

## Wnt/frizzled signaling in hepatocellular carcinoma

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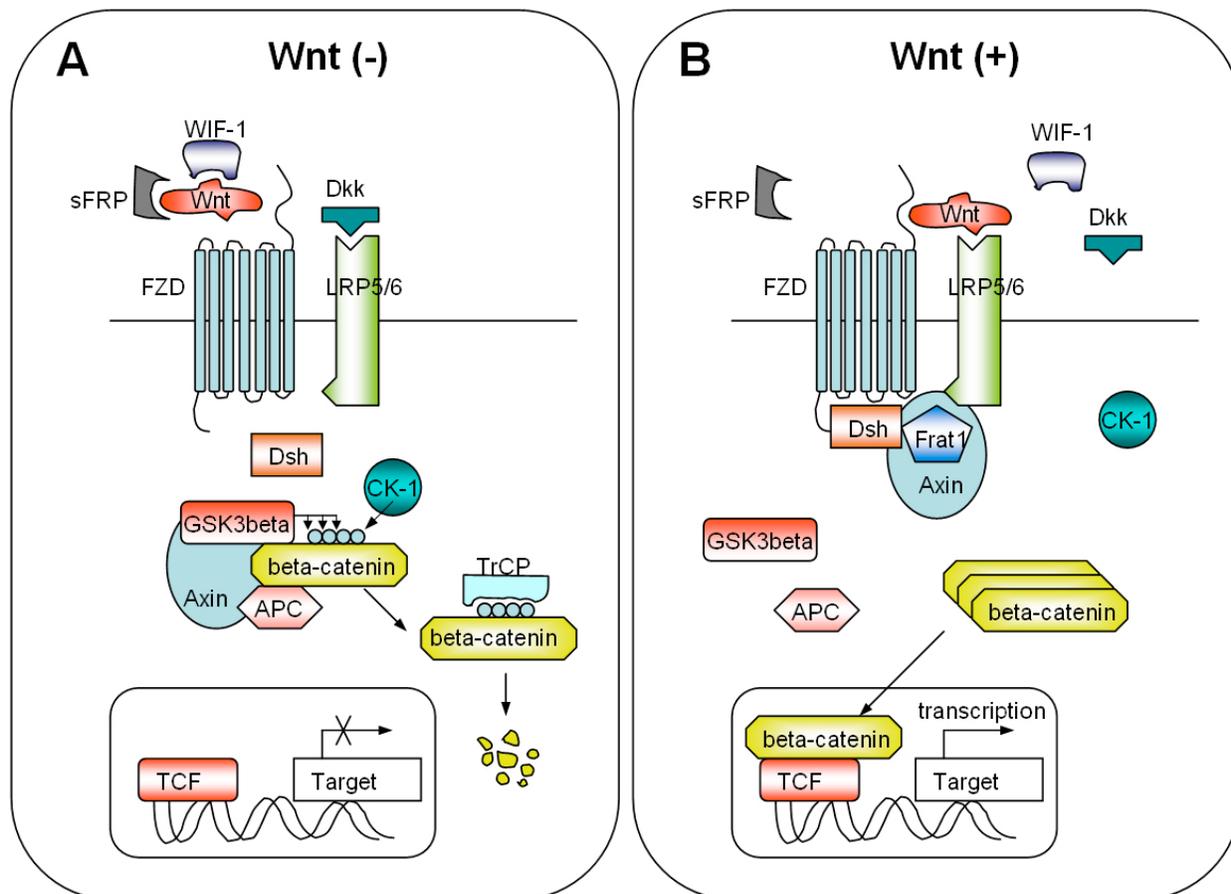
### 1. ABSTRACT

The Wnt/Frizzled (FZD) signaling cascade is important for cell fate determination during embryonic development as well as maintaining tissue homeostasis in the adult. In addition to these physiologic roles, studies have shown that deregulation of Wnt/FZD signaling occurs during carcinogenesis. As an example, over 90% of the colorectal cancers have mutations in adenomatous polyposis coli (APC) or beta-catenin genes. In addition, hepatocellular carcinoma (HCC) is another tumor with frequent aberrant activation of beta-catenin signaling. Nuclear and/or cellular beta-catenin accumulation, a hallmark of the activated canonical Wnt/FZD signaling, has been observed in 33-67% of tumors. However, mutations of APC and/or beta-catenin genes are found only in about 20-30% of HCCs, suggesting that the predominant mechanism(s) activating Wnt/FZD signaling pathway may be different from that found in colorectal cancers. There is accumulating evidence to suggest that regulatory mechanisms other than mutations involving beta-catenin or proteins in its destruction complex, many of which involve upstream components of the Wnt/FZD cascade, are important in HCC. Furthermore, information on the target genes of Wnt/FZD signaling and their roles in hepatocarcinogenesis is limited despite the recent discovery of several candidate genes. This review focuses on the alterations of Wnt/FZD signaling pathways and their relationship to the pathogenesis of HCC. A better understanding of the precise mechanisms of altered Wnt/FZD signaling may provide new molecular targets for therapy of HCC.

### 2. OVERVIEW OF THE WNT/FZD SIGNALING

#### 2.1. Wnt ligands and their receptors

The name Wnt originates as a fusion of two orthologous genes: Wingless, a *Drosophila* segment polarity gene (1) and Int-1, a mouse protooncogene (2). The Wnt family of genes encodes 350-380 amino acid proteins and 19 Wnt ligands have been identified in humans. They are a large family of secreted glycoproteins and highly conserved throughout evolution from *Drosophila* to mammals. The Wnts play essential roles in generation of cell polarity, embryonic induction, cell fate determination and tissue homeostasis. Transcription of Wnt family genes is regulated in a precise temporal and spatial manner during development (3, 4). The Wnt signaling pathways are classified into three major groups. First, in the canonical pathway, Wnt ligands activate their target genes via beta-catenin stabilization and subsequent translocation into the nucleus. This pathway is important in axis development of *Xenopus laevis*, in segment polarity and wing development of *Drosophila melanogaster* and during human carcinogenesis (3). A second pathway involves RhoA and Jun Kinase (JNK) and regulates the polar arrangement of cuticular hairs in *Drosophila* or the convergent-extension movements in *Xenopus* embryos (5). The third cascade involves intracellular calcium release and activation of protein kinase C (PKC) and calmodulin-dependent protein kinase II (6-9). According to the pathways involved, Wnt ligands can be subdivided into members of Wnt1 class (Wnt1, Wnt3, Wnt3a, Wnt7a, Wnt7b and Wnt8a) and the Wnt5a class (Wnt4, Wnt5a and Wnt11). Members of the Wnt1 class activate the canonical



**Figure 1.** Overview of the canonical Wnt/FZD signaling pathway. **A.** Under resting condition, the cytoplasmic beta-catenin is bound to its destruction complex, consisting of APC, axin/conductin, and GSK-3beta. After CK1 phosphorylates on Ser 45 residue, beta-catenin is further phosphorylated on Thr 41, Ser 37, and Ser 33 residues by GSK-3beta. Phosphorylated beta-catenin is recognized by ubiquitin ligase beta-TrCP and undergoes ubiquitylation and degradation. Therefore, the cytoplasmic level of beta-catenin is kept low in the absence of Wnt/FZD signaling. If beta-catenin is not present in the nucleus, the LEF/TCFs cannot activate the target genes. The WIF-1, sFRP, and/or Dkk can inhibit the Wnt/FZD signaling by binding to Wnt ligands or LRP5/6. **B.** When Wnt binds to both FZD and LRP5/6, Dsh is recruited and phosphorylated by FZD. Phosphorylated Dsh in turn recruits axin, which dissociates the beta-catenin destruction complex probably by Frat1-mediated mechanism. Therefore, beta-catenin escapes from phosphorylation and subsequent ubiquitylation, and accumulates in the cytoplasm. The accumulated cytoplasmic beta-catenin goes into the nucleus, where it binds to LEF/TCFs and activates the transcription of target genes.

pathway whereas those of the Wnt5a class stimulate intracellular calcium signaling (10). The Wnt ligand involved in planar cell polarity pathway is still unknown in *Drosophila*, but Wnt5a and Wnt11 have been suggested to activate the homologous pathway in vertebrates (5).

Wnt ligands bind to seven transmembrane proteins called Frizzled (FZD) receptors as shown in figure 1. At present, 10 FZDs have been discovered in humans. All FZD receptors have a highly conserved cystein-rich domain (CRD) considered to be the ligand binding motif followed by a linker region, a seven-transmembrane domain and a C-terminal cytoplasmic region that is essential for receptor signaling (11, 12). Most Wnt ligands will bind to multiple FZD receptors and *vice versa*. The pathway is probably regulated by the various affinities between FZD receptors and different Wnt ligands (13, 14).

The canonical pathway is only activated when the Wnt ligand is associated with both FZD and LRP5 or LRP6 co-receptors, which are members of the single transmembrane low-density lipoprotein receptor-related family (LRP) (15, 16). In addition to these interactions between Wnt ligands and their receptors, several other components are involved in ligand-receptor binding. Wnt-inhibitory factor-1 (WIF-1), secreted Frizzled-related protein (sFRP) and Cerberus in *Xenopus* can bind to Wnt ligands and inhibit signaling (6, 17, 18). Dickkopf (Dkk) also inhibits the canonical pathway by binding to LRP co-receptors (19) or to Kremen, which forms a tertiary complex with Dkk and LRP6 (20).

## 2.2. Canonical Wnt/FZD signaling

Beta-catenin is the key signaling molecule in the canonical Wnt/FZD pathway. Under resting conditions, most of the cellular beta-catenin binds to type I cadherins

and alpha-catenin, thereby forming adherent junctions between cells (21). In the absence of Wnt/FZD signaling, the cytoplasmic beta-catenin is bound to its destruction complex consisting of casein kinase 1 (CK1), APC, axin or its homologue conductin, glycogen synthase kinase-3beta (GSK-3beta) and diversin. CK1 phosphorylates beta-catenin at Ser 45 as a priming site followed by phosphorylation of Thr 41, Ser 37 and Ser 33 residues by GSK-3beta. Phosphorylated beta-catenin undergoes ubiquitylation and degradation by the ubiquitin ligase beta-TrCP. Therefore, the cytoplasmic level of beta-catenin is low in the absence of Wnt/FZD signaling (6).

Binding of Wnt to the FZD receptor leads to phosphorylation of the downstream molecule Dishevelled (Dsh). Phosphorylated Dsh recruits axin which results in the dissociation of the destruction complex. The precise mechanism(s) exhibited by phosphorylated Dsh is unknown although Frat1 may be involved in the dissociation of GSK-3beta from axin. Upon the dissociation of the destruction complex, beta-catenin escapes from degradation and becomes stabilized leading to accumulation in the cytoplasm. The increased free beta-catenin translocates to the nucleus and acts as an essential component for transcription factors of the lymphoid enhancing factor/T-cell factor (LEF/TCF) family (figure 1). In the absence of beta-catenin, LEF/TCFs do not activate their target genes because of the action of dominant-negative splicing variants and/or transcriptional repressors such as Groucho. Binding of beta-catenin to LEF/TCFs activates the transcription of a number of genes that regulate cell proliferation and tumor progression such as c-myc, cyclin D1, matrix metalloproteinase-7 and immunoglobulin transcription factor 2 (3, 6, 22).

### 2.3. Non-canonical Wnt/FZD signaling

In addition to the canonical pathway, Wnt ligands may activate at least two other pathways. The planar cell polarity pathway involves RhoA and JNK. Upon stimulation, JNK translocates to the nucleus and regulates the activity of multiple transcriptional factors such as c-jun, ATF2, Elk1, DPC4 and p53. This pathway controls the temporal and spatial tissue arrangement during embryonic development, but its role in carcinogenesis is uncertain (6, 22). The other pathway involves intracellular calcium signaling which is usually activated by the Wnt5a class of ligands. A gene microarray study showed that several canonical target genes were upregulated by Wnt1 but repressed by Wnt5a activity. The Wnt5a also inhibits B-cell proliferation and may be a tumor suppressor in hematopoietic malignancies (23). Another investigation reported that Wnt11 inhibited the canonical Wnt signaling pathway by multiple mechanisms including elevation of intracellular  $Ca^{2+}$  (24). However, Wnt5a was found to be overexpressed in several tumors (25, 26) and led to an increased cell motility phenotype as well as enhanced invasion of metastatic melanoma cells (27). Therefore, the role of Wnt/ $Ca^{2+}$  signaling pathway in carcinogenesis will require further study.

## 3. ALTERATIONS OF THE CANONICAL WNT/FZD SIGNALING PATHWAY IN HEPATOCELLULAR CARCINOMA

HCC is the most frequent primary malignancy of the liver and causes 500,000 to 1 million deaths annually worldwide. Although hepatitis B (HBV) and/or C viruses (HCV) are well-known major etiologic agents, any disease predisposing to liver cirrhosis can be considered as risk factor for the development of HCC. Many therapeutic options have been employed in recent years including surgical resection, percutaneous ethanol injection therapy, radiofrequency ablation, transarterial chemoembolization and orthotopic liver transplantation. However, the prognosis is still quite grim especially in patients with advanced HCC and/or severe underlying liver disease. Therefore, it is important to develop a better understanding of hepatocarcinogenesis at the molecular level since signal transduction pathways appear to play a pivotal role in the pathogenesis of this devastating disease.

Nuclear and/or cellular accumulation of beta-catenin is generally accepted as evidence of activated canonical Wnt/FZD signaling and this phenomenon may be demonstrated in tissues by immunohistochemical staining. It is a frequent event in various human tumors including colorectal, lung, breast, cervical, skin, and liver. In 33-67% of HCC, nuclear and/or cellular accumulation of beta-catenin has been described and was closely associated with the clinicopathological characteristics of the disease (28-30). Tumors with beta-catenin accumulations were generally associated with a poorly differentiated morphology (31), high proliferative activity (32), vascular invasion and dismal prognosis (30-32). There is also a significant correlation between beta-catenin accumulation and mutations (30). Although over 90% of colorectal carcinomas were reported to harbor APC or beta-catenin mutations (33), many HCCs with beta-catenin accumulation do not have either APC or beta-catenin mutations (30). These observations suggest that other elements of the cascade may be important in the upregulation of canonical Wnt/FZD signaling pathways during hepatocarcinogenesis. Indeed, there is accumulating evidence to support this hypothesis.

### 3.1. Wnt ligands and their regulatory proteins

Although many studies have investigated the role of Wnt/FZD signaling, there is scant direct evidence to suggest that Wnt ligands themselves play an important role during carcinogenesis. Several Wnt ligands were reported to be upregulated in some tumors in a tissue-specific manner. For example, Wnt1, 7a, 10b, and 13 mRNA levels were increased in squamous cell head and neck carcinomas (34) and Wnt11 overexpression was reported in prostate and gastric tumors (35, 36). However, most of the studies evaluated only mRNA and not protein levels due to the lack of available antibodies or difficulties in expressing Wnt ligands in cells. For these reasons, little is known about the types of Wnt ligand(s) expressed or if there are any alterations of their expression profiles in HCC.

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The WIF-1 protein has an N-terminal signal sequence, a highly conserved WIF domain and 5 epidermal growth factor-like repeats. Although WIF-1 is structurally unrelated to the CRD of FZD receptors or sFRP (37, 38), it binds to *Drosophila* Wingless and *Xenopus* Wnt8, and inhibits the Wnt8-FZD2 interaction. Ectopic expression of WIF-1 in human gastrointestinal cancer cells revealed that it inhibited colony formation, cell proliferation and anchorage-independent growth in soft agar and these findings are consistent with its proposed role as a tumor suppressor (39). Down-regulation of WIF-1 has been reported in many cancers including breast, lung, prostate and bladder (40, 41) and a recent study revealed that 91% of gastrointestinal cancer cell lines including all 6 HCC cell lines had decreased expression of WIF-1. The down-regulation of WIF-1 was also found in 75-80% of human esophageal, gastric, colorectal and pancreatic tumor tissues. However, there was no significant association between WIF-1 down-regulation and clinicopathological characteristics of disease suggesting that WIF-1 might be involved in an early event of tumor development. The down-regulation of WIF-1 is usually mediated by promoter hypermethylation and mRNA expression was closely correlated with protein expression level (39). However, the WIF-1 expression profile in human HCC has not been determined.

The sFRPs are soluble secreted proteins bearing homology to the CRD of FZD. Therefore, such molecules can bind to Wnt ligands and act as a competitive inhibitor for FZD mediated signaling. Consistent with their role in oncogenesis is the finding of down-regulation of sFRP expression in several esophageal, gastric and colorectal cancers. The expression of sFRP(s) is mainly regulated by promoter hypermethylation (42, 43). However, a role for these proteins in carcinogenesis is controversial. For example, sFRP was upregulated in breast tumors (44) and promoted growth of glioma cell lines (45). Based on recent titration experiments however, sFRP-1 enhanced Wnt signaling at low concentrations while at higher levels it inhibited signaling (46). Another previous study revealed that sFRP-2 resulted in the intracellular accumulation of beta-catenin and conferred anti-apoptotic properties to MCF-7 cells (47). Their role in carcinogenesis can be variable depending on the type of cells and tissues studied or expression levels. For example, sFRP-1 was downregulated in a c-myc/E2F1 transgenic mouse model of HCC (48) consistent with its role as a tumor suppressor; however, measurement of sFRP levels or methylation status has not been determined in human HCC.

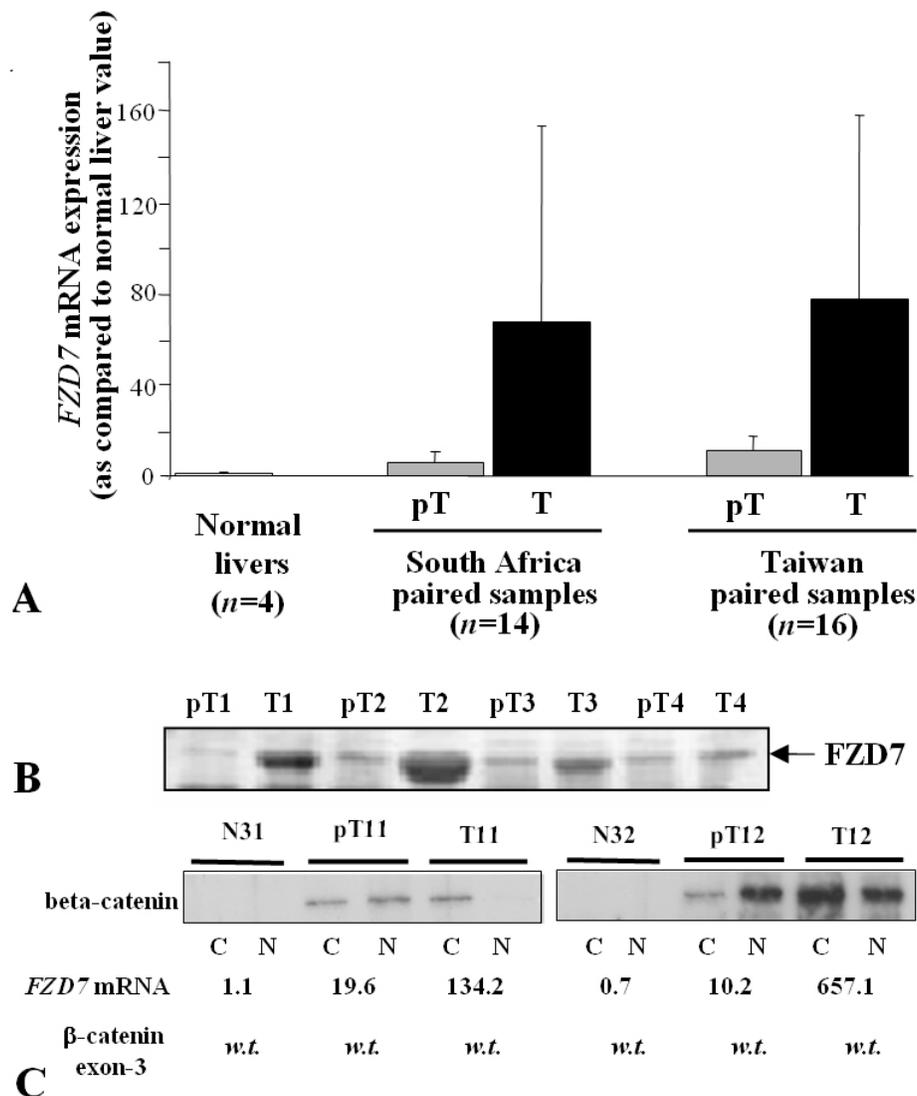
### 3.2. FZDs

The FZDs are frequently upregulated in tumor cell lines and tissues. Upregulation of FZD2, FZD3, and FZD7 were found in esophageal and gastric cancers and other FZDs such as FZD8, FZD9, and FZD10 were enhanced in gastric adenocarcinomas as well (49-51). In colorectal cancer, overexpression of FZD1 and FZD2 was associated with poorly differentiated carcinomas (52). Overexpression of FZDs was usually associated with activation of downstream beta-catenin signaling as demonstrated by increased nuclear accumulation of beta-

catenin by immunostaining. In HCC, FZD7 was markedly upregulated both in transgenic mouse models of HCC and human tumors. Recently, 4 different HCC transgenic mouse models produced by overexpression of c-myc or SV40-Tag alone or IRS-1/c-myc and HBx/c-myc double transgenes were studied. The FZD7 gene was the only FZD mRNA species that was upregulated in all 4 models of hepatocarcinogenesis as measured by real-time reverse transcription polymerase chain reaction (RT-PCR) assays. In contrast, FZD8 mRNA was downregulated. The upregulation of FZD7 protein was also confirmed by Western blot analysis. In addition, there was decreased Thr 41/Ser 45 phosphorylation of beta-catenin and increased accumulation of the protein within the cell. Interestingly, overexpression of FZD7 was observed not only in murine and human HCCs but also in surrounding peritumoral and dysplastic liver tissues and this observation suggests that it may be an early event in hepatocarcinogenesis (53, 54). In HCC cell lines, FZD7 gene expression was associated with increased nuclear accumulation of beta-catenin and increased cell motility; furthermore, expression of a dominant-negative FZD7 receptor mutant inhibited HCC cell migration and invasion (54). In human HCC tumors, FZD7 expression was increased in 90% compared to adjacent nontumorous tissue. However, 77% of nontumorous surrounding liver tissue already had displayed low level FZD7 overexpression compared to normal liver which further supports the concept of an early event in human hepatocarcinogenesis (figure 2A, 2B). Another important finding was that nuclear accumulation of beta-catenin was observed in HCC tissues containing only wild-type beta-catenin gene in the context of high-level FZD7 expression (figure 2C). This observation implies that upregulation of FZD7 was sufficient to activate the canonical Wnt/FZD signaling pathway without mutations in beta-catenin or other components of its destruction complex (54). However, the molecular mechanisms of how FZD7 is upregulated during hepatocarcinogenesis are unknown. In another c-myc/E2F1 transgenic mouse model of HCC, upregulation of FZD1 and FZD2 was demonstrated by Western blot analysis especially in tumors with nuclear accumulation of beta-catenin although the levels of FZD7 mRNA and/or proteins were not evaluated in this study (48). The expression profile of FZDs other than FZD7 has not been reported with human HCC.

### 3.3. LRP5/6 and Dkk

Shortly after the discovery of FZDs as Wnt receptors, the *Drosophila* segment polarity gene, arrow, was identified as a co-receptor for Wnt/FZD interaction. The product of arrow is a single-pass transmembrane protein that is homologous to LRP5 and LRP6 in mammals. The Wnt ligand, FZD and LRP5/6 form a ternary complex, which interacts with Dsh and axin (15, 16). Targeted disruption of LRP6 in mice showed multiple developmental anomalies that resemble a combination of several Wnt gene knockout phenotypes (55). Therefore, they are now considered as essential elements of the canonical Wnt/FZD signaling pathway and mutations of LRP5 have been linked to diseases with skeletal abnormalities (56). However, little is known about their roles in carcinogenesis. LRP6 mRNA is readily expressed in normal human tissues including



**Figure 2. A.** Quantitative real-time RT-PCR assessment of FZD7 mRNA levels in human HCC tumors (T) and the corresponding peritumoral tissues (pT), derived from Taiwan and South Africa. The mRNA levels were markedly elevated in tumor tissues. There were low level FZD7 overexpressions in peritumoral tissues compared to normal liver. The FZD7 mRNA expression levels were expressed as relative abundance of FZD7 compared to the mean value of 4 normal liver tissues. **B.** Western blot analysis of FZD7 in HCC tumors (T) and the corresponding peritumoral tissues. The FZD7 protein expression levels were also increased in HCC tumor tissues, consistent with the results of mRNA expression. **C.** Western blot analysis of beta-catenin protein accumulation in cytosolic (C) or nuclear (N) enriched fractions from 2 HCC tumors and their corresponding peritumoral tissues compared with 2 normal liver tissues. Each tumor and peritumoral region had a wild-type beta-catenin as assessed by PCR and sequencing. The experiments were performed as described in Merle *et al.*, 2004 (54).

lung, colon, kidney and small intestine as well as malignant cell lines and human tumors. It has been reported that overexpression of LRP6 altered subcellular distribution of beta-catenin and induced a decrease in the E-cadherin-bound pool with a concomitant increase of the cytosolic pool without affecting the total amount of cellular beta-catenin (57). Expression of truncated forms of LRP6 that are missing their extracellular domain can also activate this signaling pathway in both a Wnt- and FZD-independent manner (58). These mutations have not yet been demonstrated in human tumors and the role of aberrant

LRP5/6 expression during carcinogenesis needs to be better defined. However, one study revealed that LRP5 mRNA expression was significantly correlated with beta-catenin accumulation, tumor metastasis and a trend toward decreased event-free survival in patients with osteosarcoma (59).

Dkk inhibits Wnt/FZD signaling by competitive binding and/or increased clearance of LRP5/6 with Kremen. Downregulation of Dkks has been found in many cancers and was associated with promoter

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hypermethylation (60). Interestingly, Dkk-1 itself is a downstream target of beta-catenin/TCF as revealed in colon cancer cell lines thereby forming a negative feedback loop of Wnt signaling (61). While normal liver does not express Dkk-1 mRNA, studies have found it expressed in 81% of hepatoblastomas and in 10% of HCCs (62). Considering the high rate of beta-catenin accumulation and mutation in hepatoblastoma (85%), upregulation of Dkk-1 may not be an active modulator *per se* but occurs as a result of uncontrolled Wnt/FZD signaling during the development of hepatoblastomas.

### 3.4. Dsh

Dsh is an immediate downstream molecule of FZD signaling. When Wnt ligand(s) binds with FZD and LRP5/6, Dsh is phosphorylated and in turn recruits axin to dissociate the beta-catenin destruction complex by an unknown mechanism. Overexpression of Dshs has been demonstrated in non-small cell lung cancer, mesothelioma and cervical cancer and was associated with beta-catenin accumulation (63-65). Dsh1 was also upregulated in a c-myc/E2F1 transgenic mouse model of HCC especially in tumors with beta-catenin activation (48). Moreover, a recent study showed that the human homologue of Dapper 1 (HDPR1), a novel inhibitor of Dsh that can bind to the PDZ domain of Dsh was downregulated in 58% of human HCCs and this event significantly correlated with beta-catenin accumulation. Downregulation of HDPR1 was also associated with hypermethylation of the promoter region (66).

### 3.5. Beta-catenin

Beta-catenin was originally discovered as an element of the cellular adherence junction complex consisting of E-cadherin, beta- and alpha-catenins and actin. Beta-catenin has a core domain of 12 armadillo repeats, which can bind to cadherin, APC, axin/conductin and TCFs in a mutually exclusive manner. The N-terminal domain binds to alpha-catenin and has serine and threonine residues phosphorylated by GSK-3beta which are essential for recognition by ubiquitin ligase beta-TrCP. Activating mutations usually occur in or around exon 3 of the gene encoding beta-catenin (CTNNB1), affecting the putative phosphorylation sites for GSK-3beta. Thus, mutated beta-catenin can escape from phosphorylation and subsequent degradation and the net result is cytoplasmic and/or nuclear accumulation (67). Interestingly, mutations of beta-catenin and APC genes are mutually exclusive in colorectal cancers (68, 69) suggesting that just one hit of any components of beta-catenin and/or components of its destruction complex is sufficient for activation of beta-catenin/TCF signaling in the nucleus.

The reported frequency of beta-catenin mutations in human HCCs ranges from 8% to 34% (30, 70-74) and it is the most common genetic abnormality involving the Wnt/FZD signaling system. Frequent genetic alterations include missense mutations involving codons 32 and 45 (30). Several studies have shown that beta-catenin mutations are more common in HCV-associated (25% to 41%) than that HBV-associated (9%) HCCs (29, 75). A similar observation was made from an extensive analysis of

allelotypes as well as p53, axin1 and beta-catenin gene mutations. In this study, the authors suggest that HCCs could be classified into 2 groups according to the chromosome stability status. In one group, HCCs showed widespread chromosomal instability that was characterized by frequent allelic losses and these tumors were usually associated with HBV infection. The most frequent allelic losses were on chromosome 1p, 4q, 4p, 9p, 13q, 16p, 16q, and 7p. In the other group, tumors showed chromosomal stability and beta-catenin mutations associated with a chromosome 8p loss were the most common genetic alteration. Beta-catenin mutations were found in 19% of HCCs and most (88%) occurred in tumors that were unrelated to HBV induced liver disease (76). Although one study reported that mutations in CTNNB1 gene were associated with a subset of low-grade HCCs negative for HBV and with a favorable prognosis (29), it was subsequently shown not to be a predictive factor for long-term survival (30, 76). Interestingly, high mutation rates of beta-catenin gene were shown to be present in hepatoblastoma, a primary liver malignancy affecting children. This liver tumor has the highest rate of beta-catenin mutations ranging from 52% to 89% (77, 78).

### 3.6. APC

The APC gene encodes a large (312 kDa) protein with multiple diverse functions involved in cell migration, adhesion, cell cycle regulation and chromosomal stability. However, one of its most important roles involves the regulation of cellular beta-catenin levels. The APC gene is located on chromosome 5q and includes regions containing three 15-amino acid and seven 20-amino acid repeats. Both of these domains are known to bind beta-catenin and the 20-amino acid repeats have an important function in downregulating beta-catenin levels in the cell. The SAMP motifs (Ser-Ala-Met-Pro) within the 20-amino acid repeats are involved in the binding of axin/conductin. Germline mutations in the APC gene cause familial adenomatous polyposis (FAP). Patients with FAP develop numerous adenomatous polyps throughout the entire colon which can progress at any location to invasive carcinomas (3, 6, 22). APC is also the most common genetic abnormality in sporadic colorectal tumors and approximately 80% have mutations in this gene. Most of these somatic mutations are clustered between codon 1250 and 1500 in exon 15 and are frame-shift mutations or mutations that result in the generation of a premature stop codon. Typically, one allele is inactivated by a truncating or frame-shift mutation and the other is inactivated by deletion of chromosome 5q. Mutations in the APC gene are known to be involved in a very early stage of progression from adenoma to carcinoma (33, 79). Although HCC is not a tumor commonly associated with FAP, liver-targeted disruption of the APC gene activated beta-catenin signaling and led to the development of HCC in mice. There have been two case reports of HCC arising in patients with FAP (80). However, APC mutations have a surprisingly limited role in human hepatocarcinogenesis. Indeed, inactivating mutations of APC are quite rare and sporadic in human HCCs and are generally not detected even in tumors lacking beta-catenin mutations (73, 81). In contrast, hepatoblastomas have developed as an extracolonic manifestation of FAP (82).

### 3.7. Axin/Conductin

Axin1 and its homologue conductin, also called axin2, bind directly to APC, beta-catenin, GSK-3beta, CK1 and Dsh thereby acting as the scaffold of the beta-catenin destruction complex (83). The N-terminal region binds to APC while its central region binds to GSK-3beta and beta-catenin. The C-terminal region binds to Dsh and this interaction leads to dissociation of the beta-catenin destruction complex (22). In the absence of axin, GSK-3beta will phosphorylate beta-catenin at a very low rate while the addition of axin can overcome this defect (84). Axin1 mutations have been detected in some colon cancer cell lines and colorectal tumors without APC or beta-catenin mutations. These mutations are usually involved in either the Dsh- or GSK-3beta-binding sites (85). Overexpression of axin1 in mammalian cell lines results in a decrease of beta-catenin levels and results in suppression of TCF-dependent gene transcription (86). Axin1 mutations are the second most common genetic abnormality that involves beta-catenin and components of its destruction complex in HCC. About 50% of the HCC cell lines without beta-catenin mutations and 8% of human HCCs harbor axin1 mutations (70, 76). Half of axin1 mutations were point mutations whereas the remaining gene alterations involve either a small homozygous deletion or duplication. Most mutations generate a stop codon, a frame-shift, or a deletion of a large part of the axin1 gene (76). Importantly, axin1 mutations were also found to be mutually exclusive of beta-catenin mutations in human HCCs (70). A recent study analyzed the mutation patterns of beta-catenin and axin1 in 81 HCCs and 33 dysplastic nodules. Although the mutation frequencies for beta-catenin and axin1 in HCCs were 16% and 6.2% respectively, no mutations were found in 33 dysplastic nodules examined. These findings imply that mutations of beta-catenin and axin1 are late events during human hepatocarcinogenesis (87). Similarly, the axin1 mutation frequency was reported to be 7% in hepatoblastomas (74).

Conductin/axin2 has a 45% amino acid homology with axin1 and exhibits all the binding and regulatory functions of that protein. Conductin/axin2 expression can be upregulated by activation of the Wnt/FZD pathway and the promoter region contains TCF-binding sites (6). Axin2 mutations were found in 2.7% of HCCs (74) and one study reported a 37.5% axin2 mutation rate in human HCC tissues that also demonstrated nuclear translocation of beta-catenin (88). It was of interest that the mRNA and protein levels of axin2 were increased in HCC tissues compared to surrounding nontumorous liver tissues and may reflect a negative feedback loop of Wnt signaling (89).

### 3.8. GSK-3beta

The GSK-3beta protein is a serine/threonine kinase originally discovered as a component regulating glycogen metabolism (90). Unlike most cellular protein kinases, GSK-3beta is constitutively active in the resting state and preferentially phosphorylates substrates that are pre-phosphorylated by a priming kinase. Phosphorylation of substrates by GSK-3beta usually results in inhibition of their function. Several stimuli such as insulin, insulin-like growth factors (IGFs), epidermal growth factor, Wnt, or

integrin signaling cause rapid and transient suppression of GSK-3beta activity (91, 92). Inhibition of GSK-3beta activity is usually mediated by phosphorylation of the Ser 9 residue. Upon phosphorylation, it folds back onto itself, thereby preventing the active kinase site from binding to the primed substrate. Insulin and IGF-1 inhibit GSK-3beta through a phosphatidylinositol 3-kinase (PI3-K)/Akt signaling pathway. Inhibition of GSK-3beta results in dephosphorylation of glycogen synthase and eukaryotic initiation factor 2B (eIF2B) thereby increasing glycogen and protein synthesis (92, 93). In addition to its role in glycogen metabolism, GSK-3beta is involved in cell cycle, gene transcription and cell survival functions. As mentioned above, the GSK-3beta plays a central role in beta-catenin phosphorylation and degradation as a component of the canonical Wnt/FZD pathway. The phosphorylation on Ser 33 and Ser 37 residues of beta-catenin is crucial for recognition by the ubiquitin ligase beta-TrCP (94). For efficient phosphorylation of Thr 41, Ser 37, and Ser 33 residues by GSK-3beta, beta-catenin needs to be phosphorylated on Ser 45 residue by CK1. Interestingly, although CK1 acts as a priming kinase for GSK-3beta, a recent study showed that the phosphorylation status of Ser 45 was not changed upon Wnt stimulation in *Drosophila* cells (95). Therefore, the role of CK1 in this process is not fully defined. In addition to beta-catenin, axin and APC are also phosphorylated by GSK-3beta. Phosphorylation of axin and APC are important for stability and high affinity binding to beta-catenin (96-98). It is apparent that GSK-3beta is a major negative regulator of Wnt signaling and surprisingly, mutations involving the GSK-3beta gene have not been detected in human cancers (22). This finding could be due, in part, to GSK-3beta involvement in other pathways essential for cell survival.

The regulation of GSK-3beta involved in the Wnt/beta-catenin pathway is different from that found in insulin/IGF-1 signaling. Under resting conditions, Dsh interacts with axin and Frat1, while GSK-3beta binds to axin and Frat1 but not to Dsh. Therefore, there exists a quaternary complex consisting of GSK-3beta, axin, Frat1, and Dsh. Following Wnt stimulation, Dsh undergoes phosphorylation and a conformational change which in turn result in Frat1-mediated dissociation of GSK-3beta from axin. As GSK-3beta dissociates from axin, phosphorylation of beta-catenin will not occur. The effect of Ser 9 phosphorylation of GSK-3beta on Wnt signaling is controversial. In human embryonic kidney-293 cells, stimulation with Wnt decreased GSK-3beta activity; however, this reduction of activity did not correlate with Ser 9 phosphorylation (99). A similar observation was made in *Drosophila* cell experiments. Treatment with Wingless did not result in phosphorylation of Ser 9 residue on shaggy (*sgg*)/Zeste-white3, the *Drosophila* orthologue of GSK-3beta despite an increase in armadillo levels. Moreover, insulin-mediated phosphorylation of Ser 9 did not affect armadillo levels as well (100). Therefore, some authors have suggested that GSK-3beta, involved in beta-catenin regulation, is not influenced by the insulin/IGF-1 signaling pathway. In contrast, phosphorylated GSK-3beta was markedly elevated in HCC cell lines (101) and exogenous insulin and IGF-1 resulted in beta-catenin

stabilization through inhibition of GSK-3 $\beta$  activity (102). Suppression of GSK-3 $\beta$  phosphorylation also decreased beta-catenin activity (101). Increased phosphorylated GSK-3 $\beta$  was observed in several transgenic mouse models of HCCs and was prominent in HCCs with beta-catenin accumulation (48, 53). The phosphorylated form of GSK-3 $\beta$  was increased in human HCC tissues as well and correlated with cellular accumulation of beta-catenin (103). These results suggest that increased phosphorylation of GSK-3 $\beta$  may be associated with alteration of Wnt/FZD signaling during hepatocarcinogenesis and there may be a cross talk between insulin/IGF-1 and Wnt/FZD signaling pathways.

### 3.9. LEF/TCFs

Once beta-catenin translocates into the nucleus, it binds to the N-terminal region of LEF/TCFs via its armadillo repeats and activates transcription of target genes. Although beta-catenin possesses multiple transactivating elements, the protein does not have a DNA binding domain. In contrast, LEF/TCFs transcription factors can bind directly to DNA through their high-mobility group (HMG) box of around 80 amino acids but they cannot activate gene transcription. Therefore, beta-catenin serves as a co-activator of LEF/TCFs and this activity is localized to the C-terminal region of the molecule (6, 22, 67). Indeed, fusion of the C-terminal region of armadillo to TCF revealed a beta-catenin independent transcriptional activation mechanism (104). The LEF/TCFs not only provides the binding site to DNA for beta-catenin but also exerts other inhibitory functions on target genes. Introduction of artificial mutations on the TCF binding sites of many target genes including cyclin D increased their basal activities suggesting that LEF/TCFs can repress transcription of their target genes under basal conditions (105-107). These inhibitory functions are mediated by binding proteins such as the Groucho/TLE, C-terminal binding protein (CtBP) co-repressor and CREB binding protein (CBP) (67). The human LEF/TCF family consists of four members designated TCF-1, LEF-1, TCF-3, and TCF-4. All 4 monomeric proteins have a highly conserved HMG box that binds to DNA in a sequence-specific manner. The HMG box is also involved in "bending" of DNA, which opens up the binding sites for various transcription factors. The LEF/TCFs are normally expressed during embryogenesis but are downregulated in most tissues after terminal cellular differentiation. However, TCF-4 is expressed in gut epithelium throughout life and TCF-4<sup>-/-</sup> transgenic mice show a complete absence of the stem cell compartment in the crypts of the small intestine (108) implying that TCF-4 functions to maintain this early progenitor cell population. The expression of TCF-4 and LEF-1 has been reported in both liver and HCC. Interestingly, LEF/TCF mRNAs undergo extensive alternative splicing. In addition, TCF-1 and LEF-1 use two alternative promoters (109, 110). Theoretically over 100 isoforms can be produced in the case of TCF-1 although Western blot analysis revealed the predominant expression of only 8 isoforms (109). Although the functional relevance of alternative splicing is not clear, it may provide preferential activation of different target genes. In the case of alternative usage of different promoters, one protein will

be full-length but the protein derived from the other promoter will be a N-terminal truncated form lacking the beta-catenin binding domain. This protein may occupy the binding sites in DNA and inhibit beta-catenin/TCF-mediated transcriptional activation of target genes. Therefore, a decrease in the N-terminal truncated form of LEF/TCFs may be associated with carcinogenesis. Mutations of the TCF-4 gene have been described in colorectal carcinomas and cell lines with microsatellite instability (39% and 50%, respectively) and most were characterized as frame-shift mutations (111).

In HCC, one study detected mutations in exon 15 of TCF-4 gene in 2 of 32 HCCs (6.25%) using PCR-single strand conformation polymorphism; however, the functional consequences of these mutations are not known (112). Another study found no mutations of the TCF-4 gene in 34 human HCC tumors (113). Although mutations of the TCF-4 gene are rare, both studies report increased levels of TCF-4 mRNA in HCC tissues. Enhanced TCF-4 mRNA levels was closely associated with intrahepatic metastasis or c-myc overexpression. Another member of the TCF family, LEF-1 was increased in HCC tissues by immunohistochemical analyses and associated with enhanced nuclear cyclin D1 expression. However, the expression of LEF-1 was not correlated with any clinicopathological parameters of HCC (114). Therefore, it is uncertain whether overexpression of TCF-4 or LEF-1 is a primary event or merely reflects an activated Wnt/FZD signaling cascade in HCC; alterations of LEF/TCF isoform expression patterns have not yet been determined.

### 3.10. Target genes

A considerable number of the downstream target genes of Wnt/FZD signaling cascade have been described and many regulate cell cycle and proliferation. The best-known beta-catenin-regulated genes are c-myc and cyclin D1. The c-myc and cyclin D1 promoters contain TCF-binding sites which mediate the transcriptional activation by the TCF/beta-catenin complex. Dominant-negative forms of TCF have been shown to reduce c-myc and cyclin D1 expression in colorectal cancer cell lines (107, 115) confirming that both genes are regulated by the TCF/beta-catenin complex. Overexpression of c-myc has been found in a number of cancer cell lines and human tumors. However, the role of c-myc and its relationship with beta-catenin overexpression in HCC is unclear. In the immortalized murine hepatocyte cell line AML12, introduction of a mutant beta-catenin construct activated c-myc and cyclin D1 and promoted cell proliferation and survival (116). A similar observation was made in a human HCC cell line; a dominant-negative form of TCF-4 decreased the expression of c-myc and cyclin D1 and suppressed the growth of BEL-7402 cells (117). In albumin promoter regulated-SV40 T antigen expressing transgenic rats, the expression of c-myc mRNA and protein was increased in focal hepatic dysplasia and HCCs (118). However, c-myc expression was not increased in murine models of HCC induced by various chemical agents even though 43% of HCCs had beta-catenin gene mutations (119). Furthermore, although transgenic mice expressing an oncogenic form of beta-catenin developed hepatomegaly

resulting from cell proliferation, neither c-myc nor cyclin D1 was overexpressed in the liver tissues (120).

Similar conflicting results have been obtained in human HCCs. For example, Kawate et al reported c-myc gene amplification in 33% of human HCCs by differential PCR analysis and observed shorter disease-free survivals in this group of patients (121). Another study reports that amplification of the c-myc gene was more common in multi-nodular recurrent HCCs or HCCs with metastasis (122). Other immunohistochemical studies demonstrated that c-myc expression was related to early recurrence after tumor resection and correlated with a poorly differentiated morphology (123, 124). One investigation showed that overexpression and/or mutations of beta-catenin were closely correlated with c-myc overexpression in human HCCs (113). In contrast, Prange et al reports that cyclin D1 and c-myc expressions were not correlated with nuclear beta-catenin accumulation by immunohistochemical staining and were independent of the histologic grade of tumors (125). Yet in another investigation, the expression of c-myc in HCC tissues was decreased compared to the adjacent nontumorous tissues and expression was inversely correlated with the grade of differentiation (126). In a recent study that analyzed both c-myc amplification and protein expression in HCC tissues, 30% of HCCs demonstrated c-myc amplification compared to chronic liver disease and normal liver. However, even though there was c-myc gene amplification, the HCCs showed less c-myc nuclear staining than those in the liver derived from chronic diseases; more important, there was no relationship between c-myc amplification and protein expression levels. Decreased nuclear c-myc protein staining in HCC was associated with a more aggressive phenotype (127). The cyclin D1 gene regulates the G1/S transition phase of the cell cycle and is overexpressed in several human tumors. There is conflicting data regarding its role in HCC. For example, cyclin D1 expression has been shown to be present in HCCs but was not necessarily associated with the nuclear accumulation of beta-catenin (125, 128, 129).

In addition to c-myc and cyclin D1, several other genes have been identified as potential targets for beta-catenin activation in liver. These genes regulate glutamine metabolism, orphan G-protein-coupled receptor, Gpr49, epidermal growth factor receptor and leukocyte cell-derived chemotaxin 2. Expression of these target genes were discovered in transgenic murine models overexpressing wild type or mutant beta-catenin genes or were identified in human HCCs or hepatoblastomas exhibiting beta-catenin overexpression and/or mutations (130-133). The Wnt-induced secreted proteins (WISPs) are another protein family described as downstream targets of Wnt/FZD signaling which is frequently altered in human HCC. Human HCC cell lines have been found to express WISP1, WISP1v, and WISP3 mRNA and alternative spliced variants of WISP1 and WISP3 have been identified as well. Therefore, alteration of WISPs levels and/or isoform pattern expression may be associated with HCC (134).

## 4. CONCLUSIONS AND PERSPECTIVES

The role of Wnt signaling pathway has been of great interest in understanding the molecular pathogenesis of human tumors including HCC. In many tumors, the aberrant levels of beta-catenin in the cytoplasm and nucleus appears to be an important event leading to inappropriate transcription of various target genes involved in oncogenesis. The abnormal beta-catenin accumulation is mainly due to various mutations of beta-catenin, APC, and axin genes in colorectal carcinomas and other tumors. However, these mutations are relatively rare in HCC, which implies that the regulation of the Wnt pathway can be affected by the overexpression of other components such as upstream Wnt ligands and FZD receptors without these genetic alterations. Nevertheless, it is unknown which Wnt ligand(s) and/or FZD receptors are responsible for activation of the beta-catenin pathway in HCC and further studies will be required. Additional research will need to define more completely the target genes involved in the Wnt pathway in HCC. Given the general importance of this pathway in tumor development, discovery of new TCF/beta-catenin responsive target genes will be of great interest. Several components of this signaling cascade such as GSK-3beta and factors involved in the ubiquitylation degradation machinery appear to be regulated by stimuli different from the Wnt ligands and could represent entry points for a cross talk to other signaling systems. Finally, a better definition of the role of the canonical Wnt pathway during hepatocarcinogenesis may reveal new molecular targets for therapy of HCC.

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## 6. REFERENCES

1. Sharma R.P. & V.L. Chopra. Effect of the Wingless (wg1) mutation on wing and haltere development in *Drosophila melanogaster*. *Dev Biol* 48, 461-465 (1976)
2. Nusse R. & H.E. Varmus. 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)
3. Logan C.Y. & R. Nusse. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20, 781-810 (2004)
4. Strutt D. Frizzled signaling and cell polarization in *Drosophila* and vertebrates. *Development* 130, 4501-4513 (2003)
5. Veeman M.T., J.D. Axelrod & R.T. Moon. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev Cell* 5, 367-377 (2003)
6. Lustig B. & J. Behrens. The Wnt signaling pathway and its role in tumor development. *J Cancer Res Clin Oncol* 129, 199-221 (2003)

## WNT/Frizzled Signaling in HCC

7. Slusarski D.C., J. Yang-Snyder, W.B. Busa & R.T. Moon. Modulation of embryonic intracellular Ca<sup>2+</sup> signaling by Wnt-5A. *Dev Biol* 182, 114-120 (1997)
8. Sheldahl L.C., M. Park, C.C. Malbon & R.T. Moon. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr Biol* 9, 695-698 (1999)
9. Kuhl M., L.C. Sheldahl, C.C. Malbon & R.T. Moon. Ca(2+)/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. *J Biol Chem* 275, 12701-12711 (2000)
10. Du S.J., S.M. Purcell, J.L. Christian, L.L. McGrew & R.T. Moon. Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in Xenopus embryos. *Mol Cell Biol* 15, 2625-2634 (1995)
11. Hsieh J.C. Specificity of WNT-receptor interactions. *Front Biosci* 9, 1333-1338 (2004)
12. Umbhauer M., A. Djiane, C. Goisset, A. Penzo-Mendez, J.F. Riou, J.C. Boucaut & D.L. Shi. The C-terminal cytoplasmic Lys-thr-X-X-X-Trp motif in frizzled receptors mediates Wnt/beta-catenin signalling. *EMBO J* 19, 4944-4954 (2000)
13. Bhanot P., M. Brink, C.H. Samos, J.C. Hsieh, Y. Wang, J.P. Macke, D. Andrew, J. Nathans & R. Nusse. A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* 382, 225-230 (1996)
14. Rulifson E.J., C.H. Wu & R. Nusse. Pathway specificity by the bifunctional receptor frizzled is determined by affinity for wingless. *Mol Cell* 6, 117-126 (2000)
15. Wehrli M., S.T. Dougan, K. Caldwell, L. O'Keefe, S. Schwartz, D. Vaizel-Ohayon, E. Schejter, A. Tomlinson & S. DiNardo. Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* 407, 527-530 (2000)
16. Tamai K., M. Semenov, Y. Kato, R. Spokony, C. Liu, Y. Katsuyama, F. Hess, J.P. Saint-Jeannet & X. He. LDL-receptor-related proteins in Wnt signal transduction. *Nature* 407, 530-535 (2000)
17. Pennica D., T.A. Swanson, J.W. Welsh, M.A. Roy, D.A. Lawrence, J. Lee, J. Brush, L.A. Taneyhill, B. Deuel, M. Lew, C. Watanabe, R.L. Cohen, M.F. Melhem, G.G. Finley, P. Quirke, A.D. Goddard, K.J. Hillan, A.L. Gurney, D. Botstein & A.J. Levine. WISP genes are members of the connective tissue growth factor family that are up-regulated in wnt-1-transformed cells and aberrantly expressed in human colon tumors. *Proc Natl Acad Sci USA* 95, 14717-14722 (1998)
18. Jones S.E. & C. Jomary. Secreted Frizzled-related proteins: searching for relationships and patterns. *Bioessays* 24, 811-820 (2002)
19. Mao B., W. Wu, Y. Li, D. Hoppe, P. Stannek, A. Glinka & C. Niehrs. LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 411, 321-325 (2001)
20. Mao B., W. Wu, G. Davidson, J. Marhold, M. Li, B.M. Mechler, H. Delius, D. Hoppe, P. Stannek, C. Walter, A. Glinka & C. Niehrs. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature* 417, 664-667 (2002)
21. Jamora C. & E. Fuchs: Intercellular adhesion, signalling and the cytoskeleton. *Nat Cell Biol* 4, E101-E108 (2002)
22. Doucas H., G. Garcea, C.P. Neal, M.M. Manson & D.P. Berry. Changes in the Wnt signalling pathway in gastrointestinal cancers and their prognostic significance. *Eur J Cancer* 41, 365-379 (2005)
23. Liang H., Q. Chen, A.H. Coles, S.J. Anderson, G. Pihan, A. Bradley, R. Gerstein, R. Jurecic & S.N. Jones. Wnt5a inhibits B cell proliferation and functions as a tumor suppressor in hematopoietic tissue. *Cancer Cell* 4, 349-360 (2003)
24. Maye P., J. Zheng, L. Li & D. Wu. Multiple mechanisms for Wnt11-mediated repression of the canonical Wnt signaling pathway. *J Biol Chem* 279, 24659-24665 (2004)
25. Iozzo R.V., I. Eichstetter & K.G. Danielson. Aberrant expression of the growth factor Wnt-5A in human malignancy. *Cancer Res* 55, 3495-3499 (1995)
26. Lejeune S., E.L. Huguet, A. Hamby, R. Poulson & A.L. Harris. Wnt5a cloning, expression, and up-regulation in human primary breast cancers. *Clin Cancer Res* 1, 215-222 (1995)
27. Weeraratna A.T., Y. Jiang, G. Hostetter, K. Rosenblatt, P. Duray, M. Bittner & J.M. Trent. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 1, 279-288 (2002)
28. Devereux T.R., M.C. Stern, G.P. Flake, M.C. Yu, Z.Q. Zhang, S.J. London & J.A. Taylor. CTNNB1 mutations and beta-catenin protein accumulation in human hepatocellular carcinomas associated with high exposure to aflatoxin B1. *Mol Carcinog* 31, 68-73 (2001)
29. Hsu H.C., Y.M. Jeng, T.L. Mao, J.S. Chu, P.L. Lai & S.Y. Peng. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 157, 763-770 (2000)
30. Wong C.M., S.T. Fan & I.O. Ng. Beta-catenin mutation and overexpression in hepatocellular carcinoma:

## WNT/Frizzled Signaling in HCC

- clinicopathologic and prognostic significance. *Cancer* 92, 136-145 (2001)
31. Endo K., T. Ueda, J. Ueyama, T. Ohta & T. Terada. Immunoreactive E-cadherin, alpha-catenin, beta-catenin, and gamma-catenin proteins in hepatocellular carcinoma: relationships with tumor grade, clinicopathologic parameters, and patients' survival. *Hum Pathol* 31, 558-65 (2000)
32. Inagawa S., M. Itabashi, S. Adachi, T. Kawamoto, M. Hori, J. Shimazaki, F. Yoshimi & K. Fukao. Expression and prognostic roles of beta-catenin in hepatocellular carcinoma: correlation with tumor progression and postoperative survival. *Clin Cancer Res* 8, 450-456 (2002)
33. Kinzler K.W. & B. Vogelstein. Lessons from hereditary colorectal cancer. *Cell* 87, 159-170 (1996)
34. Rhee, C.S., M. Sen, D. Lu, C. Wu, L. Leoni, J. Rubin, M. Corr & D.A. Carson. Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. *Oncogene* 21, 6598-6605 (2002)
35. Kirikoshi H., H. Sekihara & M. Katoh. Molecular cloning and characterization of human WNT11. *Int J Mol Med* 8, 651-656 (2001)
36. Zhu H., M. Mazor, Y. Kawano, M.M. Walker, H.Y. Leung, K. Armstrong, J. Waxman & R.M. Kypka. Analysis of Wnt gene expression in prostate cancer: mutual inhibition by WNT11 and the androgen receptor. *Cancer Res* 64, 7918-7926 (2004)
37. Hsieh J.C., L. Kodjabachian, M.L. Rebbert, A. Rattner, P.M. Smallwood, C.H. Samos, R. Nusse, I.B. Dawid & J. Nathans. A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* 398, 431-436 (1999)
38. Kawano Y. & R. Kypka. Secreted antagonists of the Wnt signalling pathway. *J Cell Sci* 116, 2627-2634 (2003)
39. Taniguchi H., H. Yamamoto, T. Hirata, N. Miyamoto, M. Oki, K. Noshio, Y. Adachi, T. Endo, K. Imai & Y. Shinomura. Frequent epigenetic inactivation of Wnt inhibitory factor-1 in human gastrointestinal cancers. *Oncogene* (in press)
40. J. Mazieres, B. He, L., You, Z. Xu, A.Y. Lee, I. Mikami, N. Reguart, R. Rosell, F. McCormick & D.M. Jablons. Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. *Cancer Res* 64, 4717-4720 (2004)
41. Wissmann C., P.J. Wild, S. Kaiser, S. Roepcke, R. Stoehr, M. Woenckhaus, G. Kristiansen, J.C. Hsieh, F. Hofstaedter, A. Hartmann, R. Knuechel, A. Rosenthal & C. Pilarsky. WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer. *J Pathol* 201, 204-212 (2003)
42. Zou H., J.R. Molina, J.J. Harrington, N.K. Osborn, K.K. Klatt, Y. Romero, L.J. Burgart & D.A. Ahlquist. Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. *Int J Cancer* 116, 584-591 (2005)
43. Xu X.L., J. Yu, H.Y. Zhang, M.H. Sun, J. Gu, X. Du, D.R. Shi, P. Wang, Z.H. Yang & J.D. Zhu. Methylation profile of the promoter CpG islands of 31 genes that may contribute to colorectal carcinogenesis. *World J Gastroenterol* 10, 3441-3454 (2004)
44. Wong S.C., S.F. Lo, K.C. Lee, J.W. Yam, J.K. Chan & W.L. Wendy Hsiao. Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours. *J Pathol* 196, 145-153 (2002)
45. Roth W., C. Wild-Bode, M. Platten, C. Grimmel, H.S. Melkonyan, J. Dichgans & M. Weller. Secreted Frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. *Oncogene* 19, 4210-4220 (2000)
46. Uren A., F. Reichsman, V. Anest, W.G. Taylor, K. Muraiso, D.P. Bottaro, S. Cumberledge & J.S. Rubin. Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J Biol Chem* 275, 4374-4382 (2000)
47. Melkonyan H.S., W.C. Chang, J.P. Shapiro, M. Mahadevappa, P.A. Fitzpatrick, M.C. Kiefer, L.D. Tomei & S.R. Umansky. SARPs: a family of secreted apoptosis-related proteins. *Proc Natl Acad Sci U S A* 94, 13636-13641 (1997)
48. Calvisi D.F., E.A. Conner, S. Ladu, E.R. Lemmer, V.M. Factor & S.S. Thorgeirsson. Activation of the canonical Wnt/beta-catenin pathway confers growth advantages in c-myc/E2F1 transgenic mouse model of liver cancer. *J Hepatol* 42, 842-849 (2005)
49. Kirikoshi H., H. Sekihara & M. Katoh. Expression profiles of 10 members of Frizzled gene family in human gastric cancer. *Int J Oncol* 19, 767-771 (2001)
50. Kirikoshi H., H. Sekihara & M. Katoh. Up-regulation of Frizzled-7 (FZD7) in human gastric cancer. *Int J Oncol* 19, 111-115 (2001)
51. Tanaka S., T. Akiyoshi, M. Mori, J.R. Wands & K. Sugimachi. A novel frizzled gene identified in human esophageal carcinoma mediates APC/beta-catenin signals. *Proc Natl Acad Sci U S A* 95, 10164-10169 (1998)
52. Holcombe R.F., J.L. Marsh, M.L. Waterman, F. Lin, T. Milovanovic & T. Truong. Expression of Wnt ligands and Frizzled receptors in colonic mucosa and in colon carcinoma. *Mol Pathol* 55, 220-226 (2002)

53. Merle P., M. Kim, M. Herrmann, A. Gupte, L. Lefrançois, S. Califano, C. Trepo, S. Tanaka, L. Vitvitski, S. de la Monte & J.R. Wands. Oncogenic role of the frizzled-7/beta-catenin pathway in hepatocellular carcinoma. *J Hepatol* 43, 854-862 (2005)
54. Merle P., S. de la Monte, M. Kim, M. Herrmann, S. Tanaka, A. von dem Bussche, M.C. Kew, C. Trepo & J.R. Wands. Functional consequences of frizzled-7 receptor overexpression in human hepatocellular carcinoma. *Gastroenterology* 127, 1110-1122 (2004)
55. Pinson K.I., J. Brennan, S. Monkley, B.J. Avery & W.C. Skarnes. An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* 407, 535-538 (2000)
56. Kato M., M.S. Patel, R. Levasseur, I. Lobov, B.H. Chang, D.A. 2nd Glass, C. Hartmann, L. Li, T.H. Hwang, C.F. Brayton, R.A. Lang, G. Karsenty & L. Chan. Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. *J Cell Biol* 157, 303-314 (2002)
57. Li Y., W. Lu, X. He, A.L. Schwartz & G. Bu. LRP6 expression promotes cancer cell proliferation and tumorigenesis by altering beta-catenin subcellular distribution. *Oncogene* 23, 9129-9135 (2004)
58. Brennan K., J.M. Gonzalez-Sancho, L.A. Castelo-Soccio, L.R. Howe & A.M. Brown. Truncated mutants of the putative Wnt receptor LRP6/Arrow can stabilize beta-catenin independently of Frizzled proteins. *Oncogene* 23, 4873-4884 (2004)
59. Hoang B.H., T. Kubo, J.H. Healey, R. Sowers, B. Mazza, R. Yang, A.G. Huvos, P.A. Meyers & R. Gorlick. Expression of LDL receptor-related protein 5 (LRP5) as a novel marker for disease progression in high-grade osteosarcoma. *Int J Cancer* 109, 106-111 (2004)
60. Roman-Gomez J., A. Jimenez-Velasco, X. Agirre, J.A. Castillejo, G. Navarro, M. Barrios, E.J. Andreu, F. Prosper, A. Heiniger & A. Torres. Transcriptional silencing of the Dickkopf-3 (Dkk-3) gene by CpG hypermethylation in acute lymphoblastic leukaemia. *Br J Cancer* 91, 707-713 (2004)
61. Gonzalez-Sancho J.M., O. Aguilera, J.M. Garcia, N. Pendas-Franco, C. Pena, S. Cal, A. Garcia de Herreros, F. Bonilla & A. Munoz. The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. *Oncogene* 24, 1098-1103 (2005)
62. Wirths O., A. Waha, S. Weggen, P. Schirmacher, T. Kuhne, C.G. Goodyer, S. Albrecht, D. Von Schweinitz & T. Pietsch. Overexpression of human Dickkopf-1, an antagonist of wntless/WNT signaling, in human hepatoblastomas and Wilms' tumors. *Lab Invest* 83, 429-434 (2003)
63. Uematsu K., B. He, L. You, Z. Xu, F. McCormick & D.M. Jablons. Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. *Oncogene* 22, 7218-7221 (2003)
64. Uematsu K., S. Kanazawa, L. You, B. He, Z. Xu, K. Li, B.M. Peterlin, F. McCormick & D.M. Jablons. Wnt pathway activation in mesothelioma: evidence of Dishevelled overexpression and transcriptional activity of beta-catenin. *Cancer Res* 63, 4547-4551 (2003)
65. Okino K., H. Nagai, M. Hatta, T. Nagahata, K. Yoneyama, Y. Ohta, E. Jin, O. Kawanami, T. Araki & M. Emi. Up-regulation and overproduction of DVL-1, the human counterpart of the *Drosophila* dishevelled gene, in cervical squamous cell carcinoma. *Oncol Rep* 10, 1219-1223 (2003)
66. Yau T.O., C.Y. Chan, K.L. Chan, M.F. Lee, C.M. Wong, S.T. Fan & I.O. Ng. HDPR1, a novel inhibitor of the WNT/beta-catenin signaling, is frequently downregulated in hepatocellular carcinoma: involvement of methylation-mediated gene silencing. *Oncogene* 24, 1607-1614 (2005)
67. Polakis P. The oncogenic activation of beta-catenin. *Curr Opin Genet Dev* 9, 15-21 (1999)
68. Sparks A.B., P.J. Morin, B. Vogelstein & K.W. Kinzler. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res* 58, 1130-1134 (1998)
69. Iwao K., S. Nakamori, M. Kameyama, S. Imaoka, M. Kinoshita, T. Fukui, S. Ishiguro, Y. Nakamura & Y. Miyoshi. Activation of the beta-catenin gene by interstitial deletions involving exon 3 in primary colorectal carcinomas without adenomatous polyposis coli mutations. *Cancer Res* 58, 1021-1026 (1998)
70. Satoh S., Y. Daigo, Y. Furukawa, T. Kato, N. Miwa, T. Nishiwaki, T. Kawasoe, H. Ishiguro, M. Fujita, T. Tokino, Y. Sasaki, S. Imaoka, M. Murata, T. Shimano, Y. Yamaoka & Y. Nakamura. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat Genet* 24, 245-251 (2000)
71. de La Coste A., B. Romagnolo, P. Billuart, C.A. Renard, M.A. Buendia, O. Soubrane, M. Fabre, J. Chelly, C. Beldjord, A. Kahn & C. Perret. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 95, 8847-8851 (1998)
72. Miyoshi Y., K. Iwao, Y. Nagasawa, T. Aihara, Y. Sasaki, S. Imaoka, M. Murata, T. Shimano & Y. Nakamura. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 58, 2524-2527 (1998)
73. Legoix P., O. Bluteau, J. Bayer, C. Perret, C. Balabaud, J. Belghiti, D. Franco, G. Thomas, P. Laurent-Puig & J.

## WNT/Frizzled Signaling in HCC

- Zucman-Rossi. Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity. *Oncogene* 18, 4044-4046 (1999)
74. Taniguchi K., L.R. Roberts, I.N. Aderca, X. Dong, C. Qian, L.M. Murphy, D.M. Nagorney, L.J. Burgart, P.C. Roche, D.I. Smith, J.A. Ross & W. Liu. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 21, 4863-4871, 21 (2002)
75. Huang H., H. Fujii, A. Sankila, B.M. Mahler-Araujo, M. Matsuda, G. Cathomas & H. Ohgaki. Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. *Am J Pathol* 155, 1795-1801 (1999)
76. Laurent-Puig P., P. Legoix, O. Bluteau, J. Belghiti, D. Franco, F. Binot, G. Monges, G. Thomas, P. Bioulac-Sage & J. Zucman-Rossi. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 120, 1763-1773 (2001)
77. Koch A., D. Denkhaus, S. Albrecht, I. Leuschner, D. von Schweinitz & T. Pietsch. Childhood hepatoblastomas frequently carry a mutated degradation targeting box of the beta-catenin gene. *Cancer Res* 59, 269-273 (1999)
78. Wei Y., M. Fabre, S. Branchereau, F. Gauthier, G. Perilongo & M.A. Buendia. Activation of beta-catenin in epithelial and mesenchymal hepatoblastomas. *Oncogene* 19, 498-504 (2000)
79. Miyoshi Y., H. Nagase, H. Ando, A. Horii, S. Ichii, S. Nakatsuru, T. Aoki, Y. Miki, T. Mori & Y. Nakamura. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1, 229-233 (1992)
80. Colnot S., T. Decaens, M. Niwa-Kawakita, C. Godard, G. Hamard, A. Kahn, M. Giovannini & C. Perret. Liver-targeted disruption of Apc in mice activates beta-catenin signaling and leads to hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 101, 17216-17221 (2004)
81. Edamoto Y., A. Hara, W. Biernat, L. Terracciano, G. Cathomas, H.M. Riehle, M. Matsuda, H. Fujii, J.Y. Scoazec & H. Ohgaki. Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. *Int J Cancer* 106, 334-341 (2003)
82. Lal G. & S. Gallinger. Familial adenomatous polyposis. *Semin Surg Oncol* 18, 314-323 (2000)
83. Hart M.J., R. de los Santos, I.N. Albert, B. Rubinfeld & P. Polakis. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK-3beta. *Curr Biol* 8, 573-581 (1998)
84. Ikeda S., S. Kishida, H. Yamamoto, H. Murai, S. Koyama & A. Kikuchi. 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)
85. Webster M.T., M. Rozycka, E. Sara, E. Davis, M. Smalley, N. Young, T.C. Dale & R. Wooster. Sequence variants of the axin gene in breast, colon, and other cancers: an analysis of mutations that interfere with GSK3 binding. *Genes Chromosomes Cancer* 28, 443-453 (2000)
86. Sakanaka C., J.B. Weiss & L.T. Williams. Bridging of beta-catenin and glycogen synthase kinase-3beta by axin and inhibition of beta-catenin-mediated transcription. *Proc Natl Acad Sci U S A* 95, 3020-3023 (1998)
87. Park J.Y., W.S. Park, S.W. Nam, S.Y. Kim, S.H. Lee, N.J. Yoo, J.Y. Lee & C.K. Park. Mutations of beta-catenin and AXIN 1 genes are a late event in human hepatocellular carcinogenesis. *Liver Int* 25, 70-76 (2005)
88. Ishizaki Y., S. Ikeda, M. Fujimori, Y. Shimizu, T. Kurihara, T. Itamoto, A. Kikuchi, M. Okajima & T. Asahara. Immunohistochemical analysis and mutational analyses of beta-catenin, Axin family and APC genes in hepatocellular carcinomas. *Int J Oncol* 24, 1077-1083 (2004)
89. Lustig B., B. Jerchow, M. Sachs, S. Weiler, T. Pietsch, U. Karsten, M. van de Wetering, H. Clevers, P.M. Schlag, W. Birchmeier & J. Behrens. Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol Cell Biol* 22, 1184-1193 (2002)
90. Embi N., D.B. Rylatt & P. Cohen. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem* 107, 519-527 (1980)
91. Frame S. & P. Cohen. GSK3 takes centre stage more than 20 years after its discovery. *Biochem J* 359, 1-16 (2001)
92. Harwood A.J. Regulation of GSK-3: a cellular multiprocessor. *Cell* 105, 821-824 (2001)
93. Patel S., B. Doble & J.R. Woodgett. Glycogen synthase kinase-3 in insulin and Wnt signalling: a double-edged sword? *Biochem Soc Trans* 32, 803-808 (2004)
94. Fuchs S.Y., V.S. Spiegelman & K.G. Kumar. The many faces of beta-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. *Oncogene* 23, 2028-2036 (2004)
95. Matsubayashi H., S. Sese, J.S. Lee, T. Shirakawa, T. Iwatsubo, T. Tomita & S. Yanagawa. Biochemical characterization of the Drosophila wingless signaling pathway based on RNA interference. *Mol Cell Biol* 24, 2012-2024 (2004)

## WNT/Frizzled Signaling in HCC

96. Yamamoto H., S. Kishida, M. Kishida, S. Ikeda, S. Takada & A. Kikuchi. Phosphorylation of axin, a Wnt signal negative regulator, by glycogen synthase kinase-3beta regulates its stability. *J Biol Chem* 274, 10681-10684 (1999)
97. Jho E., S. Lomvardas & F. Costantini. A GSK3beta phosphorylation site in axin modulates interaction with beta-catenin and Tcf-mediated gene expression. *Biochem Biophys Res Commun* 266, 28-35 (1999)
98. Rubinfeld B., I. Albert, E. Porfiri, C. Fiol, S. Munemitsu & P. Polakis. Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 272, 1023-1026 (1996)
99. Chen R.H., W.V. Ding & F. McCormick. Wnt signaling to beta-catenin involves two interactive components. Glycogen synthase kinase-3beta inhibition and activation of protein kinase C. *J Biol Chem* 275, 17894-17899 (2000)
100. Papadopoulou D., M.W. Bianchi & M. Bourouis. Functional studies of shaggy/glycogen synthase kinase 3 phosphorylation sites in *Drosophila melanogaster*. *Mol Cell Biol* 24, 4909-4919 (2004)
101. Desbois-Mouthon C., M.J. Blivet-Van Eggelpeel, E. Beurel, M. Boissan, R. Delelo, A. Cadoret & J. Capeau. Dysregulation of glycogen synthase kinase-3beta signaling in hepatocellular carcinoma cells. *Hepatology* 36, 1528-1536 (2002)
102. Desbois-Mouthon C., A. Cadoret, M.J. Blivet-Van Eggelpeel, F. Bertrand, G. Cherqui, C. Perret & J. Capeau. Insulin and IGF-1 stimulate the beta-catenin pathway through two signalling cascades involving GSK-3beta inhibition and Ras activation. *Oncogene* 20, 252-259 (2001)
103. Ban K.C., H. Singh, R. Krishnan & H.F. Seow. GSK-3beta phosphorylation and alteration of beta-catenin in hepatocellular carcinoma. *Cancer Lett* 199, 201-208 (2003)
104. van de Wetering M., R. Cavallo, D. Dooijes, M. van Beest, J. van Es, J. Loureiro, A. Ypma, D. Hursh, T. Jones, A. Bejsovec, M. Peifer, M. Mortin & H. Clevers. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* 21, 789-799 (1997)
105. Brannon M., J.D. Brown, R. Bates, D. Kimelman & Moon RT. XTCBP is a XTcf-3 co-repressor with roles throughout *Xenopus* development. *Development* 126, 3159-3170 (1999)
106. Riese J., X. Yu, A. Munneryn, S. Eresh, S.C. Hsu, R. Grosschedl & M. Bienz. LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* 88, 777-787 (1997)
107. Tetsu O. & F. McCormick. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 398, 422-426 (1999)
108. Korinek V., N. Barker, P. Moerer, E. van Donselaar, G. Huls, P.J. Peters & H. Clevers. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 19, 379-383 (1998)
109. Van de Wetering M., J. Castrop, V. Korinek & H. Clevers. Extensive alternative splicing and dual promoter usage generate Tcf-1 protein isoforms with differential transcription control properties. *Mol Cell Biol* 16, 745-752 (1996)
110. Hovanes K., T.W. Li, J.E. Munguia, T. Truong, T. Milovanovic, J. Lawrence Marsh, R.F. Holcombe & M.L. Waterman. Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nat Genet* 28, 53-57 (2001)
111. Duval A., J. Gayet, X.P. Zhou, B. Iacopetta, G. Thomas & R. Hamelin. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. *Cancer Res* 59, 4213-4215 (1999)
112. Jiang Y., X.D. Zhou, Y.K. Liu, X. Wu & X.W. Huang. Association of hTcf-4 gene expression and mutation with clinicopathological characteristics of hepatocellular carcinoma. *World J Gastroenterol* 8, 804-807 (2002)
113. Cui J., X. Zhou, Y. Liu, Z. Tang & M. Romeih. Wnt signaling in hepatocellular carcinoma: analysis of mutation and expression of beta-catenin, T-cell factor-4 and glycogen synthase kinase 3-beta genes. *J Gastroenterol Hepatol* 18, 280-287 (2003)
114. Schmitt-Graeff A., V. Ertelt-Heitzmann, H.P. Allgaier, M. Olschewski, R. Nitschke, S. Haxelmans, K. Koelble, J. Behrens & H.E. Blum. Coordinated expression of cyclin D1 and LEF-1/TCF transcription factor is restricted to a subset of hepatocellular carcinoma. *Liver Int* 25, 839-847 (2005)
115. He T.C., A.B. Sparks, C. Rago, H. Hermeking, L. Zawel, L.T. da Costa, P.J. Morin, B. Vogelstein & K.W. Kinzler. Identification of c-myc as a target of the APC pathway. *Science* 281, 1509-1512 (1998)
116. Shang X.Z., H. Zhu, K. Lin, Z. Tu, J. Chen, D.R. Nelson & C. Liu. Stabilized beta-catenin promotes hepatocyte proliferation and inhibits TNFalpha-induced apoptosis. *Lab Invest* 84, 332-341 (2004)
117. Zhao D.H., J.J. Hong, S.Y. Guo, R.L. Yang, J. Yuan, C.Y. Wen, K.Y. Zhou & C.J. Li. Aberrant expression and function of TCF4 in the proliferation of hepatocellular carcinoma cell line BEL-7402. *Cell Res* 14, 74-80 (2004)
118. Dragan Y.P., L.M. Sargent, K. Babcock, N. Kinunen & H.C. Pitot. Alterations in specific gene expression and focal neoplastic growth during spontaneous hepatocarcinogenesis in albumin-SV40 T antigen transgenic rats. *Mol Carcinog* 40, 150-159 (2004)

119. Anna C.H., M. Iida, R.C. Sills & T.R. Devereux. Expression of potential beta-catenin targets, cyclin D1, c-Jun, c-myc, E-cadherin, and EGFR in chemically induced hepatocellular neoplasms from B6C3F1 mice. *Toxicol Appl Pharmacol* 190, 135-145 (2003)
120. Cadoret A., C. Ovejero, S. Saadi-Kheddouci, E. Souil, M. Fabre, B. Romagnolo, A. Kahn & C. Perret. Hepatomegaly in transgenic mice expressing an oncogenic form of beta-catenin. *Cancer Res* 61, 3245-3249 (2001)
121. Kawate S., T. Fukusato, S. Ohwada, A. Watanuki & Y. Morishita. Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic features, proliferative activity and p53 overexpression. *Oncology* 57, 157-163 (1999)
122. Wang Y., M.C. Wu, J.S. Sham, W. Zhang, W.Q. Wu & X.Y. Guan. Prognostic significance of c-myc and AIB1 amplification in hepatocellular carcinoma. A broad survey using high-throughput tissue microarray. *Cancer* 95, 2346-2352 (2002)
123. Niu Z.S., B.K. Li & M. Wang. Expression of p53 and c-myc genes and its clinical relevance in the hepatocellular carcinomatous and pericarcinomatous tissues. *World J Gastroenterol* 8, 822-826 (2002)
124. Cui J., B.W. Dong, P. Liang, X.L. Yu & D.J. Yu. Effect of c-myc, Ki-67, MMP-2 and VEGF expression on prognosis of hepatocellular carcinoma patients undergoing tumor resection. *World J Gastroenterol* 15, 1533-1536 (2004)
125. Prange W., K. Breuhahn, F. Fischer, C. Zilkens, T. Pietsch, K. Petmecky, R. Eilers, H.P. Dienes & P. Schirmacher. Beta-catenin accumulation in the progression of human hepatocarcinogenesis correlates with loss of E-cadherin and accumulation of p53, but not with expression of conventional WNT-1 target genes. *J Pathol* 201, 250-259 (2003)
126. Yuen M.F., P.C. Wu, V.C. Lai, J.Y. Lau & C.L. Lai. Expression of c-myc, c-Fos, and c-jun in hepatocellular carcinoma. *Cancer* 91, 106-112 (2001)
127. Chan K.L., X.Y. Guan & I.O. Ng. High-throughput tissue microarray analysis of c-myc activation in chronic liver diseases and hepatocellular carcinoma. *Hum Pathol* 35, 1324-1331 (2004)
128. Ueta T., M. Ikeguchi, Y. Hirooka, N. Kaibara & T. Terada. Beta-catenin and cyclin D1 expression in human hepatocellular carcinoma. *Oncol Rep* 9, 1197-1203 (2002)
129. Gotoh J., M. Obata, M. Yoshie, S. Kasai & K. Ogawa. Cyclin D1 over-expression correlates with beta-catenin activation, but not with H-ras mutations, and phosphorylation of Akt, GSK3 beta and ERK1/2 in mouse hepatic carcinogenesis. *Carcinogenesis* 24, 435-442 (2003)
130. Cadoret A., C. Ovejero, B. Terris, E. Souil, L. Levy, W.H. Lamers, J. Kitajewski, A. Kahn & C. Perret. New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene* 21, 8293-8301 (2002)
131. Tan X., U. Apte, A. Micsenyi, E. Kotsagrellos, J.H. Luo, S. Ranganathan, D.K. Monga, A. Bell, G.K. Michalopoulos & S.P. Monga. Epidermal Growth Factor Receptor: A Novel Target of the Wnt/beta-Catenin Pathway in Liver. *Gastroenterology* 129, 285-302 (2005)
132. Ovejero C., C. Cavard, A. Perianin, T. Hakvoort, J. Vermeulen, C. Godard, M. Fabre, P. Chafey, K. Suzuki, B. Romagnolo, S. Yamagoe & C. Perret. Identification of the leukocyte cell-derived chemotaxin 2 as a direct target gene of beta-catenin in the liver. *Hepatology* 40, 167-176 (2004)
133. Yamamoto Y., M. Sakamoto, G. Fujii, H. Tsuiji, K. Kenetaka, M. Asaka & S. Hirohashi. Overexpression of orphan G-protein-coupled receptor, Gpr49, in human hepatocellular carcinomas with beta-catenin mutations. *Hepatology* 37, 528-533 (2003)
134. Cervello M., L. Giannitrapani, M. Labbozzetta, M. Notarbartolo, N. D'Alessandro, N. Lampiasi, A. Azzolina & G. Montalto. Expression of WISPs and of their novel alternative variants in human hepatocellular carcinoma cells. *Ann N Y Acad Sci* 1028, 432-439 (2004)

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