The WNT Signaling Pathway and Its Role in Human Solid Tumors

Lin Thorstensen, Ragnhild A Lothe

Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, N-0310 Oslo, Norway (LT, RAL)

Published in Atlas Database: April 2003

DOI: 10.4267/2042/37975

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 2.0 France Licence.
© 2003 Atlas of Genetics and Cytogenetics in Oncology and Haematology

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 mammals. 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:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
</tr>
<tr>
<td>Arm</td>
<td>Armadillo</td>
</tr>
<tr>
<td>Asef</td>
<td>APC-stimulated guanine nucleotide exchange factor</td>
</tr>
<tr>
<td>β-TrCP</td>
<td>β-transducin repeat-containing protein</td>
</tr>
<tr>
<td>CBP</td>
<td>CREB binding protein</td>
</tr>
<tr>
<td>CK1, 2</td>
<td>Casein kinase 1 and 2</td>
</tr>
<tr>
<td>CtbP</td>
<td>C-terminal binding protein</td>
</tr>
<tr>
<td>DIX</td>
<td>Dishevelled homologous</td>
</tr>
<tr>
<td>Dkk</td>
<td>Dickkopf</td>
</tr>
<tr>
<td>DLG</td>
<td>Disc large tumor suppressor protein</td>
</tr>
<tr>
<td>Dsh/Dvl</td>
<td>Dishevelled</td>
</tr>
<tr>
<td>FAP</td>
<td>Familial adenomatous polyposis coli</td>
</tr>
<tr>
<td>FRAT</td>
<td>Frequently rearranged in advanced T-cell lymphomas</td>
</tr>
<tr>
<td>Fz</td>
<td>Frizzled</td>
</tr>
<tr>
<td>GBP</td>
<td>GSK-3 binding protein</td>
</tr>
<tr>
<td>GSK-3</td>
<td>Glycogen synthase kinase-3</td>
</tr>
<tr>
<td>HMG</td>
<td>High mobility group</td>
</tr>
<tr>
<td>HCC</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>ICAT</td>
<td>Inhibitor of β-catenin and TCF-4</td>
</tr>
<tr>
<td>ILK</td>
<td>Integrin-linked kinase</td>
</tr>
<tr>
<td>Lef</td>
<td>Lymphoid enhancing factor</td>
</tr>
<tr>
<td>Lgs</td>
<td>Legless</td>
</tr>
<tr>
<td>LRP</td>
<td>Low density lipoprotein receptor related protein</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>MMTV</td>
<td>Mouse mammary tumor virus</td>
</tr>
<tr>
<td>MSI</td>
<td>Microsatellite instability</td>
</tr>
</tbody>
</table>

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.

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.

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 (β-catenin-Axin-adenomatous polyposis coli (APC)-glycogen synthase kinase (GSK)-β), which targets β-catenin by phosphorylation for degradation by the proteasome. Dsh/Dvl is suggested to bind CK1 and thereby inhibiting priming of β-catenin and indirectly preventing GSK-3β phosphorylation of β-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-3β from Axin and in this way inhibits phosphorylation of β-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 β-catenin.

Stabilized β-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 β-catenin and initiation of the β-catenin phosphorylation cascade performed by GSK-3β. Phosphorylated β-catenin is then recognized by β-transducin repeat-containing protein (β-TrCP) and degraded by the proteasome, reducing the level of cytosolic β-catenin.
The first class, secreted frizzled-related proteins (sFRPs), are also called secreted apoptosis-related proteins (SAPRs) 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 LRP6 lead 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)

![Figure 2. Dsh/Dvl and binding partners](image-url)

The dishevelled homologous (DIX) domain of Dvl binds Axin. This binding inhibits Axin promoted GSK-3β dependent phosphorylation of β-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 β-catenin causing stabilization of β-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 β-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-3β, however these two components share overlapping binding sites on GSK-3β 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-3β from Axin leading to accumulation of β-catenin in the cytoplasm (Fraser et al., 2002). The human homologue of GBP is frequently rearranged in advanced T-cell lymphomas (FRAT). Newy, FRAT/GBP was shown to contain a nuclear export sequence, leading to nuclear export of itself as well as the bound GSK-3β. Thus, FRAT/GBP is involved in regulating the accessibility of cytoplasmic GSK-3β (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).
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 β-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 β-catenin (Uthoff et al., 2001). However, its exact mechanism of action remains unknown, but several binding partners have been detected (Figure 2).

4. The multiprotein complex

The stability of β-catenin (encoded by the gene CTNNB1) is regulated by a multiprotein complex consisting of β-catenin, Axin/Conductin, APC, and GSK-3β (Schwarz-Romond et al., 2002).

---

Figure 3. β-catenin and binding partners

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

GSK-3β sequentially phosphorylates threonine (Thr) 41, Ser 35, and Ser 33 of β-catenin (Liu et al., 2001; Matsuzawa and Reed, 2001). Both degradation systems require an intact APC protein (Polakis, 2001).

β-catenin and its binding partners have been shown to colocalize in the nucleus along with TCF-4 and its E-box binding partners (Rubinfeld et al., 1995). β-catenin and TCF-4 bind to the same sites on the N-terminal domain of β-catenin (encoded by the gene CTNNB1) by CK1 (Amit et al., 2002; Schwarz-Romond et al., 2002; Liu et al., 2002).

β-catenin-TCF-4 mediated transactivation of Wnt target genes is then repressed (Tago et al., 2000).
In this scaffolding complex, GSK-3β phosphorylates primed β-catenin, thus marking β-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.

β-catenin

β-catenin was first described in humans as a protein which interacts with the cytoplasmic domain of E-cadherin and with α-catenin, anchoring the cadherin complex to the actin cytoskeleton (Kemler and Ozawa, 1989). Then, the homology between β-catenin and the Armadillo (Arm) of Drosophila and β-catenin in Xenopus lead to the discovery of an additional role for mammalian β-catenin, namely as the key mediator of Wnt signaling (McCrea et al., 1991; Gumbiner, 1995).

The primary structure of β-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 β-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 γ-catenin, shares overall 70% amino acid identity with β-catenin and as much as 80% within the arm repeat domain (Huber and Weis, 2001). Plakoglobin binds E-cadherin, α-catenin, APC, Axin and Tcf/Lef transcription factors, and is involved in cell adhesion as well as Wnt signaling. However, differences between β-catenin and plakoglobin in these processes exist (Kolligs et al., 2000).

β-catenin activity is controlled by a large number of binding partners that affect the stability and localization of β-catenin (Figure 3).

APC

The first hint of the mode of action of APC came from studies showing that APC binds β-catenin (Rubinfeld et al., 1993; Su et al., 1993). Later it has been demonstrated that APC plays a central role in regulating the β-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 (Josyn et al., 1993). The holoenzyme, protein phosphatase 2A (PP2A), comprises three subunits, the structural- (A), the regulator- (B), and catalytic- (C) subunit. APC, like β-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 β-catenin degradation complex, and PP2A is suggested to dephosphorylate and activate GSK-3β, leading to degradation of β-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 Rac, 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 β-catenin (Rubinfeld et al., 1993). Phosphorylation of APC by GSK-3β increases the negative charge on APC and strengthens the interaction between APC and the positively charged arm repeat domain of β-catenin (Oving and Clevers, 2002). Down regulation of β-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 (Brehrens 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 (NESSs), located near the amino terminus (Neufeld et al., 2000a; Henderson and Fagotto, 2002). Recently, it was shown that APC binds nuclear β-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 and the stability of its nuclear import (Zumbrunn 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 EB1, another microtubul binding protein, which is required for APC-mediated attachment of microtubules to the chromosomes (Yielding et al., 1998), 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-3β (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 \( \beta \)-catenin and thereby acts as a negative regulator of Wnt signaling. Later, a homologue of Axin in mice, 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\( \beta \) is recruited to the multiprotein complex by Axin (Behrens et al., 1998) and then phosphorylates Axin and APC and increases their interaction with \( \beta \)-catenin (Peifer and Polakis, 2000). Subsequently, primed \( \beta \)-catenin bound to Axin and APC (Behrens et al., 1998), is phosphorylated by GSK-3\( \beta \), marking \( \beta \)-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\( \beta \) cooperate in \( \beta \)-catenin degradation, however diversin and GSK-3\( \beta \) use identical binding sites on Axin suggesting that a homodimeric Axin complex is present to perform phosphorylation and degradation of \( \beta \)-catenin. One of the Axin molecules binds diversin that recruits CK1, leading to priming of \( \beta \)-catenin, whereas the other Axin molecule binds GSK-3\( \beta \) causing phosphorylation of primed \( \beta \)-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 \( \beta \)-catenin (Kikuchi, 1999).

**Figure 6. Tcf/Lef and binding partners**

Nuclear \( \beta \)-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 \( \beta \)-catenin (Figure 3).

In Xenopus, Smad4, an essential component of the transforming growth factor (TGF)-\( \beta \) signaling pathway, interacts with Lef1, making a \( \beta \)-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 \( \beta \)-catenin. One of these is the Groucho (found in *Drosophila*), which interacts with a histone deacetylase resulting in a euchromatin 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, CIBP, is shown to bind and repress Tcf3 and Tcf4. TGF\( \beta \)-activated kinase (Tak)-1 acts 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 \( \beta \)-catenin-Tcf complexes from the promoter region of Wnt target genes.
GSK-3β

GSK-3β, 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-3β phosphorylates primed β-catenin prior to proteasome degradation, and it phosphorylates Axin and APC and enhances their interaction with β-catenin. Unlike most protein kinases, GSK-3β is constitutively active and phosphorylation of GSK-3β leads to inhibition of its activity (Manoukian and Woodgett, 2002). Two highly related human homologues are identified; GSK-3α and GSK-3β and these two isoforms are more than 95% identical in the protein kinase catalytic domain (Woodgett, 1990). Consequently, GSK-3α can substitute for many, but not all of the functions of GSK-3β in the Wnt signaling pathway (Manoukian and Woodgett, 2002).

5. Nuclear components

The stabilized cytosolic β-catenin will be translocated into the nucleus, but how β-catenin enters the nucleus is not yet fully understood. Nuclear β-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 β-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.

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 β-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-3β been reported mutated in human cancers. This might be explained by the central role of GSK-3β 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-3α may substitute for loss of GSK-3β 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.

β-catenin

The CTNNB1 gene encodes β-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-3β (Ser 33, 37 and Thr 41) and thus the recognition site of β-TrCP marking β-catenin for degradation. Therefore mutations within this exon increase the stability of the β-catenin protein. Indeed, somatic mutations in exon 3 have been described in a wide variety of human tumors, including colorectal carcinoma, desmoids tumor, endometrial carcinoma, HCC, hepatoblastoma, intestinal carcinoma gastric, medulloblastoma, melanoma, ovarian carcinoma, pancreatic carcinoma, pilomatrixcoma, 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 apparent in all studies and are not found in endometrial carcinomas.

---

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).
**Plakoglobin/γ-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 β-catenin, plakoglobin induces neoplastic transformation of rat epithelial cells in the absence of stabilizing mutations. The cellular transformation performed by plakoglobin is also distinct from β-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 β-catenin and these two proteins might therefore activate different target genes by recruiting different transcription co-factors to the plakoglobin/TCF and β-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 β-catenin binding and down regulation sites, in addition to the AXIN binding sites and thus making APC disable to binding and down regulation sites, in addition to the alterations of repeats in APC is required. In addition to genetic alterations of APC, inactivation through promoter hypermethylation has been found in a subset of 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 β-catenin levels, rather regulates the duration and intensity of a WNT/β-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 β-catenin. LEF1 has been suggested as a positive feedback regulator of the Wnt signaling pathway in colorectal carcinogenesis. In these tumors the β-catenin/TCF complexes selectively activate one of the promoters in LEF1 leading to expression of a full-length isoform that binds β-catenin. It has further been suggested that APC and LEF1 compete for nuclear β-catenin and that LEF1 might anchor β-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 β-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

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

TP53

Cellular responses to TP53 activation include cell-cycle arrest, apoptosis, DNA repair, senescence and differentiation. Approximately 50% of all human cancers show a 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) β-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 β 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 β-catenin, probably because the excess of cytoplasmic β-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 β-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 β-catenin/TCF transcriptional activation.

α-catenin

The CTNNA1 gene encodes α-catenin, a protein that is associated with 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 α-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-3β. Subsequently, β-catenin accumulates in the nucleus and increased expression of the WNT target gene, cyclin D1, is observed. Upon reexpression of wild-type PTEN, GSK-3β activity is elevated, leading to an increase in β-catenin phosphorylation and subsequent degradation of β-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 glioblastomas 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 β-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 β-catenin-TCF complex and thereby transcription of WNT target genes.

References

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. 1982 Nov;31(1):99-109


Kinzler KW, vogelstein B. Lessons from hereditary colorectal cancer. Cell. 1996 Oct 18;87(2):159-70


Jonkers J, Korsswagen HC, Acton D, Breuer M, Berns A. Activation of a novel proto-oncogene, Frat1, contributes to progression of mouse T-cell lymphomas. EMBO J. 1997 Feb 5;16(3):441-50


Rattner A, Hsieh JC, Smallwood PM, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc Natl Acad Sci U S A. 1997 Apr 1;94(7):2859-63


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. 1998 Mar 2;17(5):1371-84


Kikuchi A. Modulation of Wnt signaling by Axin and Axil. Cytokine Growth Factor Rev. 1999 Sep-Dec;10(3-4):255-65


Li L, Yuan H, Weaver CD, Mao J, Farr GH 3rd, Sussman DJ, Jonkers J, Kimelman D, Wu D. Axin and Frat1 interact with dvl
and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1. EMBO J. 1999 Aug 2;18(15):4233-40


The WNT Signaling Pathway and Its Role in Human Solid Tumors

Thorstensen L, Lothe RA

Atlas Genet Cytogenet Oncol Haematol. 2003; 7(2)


The WNT Signaling Pathway and Its Role in Human Solid Tumors

Thorsetsen L, Lothe RA

This article should be referenced as such: Thorsetsen L, Lothe RA. The WNT Signaling Pathway and Its Role in Human Solid Tumors. Atlas Genet Cytogenet Oncol Haematol. 2003; 7(2):146-161.

---


