deregulation in gastric and pancreatic cancer. Cell Growth Differ. 10:369-376, 1999.

14. Cagatay T, Ozturk M. P53 mutation as a source of aberrant beta-catenin accumulation in cancer cells. Oncogene 21:7971-7980, 2002.

15. Conacci-Sorrell M, Zhurinsky J, Ben Ze'ev A. The cadherin-catenin adhesion system in signaling and cancer. J.Clin.Invest 109:987-991, 2002.

16. Crawford HC, Fingleton BM, L.A., et al. The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. Oncogene 18:2883-2891, 1999.

17. Dahmen RP, Koch A, Denkhaus D, et al. Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. Cancer Res. 61:7039-7043, 2001.

18. de Lau W, Clevers H. LEF1 turns over a new leaf. Nat.Genet. 28:3-4, 2001.

19. Dimitriadis A, Vincan E, Mohammed IM, Roczo N, Phillips WA, Baindur-Hudson S. Expression of Wnt genes in human colon cancers. Cancer Lett. 166:185-191, 2001.

20. Dong SM, Kim HS, Rha SH, Sidransky D. Promoter hypermethylation of multiple genes in carcinoma of the uterine cervix. Clin.Cancer Res. 7:1982-1986, 2001.

21. Duval A, Gayet J, Zhou XP, Iacopetta B, Thomas G, Hamelin R. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. Cancer Res 59:4213-4215, 1999.

22. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. Cancer Res. 62:2447-2454, 2002.

23. Duval A, Rolland S, Tubacher E, Bui H, Thomas G, Hamelin R. The human T-cell transcription factor-4 gene: structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. Cancer Res. 60:3872-3879, 2000.

24. Fodde R. The multiple functions of tumour suppressors: it's all in APC. Nat.Cell Biol. 5:190-192, 2003.

25. Fodde R, Kuipers J, Rosenberg C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat.Cell Biol. 3:433-438, 2001.

26. Franca-Koh J, Yeo M, Fraser E, Young N, Dale TC. The regulation of glycogen synthase kinase-3 nuclear export by Frat/GBP. J.Biol.Chem. 277:43844-43848, 2002.

27. Fraser E, Young N, Dajani R, et al. Identification of the Axin and Frat binding region of glycogen synthase kinase-3. J.Biol.Chem. 277:2176-2185, 2002.

28. Gerstein AV, Almeida TA, Zhao G, et al. APC/CTNNB1 (beta-catenin) pathway alterations in human prostate cancers. Genes Chromosomes.Cancer 34:9-16, 2002.

29. Gottardi CJ, Wong E, Gumbiner BM. E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. J.Cell Biol. 153:1049-1060, 2001.

30. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66:589-600, 1991.

31. Guanti G, Resta N, Simone C, et al. Involvement of PTEN mutations in the genetic pathways of colorectal cancerogenesis. Hum Mol Genet 9:283-287, 2000.

32. Gumbiner BM. Signal transduction of beta-catenin. Curr.Opin.Cell Biol. 7:634-640, 1995.

33. Hajra KM, Fearon ER. Cadherin and catenin alterations in human cancer. Genes Chromosomes.Cancer 34:255-268, 2002.

34.He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. Science 281:1509-1512, 1998.

35.Hecht A, Kemler R. Curbing the nuclear activities of beta-catenin. Control over Wnt target gene expression. EMBO Rep. 1:24-28, 2000.

36.Henderson BR. Nuclear-cytoplasmic shuttling of APC regulates beta-catenin subcellular localization and turnover. Nat Cell Biol 2:653-660, 2000.

37.Henderson BR, Fagotto F. The ins and outs of APC and beta-catenin nuclear transport. EMBO Rep. 3:834-839, 2002.

38.Henderson BR, Galea M, Schuechner S, Leung L. Lymphoid enhancer factor-1 blocks adenomatous polyposis coli-mediated nuclear export and degradation of beta-catenin. Regulation by histone deacetylase 1. J.Biol.Chem. 277:24258-24264, 2002.

39.Hey PJ, Twells RC, Phillips MS, et al. Cloning of a novel member of the low-density lipoprotein receptor family. Gene 216:103-111, 1998.

40.Hiltunen MO, Alhonen L, Koistinaho J, et al. Hypermethylation of the APC (adenomatous polyposis coli) gene promoter region in human colorectal carcinoma. Int J Cancer 70:644-648, 1997.

41.Hirano S, Kimoto N, Shimoyama Y, Hirohashi S, Takeichi M. Identification of a neural alpha-catenin as a key regulator of cadherin function and multicellular organization. Cell 70:293-301, 1992.

42.Hovanes K, Li TW, Munguia JE, et al. Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. Nat.Genet. 28:53-57, 2001.

43.Hsieh JC, Kodjabachian L, Rebbert ML, et al. A new secreted protein that binds to Wnt proteins and inhibits their activities. Nature 398:431-436, 1999.

44.Hsu W, Zeng L, Costantini F. Identification of a domain of Axin that binds to the serine/threonine protein phosphatase 2A and a self-binding domain. J.Biol.Chem. 274:3439-3445, 1999.

45.Huber AH, Weis WI. The structure of the beta-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by beta-catenin. Cell 105:391-402, 2001.

46.Hulsken J, Birchmeier W, Behrens J. E-cadherin and APC compete for the interaction with beta-catenin and the cytoskeleton. J.Cell Biol. 127:2061-2069, 1994.

47.Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a

complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. EMBO J. 17:1371-1384, 1998.

48. Ilyas M, Tomlinson IP, Rowan A, Pignatelli M, Bodmer WF. Beta-catenin mutations in cell lines established from human colorectal cancers. Proc.Natl.Acad.Sci.U.S.A 94:10330-10334, 1997.

49. Jiao W, Miyazaki K, Kitajima Y. Inverse correlation between E-cadherin and Snail expression in hepatocellular carcinoma cell lines in vitro and in vivo. Br.J.Cancer 86:98-101, 2002.

50. Jin Z, Tamura G, Tsuchiya T, et al. Adenomatous polyposis coli (APC) gene promoter hypermethylation in primary breast cancers. Br.J.Cancer 85:69-73, 2001.

51. Jonkers J, Korswagen HC, Acton D, Breuer M, Berns A. Activation of a novel proto-oncogene, Frat1, contributes to progression of mouse T-cell lymphomas. EMBO J. 16:441-450, 1997.

52. Joslyn G, Richardson DS, White R, Alber T. Dimer formation by an N-terminal coiled coil in the APC protein. Proc.Natl.Acad.Sci.U.S.A 90:11109-11113, 1993.

53. Kang YH, Lee HS, Kim WH. Promoter methylation and silencing of PTEN in gastric carcinoma. Lab Invest 82:285-291, 2002.

54. Kaplan KB, Burds AA, Swedlow JR, Bekir SS, Sorger PK, Nathke IS. A role for the Adenomatous Polyposis Coli protein in chromosome segregation. Nat.Cell Biol. 3:429-432, 2001.

55. Kawakami K, Brabender J, Lord RV, et al. Hypermethylated APC DNA in plasma and prognosis of patients with esophageal adenocarcinoma. J.Natl.Cancer Inst. 92:1805-1811, 2000.

56. Kawasaki Y, Senda T, Ishidate T, et al. Asef, a link between the tumor suppressor APC and G-protein signaling. Science 289:1194-1197, 2000.

57. Kemler R, Ozawa M. Uvomorulin-catenin complex: cytoplasmic anchorage of a Ca2+-dependent cell adhesion molecule. Bioessays 11:88-91, 1989.

58. Kikuchi A. Modulation of Wnt signaling by Axin and Axil. Cytokine Growth Factor Rev. 10:255-265, 1999.

59. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 87:159-170, 1996.

60. Kishida S, Yamamoto H, Hino S, Ikeda S, Kishida M, Kikuchi A. DIX domains of Dvl and axin are necessary for protein interactions and their ability to regulate beta-catenin stability. Mol.Cell Biol. 19:4414-4422, 1999.

61.Kitaeva MN, Grogan L, Williams JP, et al. Mutations in beta-catenin are uncommon in colorectal cancer occurring in occasional replication error-positive tumors. Cancer Res 57:4478-4481, 1997.

62.Klingensmith J, Nusse R, Perrimon N. The Drosophila segment polarity gene dishevelled encodes a novel protein required for response to the wingless signal. Genes Dev. 8:118-130, 1994.

63.Kolligs FT, Kolligs B, Hajra KM, et al. gamma-catenin is regulated by the APC tumor suppressor and its oncogenic activity is distinct from that of beta-catenin. Genes Dev. 14:1319-1331, 2000.

64.Kong D, Suzuki A, Zou TT, et al. PTEN1 is frequently mutated in primary endometrial carcinomas. Nat.Genet. 17:143-144, 1997.

65.Kramps T, Peter O, Brunner E, et al. Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. Cell 109:47-60, 2002.

66.Krupnik VE, Sharp JD, Jiang C, et al. Functional and structural diversity of the human Dickkopf gene family. Gene 238:301-313, 1999.

67.Laurent-Puig P, Beroud C, Soussi T. APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res. 26:269-270, 1998.

68.Leung JY, Kolligs FT, Wu R, et al. Activation of AXIN2 expression by beta-catenin-T cell factor. A feedback repressor pathway regulating Wnt signaling. J.Biol.Chem. 277:21657-21665, 2002.

69.Li L, Yuan H, Weaver CD, et al. Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. EMBO J. 18:4233-4240, 1999.

70.Li X, Yost HJ, Virshup DM, Seeling JM. Protein phosphatase 2A and its B56 regulatory subunit inhibit Wnt signaling in Xenopus. EMBO J. 20:4122-4131, 2001a.

71.Li Y, Podsypanina K, Liu X, et al. Deficiency of Pten accelerates mammary oncogenesis in MMTV-Wnt-1 transgenic mice. BMC.Mol.Biol. 2:22001b.

72.Liu C, Li Y, Semenov M, et al. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108:837-847, 2002.

73.Liu J, Stevens J, Rote CA, et al. Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol.Cell 7:927-936, 2001.

74. Liu W, Dong X, Mai M, et al. Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling. Nat.Genet. 26:146-147, 2000.

75. Lovig T, Meling GI, Diep CB, et al. APC and CTNNB1 mutations in a large series of sporadic colorectal carcinomas stratified by the microsatellite instability status. Scand.J.Gastroenterol. 37:1184-1193, 2002.

76. Lustig B, Jerchow B, Sachs M, et al. Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. Mol.Cell Biol. 22:1184-1193, 2002.

77. Mai M, Qian C, Yokomizo A, Smith DI, Liu W. Cloning of the human homolog of conductin (AXIN2), a gene mapping to chromosome 17q23-q24. Genomics 55:341-344, 1999. Manoukian,A.S. and Woodgett,J.R. Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. Adv.Cancer Res. 84:203-29.:203-229, 2002.

78. Mao B, Wu W, Davidson G, et al. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. Nature 417:664-667, 2002.

79. Mao B, Wu W, Li Y, et al. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411:321-325, 2001a.

80. Mao J, Wang J, Liu B, et al. Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway. Mol.Cell 7:801-809, 2001b.

81. Matsumine A, Ogai A, Senda T, et al. Binding of APC to the human homolog of the Drosophila discs large tumor suppressor protein. Science 272:1020-1023, 1996.

82. Matsuzawa SI, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol.Cell 7:915-926, 2001.

83. McCrea PD, Turck CW, Gumbiner B. A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E-cadherin. Science 254:1359-1361, 1991.

84. Melkonyan HS, Chang WC, Shapiro JP, et al. SARPs: a family of secreted apoptosis-related proteins. Proc.Natl.Acad.Sci.U.S.A 94:13636-13641, 1997.

85. Mirabelli-Primdahl L, Gryfe R, Kim H, et al. Beta-catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. Cancer Res 59:3346-3351, 1999.

86. Miyaki M, Iijima T, Kimura J, et al. Frequent mutation of beta-catenin and APC genes in primary colorectal tumors from patients with hereditary nonpolyposis colorectal cancer. Cancer Res. 59:4506-4509, 1999.

87. Miyasaka H, Choudhury BK, Hou EW, Li SS. Molecular cloning and expression of mouse and human cDNA encoding AES and ESG proteins with strong similarity to Drosophila enhancer of split groucho protein. Eur.J.Biochem. 216:343-352, 1993.

88. Moreno-Bueno G, Hardisson D, Sanchez C, et al. Abnormalities of the APC/beta-catenin pathway in endometrial cancer. Oncogene 21:7981-7990, 2002.

89. Nagafuchi A, Ishihara S, Tsukita S. The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. J.Cell Biol. 127:235-245, 1994.

90. Neufeld KL, Nix DA, Bogerd H, et al. Adenomatous polyposis coli protein contains two nuclear export signals and shuttles between the nucleus and cytoplasm. Proc.Natl.Acad.Sci.U.S.A 97:12085-12090, 2000a.

91. Neufeld KL, Zhang F, Cullen BR, White RL. APC-mediated downregulation of beta-catenin activity involves nuclear sequestration and nuclear export. EMBO Rep. 1:519-523, 2000b.

92. Nusse R, Brown A, Papkoff J, et al. A new nomenclature for int-1 and related genes: the Wnt gene family. Cell 64:2311991.

93. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31:99-109, 1982.

94. Oving IM, Clevers HC. Molecular causes of colon cancer. Eur.J.Clin.Invest 32:448-457, 2002.

95. Pai LM, Kirkpatrick C, Blanton J, Oda H, Takeichi M, Peifer M. Drosophila alpha-catenin and E-cadherin bind to distinct regions of Drosophila Armadillo. J.Biol.Chem. 271:32411-32420, 1996.

96. Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis--a look outside the nucleus. Science 287:1606-1609, 2000.

97. Persad S, Troussard AA, McPhee TR, Mulholland DJ, Dedhar S. Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. J.Cell Biol. 153:1161-1174, 2001.

98.Pinson KI, Brennan J, Monkley S, Avery BJ, Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 407:535-538, 2000.

99.Pizzuti A, Amati F, Calabrese G, et al. cDNA characterization and chromosomal mapping of two human homologues of the Drosophila dishevelled polarity gene. Hum.Mol.Genet. 5:953-958, 1996.

100. Polakis P. Wnt signaling and cancer. Genes Dev. 14:1837-1851, 2000.

101. Polakis,P. More than one way to skin a catenin. Cell 105:563-566, 2001.

102. Poser I, Dominguez D, de Herreros AG, Varnai A, Buettner R, Bosserhoff AK. Loss of E-cadherin expression in melanoma cells involves up-regulation of the transcriptional repressor Snail. J.Biol.Chem. 276:24661-24666, 2001.

103. Rattner A, Hsieh JC, Smallwood PM, et al. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc.Natl.Acad.Sci.U.S.A 94:2859-2863, 1997.

104. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res. 57:4736-4738, 1997.

105. Roose J, Clevers H. TCF transcription factors: molecular switches in carcinogenesis. Biochim.Biophys.Acta 1424:M23-M371999.

106. Roose J, Huls G, van Beest M, et al. Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. Science 285:1923-1926, 1999.

107. Rousset R, Mack JA, Wharton KA, Jr., et al. Naked cuticle targets dishevelled to antagonize Wnt signal transduction. Genes Dev. 15:658-671, 2001.

108. Rubinfeld B, Albert I, Porfiri E, Munemitsu S, Polakis P. Loss of beta-catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene. Cancer Res. 57:4624-4630, 1997.

109. Rubinfeld B, Souza B, Albert I, et al. Association of the APC gene product with beta-catenin. Science 262:1731-1734, 1993.

110. Rubinfeld B, Souza B, Albert I, Munemitsu S, Polakis P. The APC protein and E-cadherin form similar but independent complexes with alpha-catenin, beta-catenin, and plakoglobin. J.Biol.Chem. 270:5549-5555, 1995.

111. Ryo A, Nakamura M, Wulf G, Liou YC, Lu KP. Pin1 regulates turnover and subcellular localization of beta-catenin by inhibiting its interaction with APC. Nat.Cell Biol. 3:793-801, 2001.

112. Saitoh T, Moriwaki J, Koike J, et al. Molecular cloning and characterization of FRAT2, encoding a positive regulator of the WNT signaling pathway. Biochem.Biophys.Res.Commun. 281:815-820, 2001.

113. Sakanaka C, Leong P, Xu L, Harrison SD, Williams LT. Casein kinase iepsilon in the wnt pathway: regulation of beta-catenin function. Proc.Natl.Acad.Sci.U.S.A 96:12548-12552, 1999.

114. Salahshor S, Kressner U, Pahlman L, Glimelius B, Lindmark G, Lindblom A. Colorectal cancer with and without microsatellite instability involves different genes. Genes Chromosomes.Cancer 26:247-252, 1999.

115. Salvesen HB, MacDonald N, Ryan A, et al. PTEN methylation is associated with advanced stage and microsatellite instability in endometrial carcinoma. Int.J.Cancer 91:22-26, 2001.

116. Satoh S, Daigo Y, Furukawa Y, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat.Genet. 24:245-250, 2000.

117. Savelieva E, Belair CD, Newton MA, et al. 20q gain associates with immortalization: 20q13.2 amplification correlates with genome instability in human papillomavirus 16 E7 transformed human uroepithelial cells. Oncogene 14:551-560, 1997.

118. Schwarz-Romond T, Asbrand C, Bakkers J, et al. The ankyrin repeat protein Diversin recruits Casein kinase Iepsilon to the beta-catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. Genes Dev. 16:2073-2084, 2002.

119. Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM. Regulation of beta-catenin signaling by the B56 subunit of protein phosphatase 2A. Science 283:2089-2091, 1999.

120. Shimizu Y, Ikeda S, Fujimori M, et al. Frequent alterations in the Wnt signaling pathway in colorectal cancer with microsatellite instability. Genes Chromosomes.Cancer 33:73-81, 2002.

121. Shitoh K, Furukawa T, Kojima M, et al. Frequent activation of the beta-catenin-Tcf signaling pathway in nonfamilial colorectal carcinomas with microsatellite instability. Genes Chromosomes.Cancer 30:32-37, 2001.

122. Shtutman M, Zhurinsky J, Simcha I, et al. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci U S A 96:5522-5527, 1999.

123. Sieber OM, Tomlinson IP, Lamlum H. The adenomatous polyposis coli (APC) tumour suppressor--genetics, function and disease. Mol.Med.Today 6:462-469, 2000.

124. Skotheim RI, Monni O, Mousses S, et al. New insights into testicular germ cell tumorigenesis from gene expression profiling. Cancer Res. 62:2359-2364, 2002.

125. Soria JC, Lee HY, Lee JI, et al. Lack of PTEN expression in non-small cell lung cancer could be related to promoter methylation. Clin.Cancer Res. 8:1178-1184, 2002.

126. Spiegelman VS, Slaga TJ, Pagano M, Minamoto T, Ronai Z, Fuchs SY. Wnt/beta-catenin signaling induces the expression and activity of betaTrCP ubiquitin ligase receptor. Mol.Cell 5:877-882, 2000.

127. Spink KE, Polakis P, Weis WI. Structural basis of the Axin-adenomatous polyposis coli interaction. EMBO J. 19:2270-2279, 2000.

128. Stifani S, Blaumueller CM, Redhead NJ, Hill RE, Artavanis-Tsakonas S. Human homologs of a Drosophila Enhancer of split gene product define a novel family of nuclear proteins. Nat.Genet. 2:119-127, 1992.

129. Su LK, Burrell M, Hill DE, et al. APC binds to the novel protein EB1. Cancer Res. 55:2972-2977, 1995.

130. Su LK, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. Science 262:1734-1737, 1993.

131. Tago K, Nakamura T, Nishita M, et al. Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. Genes Dev. 14:1741-1749, 2000.

132. Takemaru KI, Moon RT. The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. J.Cell Biol. 149:249-254, 2000.

133. Tamai K, Semenov M, Kato Y, et al. LDL-receptor-related proteins in Wnt signal transduction. Nature 407:530-535, 2000.

134. Taniguchi K, Roberts LR, Aderca IN, et al. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. Oncogene 21:4863-4871, 2002.

135. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398:422-426, 1999.

136. Thorstensen L, Diep CB, Meling GI, et al. WNT1 inducible signaling pathway protein 3, WISP-3, a novel target gene in colorectal carcinomas with microsatellite instability. Gastroenterology 121:1275-1280, 2001.

137. Ueda M, Gemmill RM, West J, et al. Mutations of the beta- and gamma-catenin genes are uncommon in human lung, breast, kidney, cervical and ovarian carcinomas. Br.J.Cancer 85:64-68, 2001.

138. Uthoff SM, Eichenberger MR, McAuliffe TL, Hamilton CJ, Galandiuk S. Wingless-type frizzled protein receptor signaling and its putative role in human colon cancer. Mol.Carcinog. 31:56-62, 2001.

139. van de WM, Barker N, Harkes IC, et al. Mutant E-cadherin breast cancer cells do not display constitutive Wnt signaling. Cancer Res. 61:278-284, 2001.

140. van de WM, Cavallo R, Dooijes D, et al. Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell 88:789-799, 1997.

141. van Noort M, Clevers H. TCF transcription factors, mediators of Wnt-signaling in development and cancer. Dev.Biol. 244:1-8, 2002.

142. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat.Rev.Cancer 2:594-604, 2002.

143. Wang SS, Esplin ED, Li JL, et al. Alterations of the PPP2R1B gene in human lung and colon cancer. Science 282:284-287, 1998.

144. Wang Y, Macke JP, Abella BS, et al. A large family of putative transmembrane receptors homologous to the product of the Drosophila tissue polarity gene frizzled. J.Biol.Chem. 271:4468-4476, 1996.

145. Wehrli M, Dougan ST, Caldwell K, et al. arrow encodes an LDL-receptor-related protein essential for Wingless signalling. Nature 407:527-530, 2000.

146. Wheeler JM, Kim HC, Efstathiou JA, Ilyas M, Mortensen NJ, Bodmer WF. Hypermethylation of the promoter region of the E-cadherin gene (CDH1) in sporadic and ulcerative colitis associated colorectal cancer. Gut 48:367-371, 2001.

147. Willert K, Brink M, Wodarz A, Varmus H, Nusse R. Casein kinase 2 associates with and phosphorylates dishevelled. EMBO J. 16:3089-3096, 1997.

148. Willert K, Nusse R. Beta-catenin: a key mediator of Wnt signaling. Curr.Opin.Genet.Dev. 8:95-102, 1998.

149. Wong NA, Pignatelli M. Beta-catenin--a linchpin in colorectal carcinogenesis? Am.J.Pathol. 160:389-401, 2002.

150. Woodgett JR. Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J. 9:2431-2438, 1990.

151. Wu R, Zhai Y, Fearon ER, Cho KR. Diverse mechanisms of beta-catenin deregulation in ovarian endometrioid adenocarcinomas. Cancer Res. 61:8247-8255, 2001.

152. Wu W, Glinka A, Delius H, Niehrs C. Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/beta-catenin signalling. Curr.Biol. 10:1611-1614, 2000.

153. Xu L, Corcoran RB, Welsh JW, Pennica D, Levine AJ. WISP-1 is a Wnt-1- and beta-catenin-responsive oncogene. Genes Dev 14:585-595, 2000.

154. Yamamoto H, Kishida S, Uochi T, et al. Axil, a member of the Axin family, interacts with both glycogen synthase kinase 3beta and beta-catenin and inhibits axis formation of Xenopus embryos. Mol.Cell Biol. 18:2867-2875, 1998.

155. Yan D, Wiesmann M, Rohan M, et al. Elevated expression of axin2 and hnkd mRNA provides evidence that Wnt/beta - catenin signaling is activated in human colon tumors. Proc.Natl.Acad.Sci.U.S.A 98:14973-14978, 2001.

156. Yokoyama K, Kamata N, Hayashi E, et al. Reverse correlation of E-cadherin and snail expression in oral squamous cell carcinoma cells in vitro. Oral Oncol. 37:65-71, 2001.

157. Zeng L, Fagotto F, Zhang T, et al. The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. Cell 90:181-192, 1997.

158. Zeng W, Wharton KA, Jr., Mack JA, et al. naked cuticle encodes an inducible antagonist of Wnt signalling. Nature 403:789-795, 2000.

159. Zhang F, White RL, Neufeld KL. Phosphorylation near nuclear localization signal regulates nuclear import of adenomatous polyposis coli protein. Proc.Natl.Acad.Sci.U.S.A 97:12577-12582, 2000.

160. Zhang F, White RL, Neufeld KL. Cell density and phosphorylation control the subcellular localization of adenomatous polyposis coli protein. Mol.Cell Biol. 21:8143-8156, 2001.

161. Zumbrunn J, Kinoshita K, Hyman AA, Nathke IS. Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 beta phosphorylation. Curr.Biol. 11:44-49, 2001.

162. Zysman M, Saka A, Millar A, Knight J, Chapman W, Bapat B. Methylation of adenomatous polyposis coli in endometrial cancer occurs more frequently in tumors with microsatellite instability phenotype. Cancer Res. 62:3663-3666, 2002.

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