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Regulation of Canonical Wnt Signaling During Development and Diseases

Saijun Mo[1] and Zongbin Cui[2,*]
[1]*Department of Basic Oncology, College of Basic Medical Sciences, Zhengzhou University, Zhengzhou,*
[2]*Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China*

1. Introduction

Since the discovery of first Wnt gene (*Wnt-1*) in 1982, numerous investigations have focused on the roles of different Wnt proteins in regulation of cellular proliferation, differentiation and apoptosis in a cell-specific and contextual manner (Rao & Kuhl, 2011). Wnts mainly activate intracellular biological processes through one canonical pathway and two well-characterized non-canonical pathways including Wnt/PCP (planar cell polarity) and Wnt/Ca^{2+}. The canonical pathway is also known as Wnt/β-catenin pathway due to the key roles of β-catenin in transcriptional regulation of downstream genes. These pathways play distinct roles in embryonic development, cell fate determination, cell polarity generation and cell movements, cross-talks always occur through a variety of intracellular signal molecules. In this chapter, we mainly focus on the up-to-date understanding of canonical Wnt signaling, including its functions and regulatory mechanisms both in animal development and human diseases.

2. An overview of canonical Wnt signaling

In the canonical Wnt signaling pathway, β-catenin is the key downstream effector. With the absence of Wnt ligands (Fig. 1, right), cytoplasmic β-catenin is associated with adenomatous polyposis coli (APC) and Axin proteins and phosphorylated by glycogen synthase kinase 3β (GSK-3β) and casein kinase I (CKI), resulting in its polyubiquitination and protease- mediated degradation. Under these conditions, the Lymphoid Enhancer Factor/T-cell factor (LEF/TCF) family of transcription factors in the nucleus is able to associate with transcriptional co-repressors to respress the transcription of Wnt target genes. In the presence of Wnt ligands (Fig. 1, left), the binding of a Frizzled (Fz or Fzd) receptor and a low-density lipoprotein related receptor protein (LRP5/6) 5 or 6 co-receptor, and the interaction of Fzd with cytoplasmic protein Disheveled (Dsh or Dvl) results in the phosphorylation of Dsh/Dvl by CKI and binding to GSK-3β with the involvement of Frequently Rearranged in Advanced T-cell lymphomas (FRAT) protein. These events lead to inactivation of the Axin/APC/GSK-

* Corresponding Author

3β/CKI complex and thus the inhibition of β-catenin phosphorylation and degradation, allowing the translocation and accumulation of cytosolic β-catenin into the nucleus. Nuclear β-catenin then interacts with LEF/TCF family transcription factors and several other transcriptional co-activators to initiate transcription of target genes.

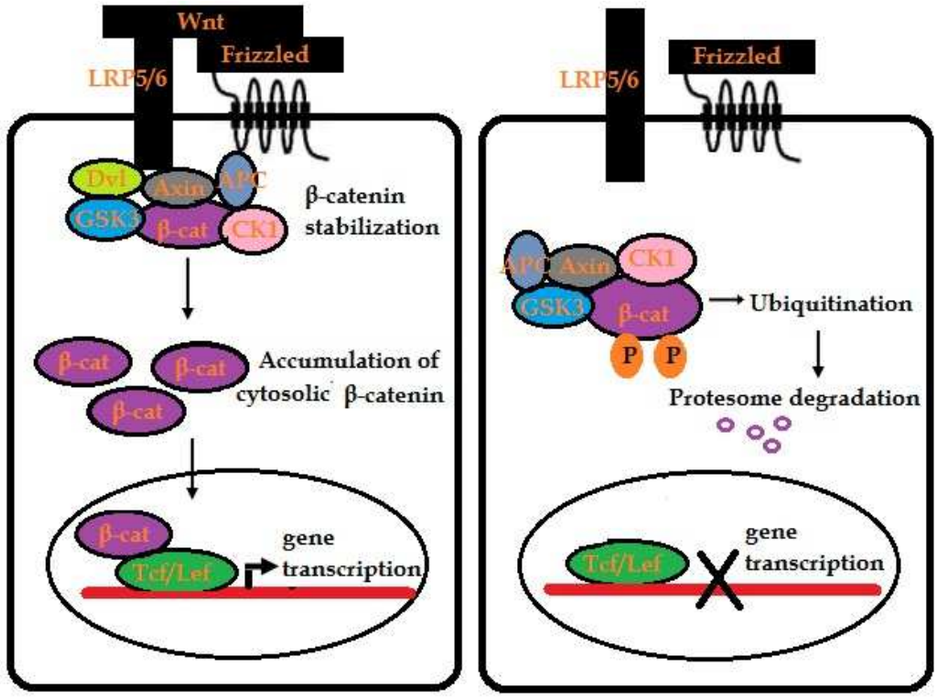


Fig. 1. An overview of Wnt/β-catenin signaling pathway (Modified from Masckauchan & Kitajewski, 2006).

2.1 Components of canonical Wnt signaling

The canonical Wnt signaling pathway mainly consists of multiple components including ligands (Wnts), receptors (Fz and LRP5/6), β-catenin complexes in cytoplasma (GSK-3β/ Axin/APC) and nucleus (TCF/LEF), Dsh (Dvls)/Frat protein, and downstream target genes. Those elements are highly conserved during evolution in species from fly to mammalian. Recent studies have expanded our knowledge of the repertoire of regulatory molecules involved in the Wnt signaling pathway, such as Caveolin-1, Norrin, R-spondin, sFRP, DKK, SOST/Sclerostin, Arrow, Ror, Krm, Lzts2, and so on.

2.1.1 The Wnt family

In general, Wnt genes encode 38- to 43-kDa glycoproteins with features of typical secreted growth factors, including a hydrophobic signal peptide, the absence of additional transmembrane domains, highly conserved cysteine residues, and the presence of *N*-linked glycosylation sites. Up to now, intensive studies have shown that Wnt genes exist in a wide range of species, including *Drosophila, Caenorhabditis elegans (C. elegans*), *Danio rerio*, *Xenopus,* mouse and human (Table 1). The Wnt family members are classically divided into canonical and non-canonical pathway components depending on their ability to transform C57MG

mammary cells or induce axis duplication in *Xenopus*. The canonical Wnts include Wnt1, Wnt2, Wnt2a, Wnt3, Wnt3a, Wnt7a, Wnt7b, Wnt-8a, Wnt10a and Wnt10b while Wnt4, Wnt5a and Wnt11 activate the noncanonical Wnt pathways. Generally speaking, the distinction between two groups of ligands is as follows: Canonical Wnts bound to Frizzled and activate the β-catenin/TCF-mediated transcription, whereas non-canonical Wnts bound to Frizzled and activate small Rho GTPases, c-Jun N-terminal kinase (Jnk) and other β-catenin-independent signaling events. However, an increasing number of recent studies have indicated that the two types of Wnts can regulate the canonical and noncanonical pathways each other.

	Wnt proteins	**Frizzled proteins**
Mouse and human	Wnt1, 2, 2B, 3, 3A, 4, 5A, 5B, 6, 7A7B, 8A, 8B, 9A, 9B, 10A, 10B, 11, 16	FZD1–10, Fz, SMO
X. laevis	XWnt1, 2, 2B, 3, 3A, 4, 5A, 5B, 6, 7A, 7B, 7C, 8A, 8B, 10A, 10B, 11, 16	XFz1-9, 10A, 10B, SMO
Danio rerio	Wnt1, 2, 2Bα, 3A, 4A, 4B, 5A, 5B, 7Aα, 7Bα, 8A, 8B, 9A, 9B,10A,10B, 11,16	Fz1-6, 7a, 7b, 8a, 8b, 8c, 9, 10, SMO
C. elegans	CWN-1, 2 and EGL-20	MOM-5, LIN-17, CFZ-2, MIG-1
D. melanogaster	Wg, DWntD, DWnt2, 4, 5, 6, 10	Dfz2, Dfz3, Dfz4, SMO

Table 1. Wnt and Frizzled proteins in mammals (mouse and human), *D. melanogaster*, *X. laevis*, *Danio rerio* (zebrafish) and *C. elegans*. Fz/Dfz, Fz in *D. melanogaster*; XFz, Fz in *X. laevis*; MOM-5, more of mesoderm (MS) family member-5; LIN-17, abnormal cell Lineage family member-17; CFZ-2, *C. elegans* Frizzled homolog family member-2; MIG-1, abnormal cell migration family member-1. For more details, see (van Amerongen & Nusse, 2009; http://www.stanford.edu/ ~rnusse/Wntgenes/zebraf Wnt.html)

2.1.2 Receptors

Both canonical and noncanonical classes of Wnt ligands transduce signals through membrane receptors including FZD1-10, LRP5, LRP6, ROR1, ROR2 and RYK.

2.1.2.1 Frizzled (Fz, FZD) family

The Fz family consists of seven-pass transmembrane proteins that are similar to G protein-coupled receptors. Fz exist in *Drosophila, C. elegans*, *Danio rerio*, *Xenopus,* mouse and human (Table 1). Fz exhibit a number of typical features (Huang & Klein 2004): (i) a highly conserved cysteine-rich domain (CRD), which may constitute the orthosteric binding site for Wnts; (ii) a linker region that shows little sequence similarity among family members; (iii) a highly conserved seven- transmembrane domain; and (iv) a cytoplasmic domain of variable size and little sequence homology among family members.

2.1.2.2 LRP5/6 co-receptor and Arrow

The LRP5/6, additional single-pass transmembrane proteins, is low-density lipoprotein receptor-related protein 5/6. *Drosophila* Arrow is homologous to mouse LRP5 and LRP6. LRP5 and 6 contain three ligand-binding repeats, four β-propeller regions and the flanking epidermal growth factor (EGF) repeats. The intracellular domain of LRP5/6 can bind to Axin. The LRP5/6 interaction domain of Dkk has been mapped to the C-terminal domain.

2.1.2.3 Ryk and Ror

Ryk and Ror, also single transmembrane domain Wnt receptors, are not required for, but in some cases may antagonize Wnt/β-catenin signaling. The receptor tyrosine kinases Ror1/2 (Ror in *D. melanogaster*; Cam-1, CAN cell migration defective in *C. elegans*) and Ryk (called Derailed in *D. melanogaster*; Lin-18 in *C. elegans*) should be seen as autonomous Wnt receptors, Fzd coreceptors, or possibly both (Schulte, 2010).

2.1.3 β-catenin complex

2.1.3.1 β-catenin

β-catenin is an essential transcriptional co-activator in the canonical Wnt pathway and exists as an unstable monomer in the cytoplasm. Cytoplasmic β-catenin (not binging to Wnts) is rapidly turned over through the action of multi-component protein phosphorylation machinery consisting of GSK-3β, Axin, and APC protein (formed GSK-3β/Axin/APC complex). Phosphorylated β-catenin is targeted for degradation by proteosome. The binding of Wnts to their receptors results in the nuclear translocation and accumulation of β-catenin and thus activation of LEF/TCF transcription factors by formation of the LEF/TCF complex to initiate the expression of target genes. β-catenin homolog in *Drosophila* is called Armadillo. The Armadillo and β-catenin consist of 13 Armadilllo (Arm) repeat domains, which are essential for interaction with other proteins. Vertebrates have two Armadillo/β-catenin homologs, β-catenin and plakoglobin (also called gamma-catenin).

2.1.3.2 GSK-3β/Axin/APC complex

GSK-3 was first identified as a consequence of its phosphorylation activity toward glycogen synthase. The mammalian GSK3 contains two members GSK-3α and GSK-3β. GSK-3β, also known as human tau protein kinase (TPK I), is a multifunctional serine-threonine kinase discovered in 1980 and originally identified as a regulator of glycogen metabolism. Phosphorylation of the tyrosine 216 residue results in the constitutive activity of GSK-3β, suggesting this residue is important for signal transduction. GSK-3β contains three groups of binding sites: ATP site, Axin binding site and Priming site.

There are two vertebrate Axin genes**.** Axin1 is constutively expressed, but Axin2 (also called Conductin or Axil) is induced by activation of Wnt signaling and therefore functions in a negative feedback loop. Several functional domains in Axin have been mapped, including an RGS-box (or RGS domain) for the Axin and APC interaction, two binding domains for β-catenin and GSK, and a DIX domain for Axin and Dishevelled interacion**.** Axin also binds to the phosphatase PP2A**.**

Both mammals and *Drosophila* carry two APC genes: APC and APC2 (APCL) in mammals, and dAPC1 and dAPC2/E-APC in Drosophila. There is a natural mouse apc1 mutant called *min1*. Mammalian APC contains multiple binding sites for numerous proteins (Aoki & Taketo, 2007), including microtubules (a basic domain), β**-**catenin (15-aa or 20-aa repeats), Axin (SAMP repeats), cytoskeletal regulators EB1 and IQGAP1 (C-terminal domains), and the Rac guanine-nucleotide-exchange factor (GEF) Asef1 (an armadillo repeat-domain). An oligomerization domain is also found at the N-terminal of APC.

2.1.3.3 TCF/LEF complex

In invertebrates, there is one TCF gene rarely displaying alternative transcripts. However, vertebrates have four TCF genes (*Tcf-1*, *Lef-1*, *Tcf-3* and *Tcf-4*), and each of them gives rise to a variety of alternative transcripts (Nusse, 1999; Arce et al, 2006). Four domains are existed in an invertebrate TCF: (i) an N-terminal β-catenin-binding domain (BCBD); (ii) a central domain; (iii) a well-conserved high-mobility group (HMG) DNA-binding domain, including a nuclear localization signal (NLS); and (iv) a long C-terminal tail. The general structures are conserved in vertebrate TCF/LEFs: TCF-1E isoforms are remarkably similar in overall domain structure to invertebrate TCFs; other vertebrate TCF isoforms have lost parts of these domains and/or included novel peptide motifs.

2.1.4 Dishevelled (Dvl/Dsh)

Three Dsh proteins Dsh-1, Dsh-2, and Dsh-3 have been found in mammals, while only Dsh in *Drosophila* and mig-5 in *C.elegans*. The Dsh family members in all organisms are comprised of three highly conserved domains (Habas & Dawid, 2005): (i) an amino-terminal DIX domain (named for Dsh and Axin), which is essential for the interaction between Dsh and Axin; (ii) a central PDZ domain (named for Postsynaptic density-95, Discs-large and Zonula occludens-1); and (iii) a carboxy-terminal DEP domain (for Dsh, Egl-10 and Pleckstrin).

2.1.5 Target genes (http://www.stanford.edu)

The canonical Wnt pathway controls biological processes via the regulation of target gene expression, including direct and indirect target genes. The expression of direct Wnt target genes, e.g. cyclin D1 and Myc, multidrug transporter P-glycoprotein (MDR1/ABCB1), is activated by the transcription factor TCF, which binds to specific sequence motifs in the promoter. Indirect target genes are regulated via transcription regulators, which are also targets of the Wnt pathway. Wnt signaling can promote the expression of several Wnt pathway components, including Fz, LRP5/6, DKK, Axin, Tcf, Lef and so on. The results indicate that feedback controls are key features of Wnt signaling regulation.

2.1.6 Other factors

1. β-arrestin

β-arrestins are originally identified as negative regulators of G protein-coupled receptors (GPCR). β-arrestin-1 and -2 are required for cellular communication by means of Wnts and FZDs. In canonical pathway, β-arrestins participate in the formation of a ternary complex composed of phosphorylated Dvl, β-arrestin and Axin, and affect the transcriptional activity of TCF/LEF (Schulte et al, 2010). It is not clear whether β-arrestin serves only as a scaffolding protein or whether β-arrestin-dependent endocytosis is required.

2. CKI

The CKI family is highly conserved monomeric serine–threonine protein kinases. Mammalian contain several CKI isoforms, which are α, β, γ, δ and ε. CKI prefer substrates primed by prior phosphorylation and works closely with other kinases in the Wnt pathway (Cheong & Virshup, 2011): First, CKI is itself regulated by posttranslational modification

including autophosphorylation; Second, CKI plays a role in phosphorylation of Dvl in the Wnt signaling pathway; Finally, CKI also regulate the Wnt signaling pathway by interacting with the Wnt receptor LRP.

2.2 The regulatory mechanism of canonical Wnt signaling

The regulatory mechanisms of canonical Wnt signaling pathway are very complicated. It is well established that components of Wnt/β-catenin pathway include Wnts, Fz, LRP5/6, APC, Axin, Dvl, GSk3β, CKI, TCF/LEF and so on. These components can form different complex and play distinct regulatory roles in canonical Wnt signaling: some components exert their roles as activators, such as canonical Wnts, Dvl, while some components act as inhibitors, such as GSK-3β. The canonical Wnt pathway can be regulated by other molecules such as R-spondins, Dkk, Wise, Caveolin-1, Neucrin, sFRP, and Wif and this pathway also can crosstalk with multiple signaling pathways including BMP, TGF-β, Notch, FGF signaling, and so on.

2.2.1 Activators of canonical Wnt signaling pathway

Many signal molecules can activate the canonical Wnt signaling pathway, leading to stabilization of β-catenin (Nusse, 1999; Bejsovec, 2005; http://www.stanford.edu/group/nusselab/cgi-bin/Wnt/activators_detectors). Approaches to activate this pathway include: (i) increased expression of Wnt ligands, receptors, β-catenin and Axin; (ii) phosphorylation of Dvl and LRP tail; (iii) inhibition of GSK-3β activity by factors such as LiCl and Akt; (iv) blocking the negative regulators of Wnt signaling, such as Axin and APC; (v) increased expression of Dsh (Dvls) to inhibit the function of the degradation complex and phosphorylation of β-catenin through its binding to GSK-3β and then promote the target gene transcription. Some other activators are described below.

1. Norrin

Norrin serves as a ligand and binds to FZD4 to activate the Wnt signaling pathway dependenting on the presence of cell surface LRP5. The CRD of FZD4 has been shown to play a critical role in Norrin-FZD4 binding and is associated with canonical Wnt signaling.

2. R-spondins

R-spondins are a family of cysteine-rich secreted proteins and consist of four homologs (Rspo-1, 2, 3, and 4) in vertebrates. No representative is found in *C elegans*, *D. melanogaster*, or *Saccharomyces cerevisiae*. All R-spondins contain two furin-like cysteine-rich domains at the N-terminus followed by a thrombospondin domain and a basic charged C-terminal tail. The furin domain of R-spondins is sufficient to synergize with Wnt3a and antagonize DKK1 function. Similar to the activity of Wnts, R-spondins activate Wnt/β-catenin signaling through binding to LRP6, inducing its phosphorylation, and promoting β-catenin stabilization. However, R-spondins do not directly activate LRP6 and require the presence of Wnts to block Dkk-induced endocytosis of LRP6 and thus ensure an appropriate receptor density in the membrane for Wnt signaling. Although all four R-spondins activate the canonical Wnt pathway, R-spondin-2 and -3 are more potent than R-spondin1, whereas R-spondin-4 is relatively inactive. In addition to LRP6, R-spondin-2 interacts with FZD8 to activate the canonical Wnt signaling (Kim et al, 2008).

3. CBP (CREB–binding protein) and P300

CBP or its closely related homolog p300, contains multiple functional domains including CREB binding domain, Bromo-domain, three zinc finger, Gluarrine-rich domain and HAT. Despite the high degree of homology, CBP and p300 are not completely redundant and have unique critical roles: CBP but not p300 is essential for hematopoietic stem cell self-renewal, whereas p300 is critical for proper hematopoietic differentiation (Teo & Kahn, 2010). The C-terminal domain of β-catenin has been found to interact with the histone acetyltransferases CBP/p300, which have distinct functions in the regulation of TCF/β-catenin–mediated survivin/BIRC5 transcription. ICG-001, a selective CBP/Catenin Antagonist, can modulate the canonical Wnt Signaling.

4. Ubiquitin ligase RNF146 (RING finger protein 146)

RNF146 is a RING-domain E3 ubiquitin ligase. RNF146 can act as a positive regulator of Wnt signaling through ubiquitylating and destabilizing Axin and tankyrase (Callow et al, 2011).

5. C/EBPβ, Shikonin and Testosterone

CCAAT/enhancer binding protein β (C/EBPβ) is rapidly induced in early stages of adipogenesis and is responsible for transcriptional induction of Peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPα by maintaining active Wnt/β-catenin signaling, after addition of adipogenic inducers. C/EBPβ is involved in the expression of Wnt10b, a major Wnt ligand in preadipocytes, while C/EBPβ is not an essential factor for the regulation of Wnt10b expression during adipogenesis.

Shikonin is a natural naphthoquinone compound and inhibits adipogenesis through the activation of the Wnt/β-catenin pathway. Shikonin induces the upregulation and nuclear translocation of β-catenin.

Testosterone supplementation in men decreases fat mass. Testosterone and dihydrotestosterone inhibit adipocyte differentiation *in vitro* through an AR-mediated nuclear translocation of β-catenin and activation of downstream Wnt signaling.

6. Frat protein/GBP

Three homologs (Frat1, Frat2 and Frat3) are found in vertebrates. The Frat homolog is called GBP in *Xenopus*, which is essential for embryonic axis formation. No protein similar to FRAT/GBP has been found in *Drosophila*. Frat proteins are potent activators of canonical Wnt-signal transduction: First, the binding of Frat to GSK3 can induce signaling through β-catenin/TCF; Second, Frat can bind to Dishevelled and advocate as the "missing link" that bridges signaling from Dishevelled to GSK3 in the canonical Wnt pathway.

2.2.2 Antagonists of canonical Wnt signaling pathway

2.2.2.1 Antagonists that bind to Wnt ligands

When antagonists bind to Wnt, they prevent Wnts from binding their receptors and presumably block the activity of Wnt signaling pathway. Antagonists in this group include sFRP, WIF-1 and Cerberus (Rubin et al, 2006).

1. Soluble Frizzled-related proteins (sFRPs)

The sFRPs family consists of a group of Wnt binding proteins including a frizzled-type cysteine-rich domain (CRD). Unlike the Frizzled, the C-terminus of sFRPs contains a netrin (NTR) domain and has no transmembrane segments. The sFRPs are encoded by FRP/FrzB genes, including sFRP1-2, FrzB, sFRP4-5 and Sizzled. sFRP1 and sFRP2 are identified to antagonize the Wnt activity. FrzB interacts with Wnt-8 and block the Wnt-8 signaling in *Xenopus* embryos development. In mammalian cells, FrzB can bind to Wnt1 and inhibit the β-catenin accumulation induced by Wnt1.

2. Wnt inhibitory factor-1 (WIF-1)

WIF-1 is a unique Wnt antagonist with differences in structure from sFRP and Dkk families. WIF-1 contains a highly conserved N-terminal domain named WIF domain (WD) and five epidermal growth factor repeats and the WD domain is sufficient for Wnt binding and signaling inhibition.

3. Cerberus

Cerberus belongs to the Cerberus/Dan gene family and lacks the FZD-CRD and WD. The identified members of Cerberus include mouse cerberus-like gene (mCer-1) and cerberus-like-2 (mCer2), chick Cerberus (ccer), *Xenopus* Cerberus (Xcer) and Coco, zebrafish Charon. However, the mCer-1 does not encode a Wnt antagonist and the antagonist activity of mammalian Coco has not been confirmed.

4. Wingful (Wf)/Notum

Notum, formerly called Wf in *Drosophila*, is a secreted hydrolase and has orthologs in mice and human. A number of studies have shown that Notum can also regulate Wnt signaling. For example, overexpression of *Drosophila* Wf severely inhibits Wg signaling activity and serves as a potent feedback inhibitor of Wg and complements the embryonic Naked cuticle (Nkd) system. In addition, Notum is a novel target of β-catenin/TCF4 and high levels of Notum are significantly associated with intracellular (nuclear or cytoplasmic) accumulation of β-catenin protein.

2.2.2.2 Antagonists that bind to LRP5/6

1. Dickkopfs (DKK) family

DKKs were the first glycoproteins reported to block the β-catenin pathway by binding to LRP5/6 and disrupting the formation of LRP5/6–FZD complexes. DKKs include DKK1–4 (no counterpart in *D. melanogaster*) and the DKK-like protein 1 (Dkk-3-related protein which is named Soggy in *D. melanogaster*). There are two conserved cysteine-rich domains (Cys-1 and Cys-2) in DKKs, while Sgy lacks cysteine-rich domains.

DKK-1 and DKK-4 display a Wnt antagonist mechanism, while the mechanism for the antagonizing effect of DKK-1 or -4 on LRP6 and Wnt/β-catenin pathway remains unclear. Results from several studies indicate that the Cys-2 domain of DKK-1 binds to LRP6 and Krm, forming a ternary complex and inducing LRP6 internalization. However, another reseach group has reported that DKK-1 blocks Wnt signaling but does not promote LRP6 internalization and degradation. The mechanism for DKK-2 activity is also disputable. Two groups have reported that DKK-2 is a poor inhibitor of Wnt signaling similar to DKK-1,

while another group has found the Wnt antagonizing activity of Dkk-2. There are two possible explanations for this discrepancy: (i) DKK-2 binds to LRP6 with a lower affinity and the binding Kd value is approximately 2 folds of that for DKK-1 (0.73 nM vs 0.34 nM); (ii) Dkk-2 is suggested to play the role as an agonist in low-Wnt/high-LRP6 condition, and acts as an antagonist in environment with high-Wnt levels. DKK-3 does not bind to LRPs or Krm1/2 and can not inhibit Wnt signaling.

2. Neucrin

Neucrin consists of a cysteine-rich domain in its carboxyl terminal region, similar to two domains of DKKs. Neucrin as well as DKKs bind to LRP6 and inhibit the stabilization of cytosolic β-catenin, indicating that Neucrin is also an antagonist of canonical Wnt signaling.

3. Wise/sclerostin

Wise (also known as *Sostdc1*, *ectodin* and *USAG-1*) is a member of Dan family of glycoproteins including Cerberus, gremlin, Dan, Coco, and protein-related-to-Dan-and -Cerberus. Recently, Wise and the related protein sclerostin were identified as inhibitors since they bind to the extracellular domain of the Wnt co-receptors LRP5 and LRP6. Sost shares 36% amino acids identity with Wise. They share a "cysteine knot" domain that occupies the central part of proteins. Sost behaves exclusively as an antagonist for Wnt/LRP5/6 signaling in mammalian cells and *Xenopus* embryos, whereas Wise alone can function as a weak agonist to activate β-catenin signaling to a limited extent. Unlike DKK1, Sost inhibition of Wnt signaling is insensitive to the presence of Krm2, a transmembrane protein that binds to DKK1.

4. CTGF (Connective tissue growth factor)

CTGF, a CCN family member, is a multi-domain protein and each domain can interact with several ligands, such as growth factors (e.g. TGF-β, BMP-4), cell surface proteins (e.g. LRP) and extracellular matrix proteins. CTGF can suppress Wnt signaling through binding to LRP6. This interaction is likely to occur through the C-terminal domain of CTGF.

5. SERPINA3K

SERPINA3K is a member of the serine proteinase inhibitor (SERPIN) family. The interaction between SERPINA3K and the extracellular domain of LRP6 blocks the Fz/LRP6 (receptor/co-receptor) dimerization induced by a Wnt ligand. Reseachers have also found that SERPINA3K binds to LRP6 with a Kd of 10 nM.

6. Adenomatosis polyposis coli down-regulated 1 protein (APCDD1)

APCDD1 is a membrane-bound glycoprotein that is abundantly expressed in human hair follicles. Former studies have found two Tcf-binding motifs in the 5'-flanking region of *APCDD1* and indicated that APCDD1 is directly regulated by the β-catenin/Tcf4 complex. However, recent functional studies show that APCDD1 inhibits Wnt signaling in a cell-autonomous manner and functions upstream of β-catenin. *In vitro* analysis indicates that APCDD1 can interact with Wnt3a and LRP5, two essential components of Wnt signalling. These results suggest that APCDD1 is a novel Wnt inhibitor.

2.2.2.3 Factors binding to Fz

1. Shisa

Homologues of Shisa are found in human, rat and chick, *Xenopus* and Zebrafish. However, no Shisa homologues are identified in *Ciona intestinalis*, *C.elegans* or *Drosophila*. All Shisa proteins contain two CRD and an N-terminal signal peptide. Shisa proteins represent a distinct family of Wnt antagonists, which trap Fz proteins in the ER and prevent Fz from reaching the cell surface.

2. Other factors

sFRP and Insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) can also antagonize Wnt signaling via binding to both Fz and LRP6 (MacDonald et al, 2009).

2.2.2.4 Factors binding to β-catenin

1. Chibby (Cby)

Cby physically interact with β-catenin and compete with the TCF/LEF family for binding to β-catenin. The coiled-coil motif of Cby is responsible for its specific binding to the armadillo repeats 10−12 and the C-terminal region of β-catenin. And phosphorylated Cby plays an essential role in the intracellular distribution of β-catenin in conjunction with 14-3-3 protein.

2. ICAT (Inhibitor of β-catenin and TCF4)

Orthologs of ICAT are highly conserved in vertebrate except frogs. ICAT inhibit β-catenin to bind to TCF/LEF and functions as a negative regulator of Wnt signaling. The crystal structure data indicate that ICAT bound to the armadillo repeat domain of β-catenin, since ICAT contains an N-terminal helilical domain that binds to repeats 11 and 12 of β-catenin, and an extended C-terminal region that binds to repeats 5-10 in a manner similar to that of Tcfs and other β-catenin ligands.

3. Caveolin-1 (Cav-1)

Cav-1 is an integral membrane proteins and accumulates of β-catenin within caveolae membranes and thus inhibits the β-catenin/Lef-1 signaling activated by Wnt-1 or the overexpression of β-catenin itself. Recent findings indicate that Cav-1 inhibits Wnt signaling by directly interacting with β-catenin depending on its scaffolding domains (Mo et al, 2010).

4. Lzts2

Lzts2 previuosly called LAPSER1 is a putative tumor suppressor that can directly interact with and mediate the nuclear export of β-catenin. We have recently shown that Lzts2 plays important roles in the dorsoventral patterning and embryonic cell movements in zebrafish (Li et al, 2011).

2.2.2.5 Factors associated with LEF/TCF

1. Groucho

Long Groucho/TLEs are transducin-like-enhancer of Split orthologs that function as the inhibitor of canonical Wnt pathway. The β-catenin and LEF/TCFs activation complexes are opposed by the LEF/TCF•Groucho repressor complexes. The C-terminal WD repeat domain in Groucho/TLE is responsible for binding to LEF/TCFs.

2. Endostatin

Endostatin is a C-terminal fragment of collagen XVIII and blocks the canonical Wnt mediated transcription depending on TCF.

2.2.2.6 Dvl inhibitors

1. Naked cuticle (Nkd)

The insects typically have a single Nkd gene, whereas there are two Nkd genes, Nkd1 and Nkd2, in human, mouse and zebrafish (have additional homology Nkd3). Nkd1 and Nkd2 contain a most conserved region of the EFX domain in species from fly to vertebrate. The EFX domain is required for the interaction of Nkd with the basic/PDZ domains of Dsh or Dvl in fly and vertebrate, thus inhibiting Wnt/β–catenin signaling.

2. Protease-activated receptors (PARs)

PARs belong to a large family of seven-transmembrane-spanning G protein-coupled receptors (GPCRs), which can couple to $G\alpha_{i/o}$, $G\alpha_{q}$, or $G\alpha_{12/13}$ within the same cell type. PAR1-$G\alpha_{13}$ associations inhibit the canonical Wnt signaling pathway by the recruitment of Dvl, an upstream Wnt signaling protein via the DIX domain.

3. Dapper/Frodo

Dapper (Dpr) is also called Frodo or Dact. A conserved C-terminal PDZ-binding motif in Dpr is responsible for the interaction with the PDZ domain of Dvl**.** This interaction depends on the phosphorylation of Dpr by CKIδ/ε.

2.2.3 Context-dependent agonists/antagonists

1. CKI

CKI family plays a complicated role in Wnt/β-catenin signaling in a context-dependent manner: CKIα acts as a potent negative regulator of β-catenin for interacting with Axin and phosphorylates serine 45 of β-catenin, while CKIε and CKIδ are found to be positive regulators and act upstream of Axin and GSK3 to stabilize β-catenin.

2. sFRPs

Indeed, biphasic effects of SFRPs were reported: low sFRP1 concentrations promote, whereas high sFRP1 concentrations decrease Wingless-induced β-catenin stabilization.

3. DKK-2

The N-terminal domains in DKK-1 and DKK-2 have different functions. The N-terminal fragment of DKK-2 synergies with LRP6 to induce Wnt signaling activation, while the N-terminal domain of DKK-1 appears to have no such function. Together with other evidence, DKK-2 is suggested to play the role as an agonist in low-Wnt/high-LRP6 condition, and acts as an antagonist in environment with high-Wnt levels.

4. Wise

It is known that Wise is an inhibitor of canonical Wnt pathway and first identified as its ability to alter the antero-posterior characterstic of neuralized *Xenopus* animal caps by

promoting the activity of the Wnt pathway. Thus, Wise appears to have a dual role in modulating Wnt pathway. It remains unclear how the Wise interacts with components in the Wnt signaling. One explanation is that Wise competes with Wnts for binding to LRP6 in the presence of Wnts, Wise and Dkk, and results in a weak Wnt-dependent activity or a complete block of receptor activity.

5. CBP (cAMP response-element binding protein)/P300

CBP and P300 are bimodal Wnt regulators with conserved roles in organisms from flies to vertebrates (Li et al, 2007). CBP/P300 can negatively regulate canonical Wnt signaling through directly binding and acetylating TCF, thus reducing TCF ability to bind with β-catenin. In contrast, CBP acts as a co-activator by directly interacting with the β-cateinin (Arm in fly). The interaction domain has been mapped to the N-terminal region of CBP and the C-terminal region of β-catenin. A recent study has identified that the phosphorylation of a Proline-directed Serine 92 residue modulates the selective binding of CBP with β-catenin.

2.2.4 Epigenetic regulation

Epigenetic regulation including DNA methylation of promoter CpG islands and/or histone modification often leads to the activation or amplification of aberrant Wnt/β-catenin signaling. Many genes endocing components in this pathway can be modified by DNA hypermethylation, thus being closely associated with tumorgenesis (Fig.2). In addition,

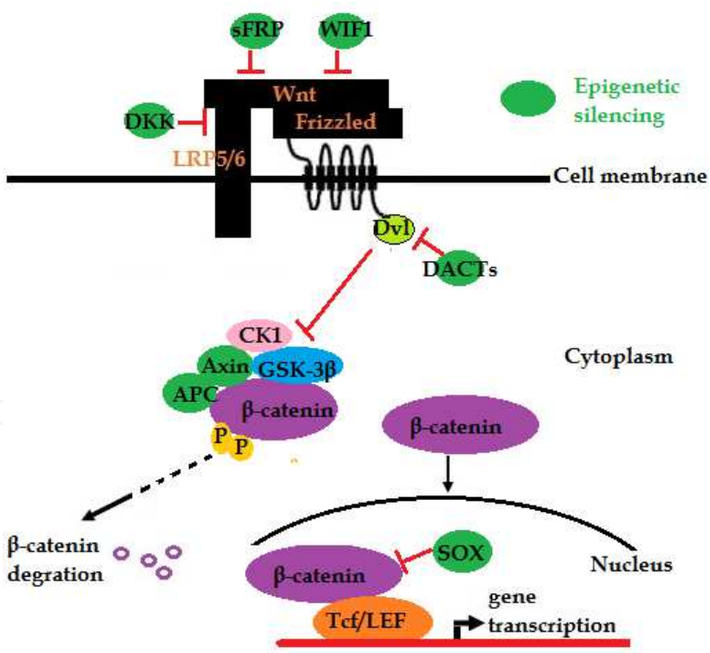


Fig. 2. Several Epigenetic silencing of regulators contribute to the aberrant activation of Wnt/β-catenin signaling in human cancers. Through promoter methylation or histone modification, epigenetic silencing of certain nuclear proteins (SOX) and many antagonists (SFRP, WIF, Dkk, APC and DACT) disrupt individual levels of the Wnt/β-catenin pathway, resulting in constitutive activation of TCF/LEF-β-catenin-dependent transcription of target genes. (Ying & Tao, 2009)

methylation or histone modification of promoters for some inhibitors including Long Groucho/TLEs and Pygopus also affect the activity of canonical Wnt signaling.

2.2.5 Crosstalk among signaling pathways

2.2.5.1 Crosstalk with noncanonical Wnt pathway

There are a number of noncanonical Wnt signaling pathways such as Wnt/PCP, Wnt/Ca^{2+}, Wnt/ROR and Wnt/RYK. Among these pathways, Wnt/PCP and Wnt/Ca^{2+} are well characterized and ligands activating the non-canonical pathways mainly include Wnt4, Wnt5a and Wnt11. The components of canonical Wnt pathway such as Wnts, β-catenin, Fz and Dsh, play roles both in canonical and non-canonical Wnt pathways through distinct mechanisms (Grumolato et al, 2010). The molecular mechanisms underlying the interatction of two Wnt signaling pathways are shown by a model in Fig.3. The canonical and noncanonical Wnts exert reciprocal inhibition at the cell surface by competition for Fzd binding: canonical Wnt3a and noncanonical Wnt5a ligands specifically trigger completely unrelated endogenous coreceptors LRP5/6 and Ror1/2, respectively through a common mechanism that involves their Wnt-dependent coupling to the Frizzled (Fzd) coreceptor and recruitment of shared components, including Dvl, Axin and GSK3.

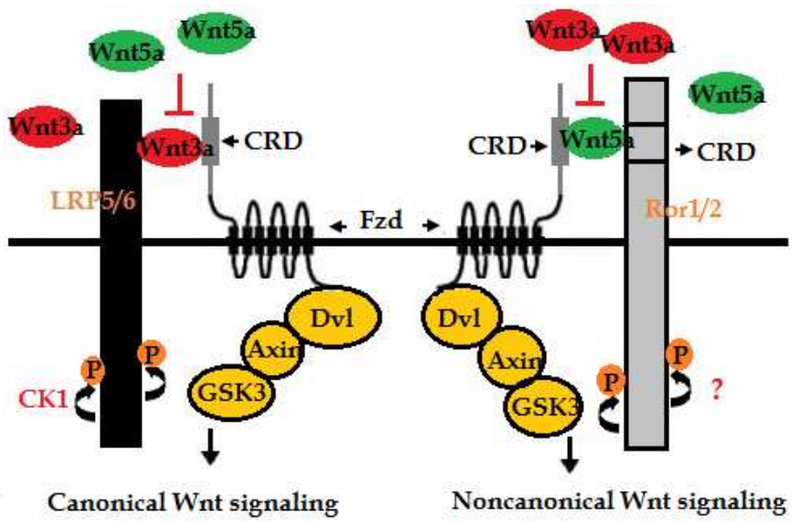


Fig. 3. A model for molecular mechanisms underlying the interatction between canonical and noncanonical Wnt signaling. (Details in Grumolato et al, 2010)

2.2.5.2 TGF-β (Transforming growth factor-β) signaling pathway

TGF-β signaling pathway includes subfamilies of TGF-β, BMPs, Nodal and activin/inhibin . Wnt and BMP pathways cooperate or attenuate each other, thus causing effects that cannot be achieved by either alone in many biological events. The componets of Wnt signaling, including CK, Wise, sFRPs, are associated with their interaction. For example, the molecular mechanisms underlying the interaction between Wnt and BMP signaling are very complex (Itasaki & Hoppler, 2010). First, by mutual regulation of each other's gene expression, activation of the Wnt pathway leads to up- or down-regulation of BMP pathway components, or vice versa. The second mechanism is, extracellular signaling of both

pathways can cause either activation or inhibition of signaling; several secreted molecules, including Wise, sFRPs, CK and Cerberus, can bind to extracellular components of both the BMP and Wnt pathways. Third, the interactions between signal transduction components of the pathways can interfer with or enhance one pathway by signal transduction components of the other pathway; the components include Dvl and Smad1 (or Smad3), GSK3 and Smad1, β-catenin and Smad, Smad7 and Axin. The forth mechanism is the regulation at the promoter or enhancer level.

2.2.5.3 Crosstalk with Notch signaling

Notch signaling pathway possesses four different Notch receptors, including Notch1, 2, 3 and 4. During somite differentiation, the interaction of Wnt and Notch signaling are required for activation of the downstream gene *cMESO1/mesp2*. Notch intracellular domain (Notch ICD or NICD) can directly or indirectly interact with several Wnt components including Dvl, β-catenin, APC, Axin and GSK-3β, thus controlling the activity of Wnt signaling (Andersson et al, 2011). Furthermore, the crosstalk is also seen between sFRPs and Notch signaling. The sFRPs bind to ADAM10, downregulating its activity and thus inhibiting Notch signaling.

2.2.5.4 Crosstalk with FGF signaling pathway

FGFs (22 members) signal enter into the nucleus by binding to FGFR (*fgfr1-4*) and activate multiple signal transduction pathways. There are several models of Wnt-FGF signaling interactions. Canonical Wnt pathway can meidiate the expression of FGF16, FGF18 and FGF20 genes as well as the regulation of SPRY4 gene transcription. Within the 5'-promoter region of human *SPRY4* gene, double TCF/LEF binding sites were identified. FGF signaling also can affect Wnt signaling. For example, the activation of Wnt pathway by FGF-2 is mediated by PI3K/Akt signaling to maintain undifferentiated hESC. In addition, Wnt componets, including β-catenin, GSK-3β, Axin, antagonist of Wise, are associated with the interaction between canonical Wnt and FGF pathways.

2.2.5.5 Crosstalk with TNF (Tumor necrosis factor) signaling pathway

TNF is a cytokine involved in systemic inflammation and is a member of cytokines that stimulate the acute phase reaction. The cooperation of Wnt and TNF signaling pathways play important roles in the regulation of many biological events. Early signals induced by TNF-α via the death domain of TNFR1 are required for the mediation of downstream effects on β-catenin/TCF4 activity and for TNF-α-induced antiadipogenesis. A recent study has indicated TNF-α enhances the Wnt/β-catenin signaling by induction of Msx2 expression. In addition, TNF signaling regulated by Wnt pathway is essential for tooth organogenesis.

2.2.5.6 Crosstalk with Hedgehog (Hh) signaling

The vertebrate Hh family is represented by at least three members: Desert Hh (Dhh), Indian Hh (Ihh) and Sonic Hh (Shh). The Hh pathway is able to interact with canonical Wnt pathway. Hh signaling inhibits the canonical Wnt signaling and proliferation in intestinal epithelial cells meidiated by Hh repressor Gli1. However, different mechanisms are found in the development of spinal cord: dorsal Gli3 expression might be directly regulated by canonical Wnt activity; In turn, Gli3, by acting as a transcriptional repressor (mediated by TCf), restrictes graded Shh/Gli ventral activity to properly pattern the spinal cord.

Although Shh is an inhibitor of the Wnt/β-catenin pathway, activation of Wnt/β-catenin signaling upregulates Shh expression during normal development of fungiform papillae. Thus, the positive and negative feedback loop that coordinates Wnt and Shh pathways is essential for fungiform papillae development. Recent findings indicate that Shh is a downstream target of Wnt signaling and acts as a negative-feedback regulator of Wnt signaling via *Dkk1* and other targets.

2.2.5.7 Crosstalk with retinoic acid (RA) signaling

RA is a lipophilic molecule and a metabolite of vitamin-A (all-trans-retinol). Most of studies have suggested that RA can inhibit canonical Wnt signaling pathway. For example, the RA activity in the perioptic mesenchyme is required for expression of Pitx2 and Dkk2, which affects Wnt/β-catenin signaling during eye development. Retinoic acid can downregulate the expression of Wnt-3a in mouse development and repress the expression of Wnt8a and Wnt3a in the developing trunk. Depending on the presence of RA or not, recent findings suggest a dual activity for RA interaction with Wnt signaling: RARγ regulates Wnt/β-catenin signaling in chondrocytes positively or negatively depending on retinoid ligand availability. RARγ enhances the Wnt/β-catenin signaling under retinoid-free conditions, but inhibits the signaling in RA-treated cells.

3. Roles of canonical Wnt signaling in animal development

Canonical Wnt signaling is a highly conserved pathway involved in a variety of biological processes for animal development and homeostasis. Here, we mainly focus on three aspects: (i) Its roles in the embryogenesis, especially the establishment of Spemann organizer and the patterning of body axes. (ii) Its roles in the regulation of mammalian stem cell fate and somatic cell reprogramming. (iii) Its roles in the development of organs, such as nervous system, cardiovascular system, reproductive system, digestive system and skeletal system.

3.1 Invertebrate development

3.1.1 *Drosophila* development

In the *Drosophila* embryo, *wg* is required for formation of parasegment boundaries and for maintenance of *engrailed* (*en*) expression in adjacent cells (Wodarz & Nusse, 1998). Embryos with mutations in genes *porcupine* (*porc*), *dsh*, *armadillo* (*arm*) and *pangolin* (*pan*), exhibit a very similar phenotype. By contrast, mutations in *zeste-white 3* (*zw3*) demonstrate an opposite phenotype, a naked cuticle.

3.1.2 *C.elegans* development

In the *C. elegans* embryo, molecular analysis of three mutants revealed that they encode proteins similar to *porc* (*mom-1*), *mom-2*, and *frizzled* (fz), a Wnt receptor (*mom-5*). Similar to the *pan* mutant, mutation of *pop-1*, an HMG box protein results in an opposite effect of *mom* mutations: both EMS daughters adopt the E fate and produce exclusively gut (Wodarz & Nusse, 1998).

3.1.3 Cnidarians development

Wnt signaling is also essential for cnidarian embryogenesis (Guder et al, 2006). Recent works have revealed that almost all bilaterian Wnt gene subfamilies (except Wnt9) are

present in cnidarians. An additional Wnt (WntA) is present in cnidarians. Therefore, the hydroid and sea anemone are used to discuss the roles of canonical Wnt signaling in cnidarians development. The important example is that the formation of *Hydra* head organizer is known to be activated by Wnts. HyWnt3a is expressed in a small cluster of ectodermal and endodermal epithelial cells at the apical tip of the hypostome and at the site of the head organizer. HyTcf is expressed in the hypostome, but in a broader domain than Wnt3a and shows a graded distribution highest at the apex. HyDsh and hyGsk-3β are uniformly expressed throughout the polyp at low levels, although hyGsk-3β transcripts are absent in the foot region cells. The hypostome have much higher levels of nuclear β-catenin than cells in the body column, indicating that Wnt signaling is active in the hypostome.

3.2 Vertebrate development

3.2.1 Embryogenesis

The role of Wnt/β-catenin signaling during embryogenesis has been well characterized in *Xenopus,* zebrafish and mouse.

3.2.1.1 The fate of *Xenopus* body axis

In *Xenopus*, the canonical Wnt signaling acting through β-catenin functions both in establishing the dorsoventral axis and in patterning the anterior–posterior axis. During early cleavage, preferential localization of maternal β-catenin to nuclei of cells on the future dorsal side of the embryo establishes the dorso-ventral axis. However, this nuclear localization of β-catenin, which sends a transient dorsal signal to neighboring cells, disappears briefly about the time the blastopore begins to form. While several maternally expressed Wnts (*XWnt-5a*, *XWnt-7b*, *XWnt-8b*, *XWnt-11*) are not required for this dorsal β-catenin signal. The dorsalizing activity of Wnt ligands, however, is lost at or shortly after the midblastula transition (MBT) around 7–8 h of development. Soon afterward, during gastrulation, Wnt signaling is thought to play roles in nervous system patterning and notochord-somite boundary formation, and perhaps in suppressing dorsal axis formation on the ventral side.

The components of canonical Wnt pathway play different roles in the body axis formation of *Xenopus.* In embryos, ectopic expression of Wnts such as Wnt1 or Wnt8a, can induce the formation of a secondary body axis. However, XWnt-3a plays a major role in anterior–posterior patterning of the neuroectoderm and mesoderm. The β-catenin is required for axis formation and enriched dorsally by the two-cell stage in a manner dependent on cortical rotation. XTcf-3 is required for early Wnt signaling to establish the dorsal embryonic axis and closely related Xlef-1 is required for Wnt signaling to pattern the mesoderm after the onset of zygotic transcription. A number of studies have indicated that CKII has a critical role in the establishment of the dorsal embryonic axis. Dvl has been shown recently to be enriched dorsally in one-cell embryos, and ectopic GFP-tagged Dvl is transported along the microtubule array during cortical rotation. The activity of GSK-3 plays dual roles in *Xenopus* axis formation depending on its distribution and association with two GSK-3 binding proteins, GBP and Axin.

3.2.1.2 The development of zebrafish body axes

In zebrafish, maternally Wnt/β-catenin signaling is essential for the formation of organizer (also known as ‘‘shield’’). The zygotic Wnt/β-catenin signaling is activated by Wnt ligands

after MBT to antagonize the organizer and be involved in anterior-posterior patterning of the neural axis. In the zebrafish embryo, β-catenin accumulates specifically in nuclei of dorsal margin blastomeres at as early as the 128-cell stage. This asymmetric nuclear localization of β-catenin is an early marker of the dorsoventral axis. Soon after the MBT, β-catenin activates the expression of a number of zygotic genes, including *bozozok*, *chordin*, *Dkk1*, *squint* (*sqt*) and FGF signals. These β-catenin targets act to inhibit the action of ventralizing factors or, in the case of Sqt, induce mesendodermal fates at the dorsal margin. However, genetic studies in zebrafish have shown that Wnt8 signals are essential for the establishment of ventral and posterior fates. During gastrulation, Wnt8 mRNA and strong activity of a Wnt/β-catenin responsive reporter are evident at the ventrolateral margin. Simultaneous reduction of Wnt3a and Wnt8 activities results in a stronger expansion of dorsoanterior fates, indicating that these two Wnts have overlapping functions. Wnt inhibitors, such as Cerberus, Frzb1 and Dkk1, can function as head inducers. The Wnt antagonist Dkk1 is expressed early in the dorsal margin and dorsal yolk syncytial layer and during gastrulation in the developing prechordal plate, where it could function to counteract the ventralizing and posteriorizing effects of canonical Wnt signaling. Another Wnt antagonist Caveolin-1 (Cav-1) can maternally regulate dorsoventral patterning by limiting nuclear translocation of active β-catenin in zebrafish (Mo et al, 2010).

3.2.1.3 The fate of mouse body axis

Wnt/β-catenin signaling also plays multiple roles in the production and patterning of the mouse primary axes similar to that in frog and fish. The Wnt/β-catenin signaling precedes primitive streak formation and is present in epiblast cells that will go on to form the primitive streak. The N-terminally nonphosphorylated form of β-catenin as well as Wnt/β-catenin signaling is first detectable in the extraembryonic visceral endoderm in day-5.5 embryos. Before the initiation of gastrulation at day 6.0, Wnt/β-catenin signaling is asymmetrically distributed within the epiblast and is localized to a small group of cells adjacent to the embryonic–extraembryonic junction. At day 6.5 and onward, Wnt/β-catenin signaling was detected in the primitive streak and mature node. The expression of Wnt3, high levels of β-catenin and TCF-responsive promoter activity are detected at the site of primitive streak formation in the embryo posterior; conversely, the Wnt inhibitor *Dkk1* was expressed in anterior visceral endoderm. Extensive studies have indicated that *Wnt3* and *β-catenin* knockout mice fail to form the primitive streak, whereas knockout of the Wnt inhibitor Dkk1 results in an anterior truncation. In this way, the role for Wnt in early head-tail development within the mouse embryo could be viewed as similar to that for A-P patterning in frogs (Marikawa, 2006). In the mouse embryo, Axin is a maternal protein present throughout development and *Axin* mutations lead to axis duplication, similar to the effects of ectopic Wnt8 expression.

3.2.2 Embryonic stem cell (ESC)

Canonical Wnt signaling has been implicated in the control of various types of stem cells and may act as a niche factor to maintain stem cells in a self-renewing state. The stem cells contain tissue-specific somatic stem cells, tumor (or cancer) stem cells and ESC (Reya & Clevers, 2005; Nusse, 2008; Valkenburg et al, 2011), and the details of the former two types of stem cells are showed in Section 3.2.3 and 4.1, respectively. Mammalian ESCs provides an excellent model system for studying cell fate determination in early development of mouse

and human. Studies have shown that activation of Wnt/β-catenin signaling in human and mouse ES cells enhance the expression of pluripotency genes and may facilitate the self-renewal of stem cells. For example, in ESCs, overexpression of Wnt1 or stabilized β-catenin or lack of APC results in the inhibition of neural differentiation and in the activation of downstream targets of Wnt signaling, including cyclins and c-myc.

Most of Wnt signaling components are involved in the control of ESC differentiation. For example, ESCs cultured in Wnt3a-conditioned medium undergo mesendoderm differentiation. Under the condition of elevated β-catenin activity, cultured mouse ESCs at high density embark on neural differentiation. Inhibition of GSK-3β transiently enhances the maintenance of ESCs. In addition, in mouse ESCs, loss of Tcf3 function promotes self-renewal in the absence of leukemia inhibitory factor (LIF), but these cells cannot form embryoid bodies. Canonical Wnt/β-catenin signal is reported to regulate nuclear orphan receptor Nr5a2 (also known as liver receptor homologue-1, Lrh-1) expression. β-catenin and Tcf3 are targeted to Nr5a2 and Nr5a2 and in turn directly activate the expression of Tbx3, Nanog, and Oct3⁄4, which are components of the core pluripotency network. Based on these findings, a model has been proposed for the effects of canonical Wnt signaling pathway on the ESCs (Tanaka et al, 2011). Moreover, an elevated level of Wnt signaling activity can promote the maintenance of pluripotency, but the normal level of Wnt⁄ β-catenin signaling has no apparent impact on ESCs.

Reseaches identified Wnt ligands expressed in human ESCs or pluripotent stem cells. For instance, *Wnt3*, *5a* and *10b* mRNAs are expressed in undifferentiated human ES cells; *Wnt5a* and *8b* mRNAs in embryoid body; *Wnt6*, *8b* and *10b* mRNAs in ESC-derived endoderm precursor cells; *Wnt4*, *5a*, *6, 7a*, *7b* and *10a* mRNAs in ES cells-derived neural precursor cells. The expression patterns of Wnt ligands indicate that canonical Wnt signaling plays important roles in maintenance and differentiation of human ESCs. However, it appears that roles of Wnt signaling in the maintenance and differentiation of human and mouse ESCs are controversial. For example, human ESCs cannot be maintained by supplementation of Wnt3a in the absence of feeder cells, raising the possibility that feeder cells produce factors that synergize with Wnt signals to support the self-renewal of ESCs.

3.2.3 Organogenesis and development

Single molecules in canonical Wnt pathway are important regulators in animal development and implicated in tissue homeostasis of adult organisms. In adult animals, there are tissue-specific somatic stem cells (SCs) niches in adults have been found in mesenchymal, hematopoietic, neural, epidermal and gastrointestinal tissues. It is well known that many signaling pathways and their signaling networks, including Wnt, FGF, Notch, Hedgehog, and TGFβ/BMP, are essential for animal development. In this section, we mainly discuss the roles and molecular mechanisms of canonical Wnt pathway in the control of animal organgenesis and in the fate determination of somatic stem cells.

3.2.3.1 Cardiovascular system development

1. Cardiomyocytes differentiation

The Wnt/β-catenin signaling is essential for cardiac differentiation (Cohen et al, 2008). Canonical signaling through Wnt1 and Wnt3a expression in the anterior mesoderm inhibits

the expression of early cardiac genes in the cardiac crescent of chick and frog embryos, including Nkx2.5 and GATA4. However, conditional deletion of β-catenin1 by cytokeratin 19 (Krt19)-Cre results in formation of ectopic heart tissues in the endoderm, suggesting that downregulation of β-catenin activity promotes cardiac differentiation. Although lots of Wnts are expressed during cardiac specification of mouse embryo, but their molecular mechanism remains unclear. It has been shown that a bi-phasic regulation of Wnt/β-catenin signaling is existed in mouse cardiac differentiation: activation of Wnt/β-catenin signaling pathway in the early phase; inhibition of this pathway and activation of the noncanonical pathway in later phases. The biphasic role for this pathway is also found in zebrafish cardiac differentiation. However, Wnt11, a non-canonical Wnt signaling ligand that can activate the caspase 3⁄8 to degrade β-catenin and thereby inhibits canonical Wnt signaling, is required at later stages of cardiac differentiation. These data suggest that Wnt/β-catenin signaling mainly plays a suppressive role in the cardiogenesis.

2. Vascular development and remodeling

Wnt/β-catenin signaling has been shown to play important roles in vascular development and remodeling (Tian et al, 2010; van de Schans et al, 2008). Loss of β-catenin also leads to defective endocardial cushion/cardiac valve development through defective endothelial-mesenchymal transformation. Several Wnt ligands, such as Wnt2a, Fzd5, have been implicated in regulating EC development and associated with abnormal placental vascular development. Inhibition of canonical Wnt signaling often results in decreased vascular smooth muscle cells (VSMCs) proliferation and *cyclin D1* expression.

3.2.3.2 Hematopoietic system development

1. Hematopoiesis

Hematopoietic stem cells (HSCs) have the ability to generate all lineages of blood cells, including red blood cells, platelets, lymphocytes, monocytes, and macrophages. Wnt2b is a key factor for hematopoietic stem or progenitor cells. Purified Wnt3a proteins promote the self-renewal of HSCs derived from Bcl2-transgenic mice. Therefore, the self-renewal of HSCs is likely promoted by the canonical Wnt signaling activation and Wnt signals can provide signals for HSC fate determination in the stem cell niche (Staal & Luis, 2010). However, some studies have shown that constitutive activation of β-catenin impairs multilineage differentiation and causes exhaustion of the HSC pool. The controversial results may be resulted from: (i) different levels of Wnt/β-catenin signaling activation; (ii) dosage responses of Wnt signaling required; (iii) the interference by other signals in the context of Wnt activation; and (iiii) Wnt proteins in various blood cell types.

2. Lymphopoiesis

The canonical Wnt signaling plays a crucial role in Lymphopoiesis, such as T cell and B cell development. The canonical Wnt signaling is aasociated with most immature stages of T cell development. Overexpression of cell autonomous inhibitors of β-catenin and Tcf (ICAT) blocks the development of earliest stage T cells in the thymus. Similarly, the secreted Wnt inhibitor DKK1 can inhibit the thymocyte differentiation at the most immature stages. On the contrary, overexpressing activated forms of β-catenin leads to the generation of more thymocytes and activates proliferation-associated genes in immature thymocytes. The canonical Wnt signaling can regulate B cell development. *Lef1*-deficient mice have a mild

block of B lymphopoiesis in fetal but not in adult, and show defects in B cell proliferation. Depletion of Fz9 leds to pronounced splenomegaly, thymic atrophy, and lymphadenopathy with age, with accumulation of plasma cells in lymph nodes during mouse development. In addition, treatment of human B cell progenitors with Wnt3a in the stromal cell co-culture assays negatively regulates the cell proliferation.

3.2.3.3 Nervous system development

1. Neural crest stem cells (NCSCs)

Wnt/β-catenin can induce sensory neurogenesis by acting instructively on embryonic neural crest stem cell (NCSCs) (Toledo et al, 2008). In the central nervous system (CNS), the activation of β-catenin leads to the amplification of the neural progenitor pool. Constitutive expression of β-catenin in neural stem/progenitor cells results in the expansion of the entire neural tube. In addition, Wnt ligands, such as Wnt3a, promote the differentiation of neural SCs in the neocortex at E11.5 at the expense of neural SC expansion. Continuous neurogenesis in adult happens only in two specialized niches of the adult CNS, subventricular zone (SVZ) and subgranular zone (SGZ).

2. Neural crest formation

Canonical Wnt signaling has been found in early stages of neural crest development, such as neural crest induction and melanocyte formation. For example, ablation of β-catenin results in a decrease of tissue mass in the spinal cord and brain, and the neuronal precursor population, and a lack of melanocytes and sensory neural cells in dorsal root ganglia.

3. Neuronal differentiation

Numerous components of canonical Wnt signaling have been shown to regulate the precise patternings of developmenting neural tissue. Wnt1 acts as a mid-hindbrain organizer and the ablation of Wnt-1 causes severe deficiencies during mid–hindbrain formation in mice; Wnt-3, -3a, -7b and -8b, can participate in the development of the forebrain (gives rise to hippocampus). The functions of Wnts in neuronal differentiation depend on signals with temp-spatial distribution, triggering the differentiation of precursor cells to neurons.

4. The development of dopaminergic (DA) neurons

DA precursors can respond to canonical Wnts. For example, before the appearance of DA neurons, the expression of Wnt-1 and Wnt-3a is detected in the developing ventral midbrain (VM). In addition, the GSK-3β-specific inhibitor kenpaullone increases the DA differentiation through stabilizing β-catenin in ventral mesencephalic precursors. There are 13 Wnt ligands, all 10 Fzds receptors, and several intracellular Wnt signaling modulators are identified to developmentally regulate the development of midbrain and the DA precursors respond to Wnts in a very specific/temporal manner.

5. Synapses

The function of Wnt signaling pathway in synapses has been characterized during neuronal development. Wnts play roles in the formation of the sensory–motor connections in mouse spinal cord. Wnt-3 and 7a can promote synaptogenesis inducing the clustering of synapsin I, a presynaptic protein involved in synapse formation and function. This effect is controlled by the canonical pathway and can be mimicked by the GSK-3β inhibition induced by

lithium. Interestingly, the Dvl-1 is found to present in synaptosomes of adult mice and it also co-localized with the presynaptic markers synaptophysin, bassoon and VAMP-2.

6. Sympathetic nervous system development

A recent study has shown that Fz3 acts at early developmental stages to maintain a pool of dividing sympathetic precursors, likely via activation of β-catenin, and Fz3 functions at later stages to promote innervation of final peripheral targets by post-mitotic sympathetic neurons.

7. Canonical Wnt signaling in brain

It is known that the expression of specific Wnt ligands occurs in distinct regions of developing human brain (Malaterre et al, 2007). The major role of Wnt1 is to regulate the proliferation of precursor populations in the developing mid-/hindbrain region; Wnt7b is expressed in cerebral cortical and diencephalic progenitor cells during early human development; Wnt3a appears to have a very specific role in the development of the hippocampus, a structure involved in integrating many of the higher order tasks, such as memory and learning. β-catenin in the E9.5 telencephalon is highly enriched at the apical end of the neural precursor cells and colocalized with N-cadherin at adherens junctions, implying that the main role of β-catenin at this stage of telencephalic specification is to promote neuroepithelial adhesion. Other canonical Wnt pathway-related factors are also implicated in a number of aspects of brain development. GSK-3β and β-catenin transcriptional partners LEF1 and TCF4 are expressed during brain development in mouse. These results suggested that brain developent respond to canonica Wnt signaling pathway in a very specific and temporal manner.

3.2.3.4 Reproductive system development

1. Gonadal development

Many Wnt genes are expressed in gonads. Wnt1, 3 and 7a are specifically expressed in the testis; Wnt5a, Wnt6 and Wnt9a are specifically expressed in the ovary; Wnt4 is expressed in mouse gonads in both sexes at embryonic day 9.5 (E9.5) and becomes ovary-specific at the time of sex determination around E11.5. These expression patterns of multiple Wnt genes in the gonads suggest that canonical Wnt signaling pathway is essential for gonadal development. Activation of Wnt/β-catenin signaling is required for female differentiation (Liu et al, 2009). Although β-catenin is present in gonads of both sexes, it is necessary for ovarian differentiation but dispensable for testis development. Lacking β-catenin, defects in ovaries are strikingly similar to those found in the R-spondin1 (Rspo1) and Wnt4 knockout mouse ovaries, including formation of testis-specific coelomic vessel, appearance of androgen-producing adrenal-like cells and loss of female germ cells. Studie have found that activation of β-catenin in otherwise normal XY mice effectively disrupts the male program and results in male-to-female sex-reversal.

The expression of sex determining gene Sry (sex-determining region Y) within the initially bipotential gonad is sufficient to induce the male developmental program. Both human *SRY* and mouse *Sry* are capable of repressing the Rspo1/Wnt/β-catenin signaling, thereby switching on testis determination. Interestingly, the HMG box of human SRY can bind directly to β-catenin while the mouse Sry binds to β-catenin *via* its HMG box and glutamine-rich domain.

2. Mammary stem cells and Mammary gland development

Several lines of evidence suggest that canonical Wnt signaling is involved in the maintenance of the stem/progenitor pool in the mammary gland. The stem/progenitor fraction is increased in the hyperplastic mammary glands of MMTV–Wnt-1 and MMTV–ΔNβ-catenin transgenic mice and in primary cultures of mammary epithelial cells treated with Wnt-3a. In addition, Lrp5 is expressed in the basal epithelium and cells with high expression have a 200-fold greater ability to regenerate a mammary tree when transplanted into cleared mammary fatpads. Embryos overexpressing the Wnt antagonist Dkk1, as well as animals deficient for Lrp6 or Lef-1, fail to form mammary placodes. These findings validate the importance of Wnt/β-catenin in mediating the activity of mammary stem cells.

The canonical Wnt signaling is essential for specification and morphogenesis of the mammary gland. Numerous components of the Wnt signaling cascade are expressed during embryonic mammary morphogenesis, including Wnt ligands (*i.e.*, Wnt1, 2, 3, 3a, 5a, 5b, 6, 7b, 10a, 10b, 11), receptors (*i.e.*, Fzd1-9, LRP5, LRP6), and downstream DNA-binding proteins (*i.e.*, Tcf1, 3, and 4 and Lef1). Wnt2, Wnt5a and Wnt7b are enriched in the terminal end bud microenvironment. Targeting other positive acting elements of the Wnt pathway, such as Lrp6, Lrp5, Lef1 and Pygo2, can result in placodal impairments, ranging from loss to reduced size and degeneration, while stimulating β-catenin signaling produces the converse effect – acceleration, expansion and induction of placodes and placodal markers. During mammary development, Wnt5a is considered to negatively regulate the Wnt/β-catenin pathway. Constitutive expression of the canonical Wnt4 leads to more highly branched ducts in virgin females, similar to what occurs during early pregnancy.

3. Prostate stem cells (PSCs) and Prostate gland development

Evidence of canonical Wnt signaling involvement in prostate stem cells is based on limited studies. A few findings suggest that Wnt signaling regulates the terminal differentiation of basal cells into luminal cells by controlling the proliferation and/or maintenance of epithelial progenitor cells. For example, more p63 (basal cell marker) positivity is seen in the ductal region of Wnt3a-treated cultures while fewer p63 positive cells are present in Dkk1-treated cultures. These results are supported by the CK8 (luminal cell marker) immunostaining. The expression of many Wnt signaling molecules such as Fzd6 and Wnt2 is increased in both fetal and adult PSC. This result means that adult PSCs acquire characteristics of self-renewing primitive fetal prostate stem cells, which in turn might also be characteristic of oncogenesis.

The canonical Wnt pathway has been implicated in prostate development (Kharaishvili et al, 2011). Wnt antagonist sFRP2 is highly expressed early in prostate development and down-regulated at later time points. It is indicated that both enhancement and reduction of canonical Wnt signaling can adversely affect branching morphogenesis in the developing rat prostate model. Treated with Wnt3a, rat ventral prostate cultures at postnatal day 2 (P2) show blunted and enlarged ductal tips at 7th day, while Dkk-treated prostates exhibit poor epithelial branching. The highest level of Axin2 is detected on P2, consistent with a higher progenitor cell population; while the level declines over time according to prostate maturation when the majority of the epithelial cells are terminally differentiated luminal cells. Other Wnts and components including three canonical Wnts (Wnt2, Wnt2b and Wnt7b), Fzd2 and 4 and Dvl are also highly expressed on P3 in ventral lobes. Except for

Wnt7b, all of them show a high expression at birth with levels declining during and after the completion of morphogenesis. Those results suggest that the canonical Wnt signaling is temporally regulated during prostate development.

3.2.3.5 Skeletal development

1. Mesenchymal stem cells (MSCs)

The MSCs can differentiate into mesoderm-derived chondrocytes, osteocytes, adipocytes, fibroblasts, myocytes as well as nonmesoderm-derived hepatocytes, and neurons. Canonical Wnt signals are required for maintenance of undifferentiated MSCs, inhibition of adipocyte maturation, dedifferentiation of adipocytes, and inhibition of osteoblastic differentiation (Ling et al, 2009). MSCs express a number of Wnt ligands including Wnt2, Wnt4, Wnt5a, Wnt11 and Wnt16, and several Wnt receptors including FZD2, 3, 4, 5 and 6, as well as various coreceptors and Wnt inhibitors. Exogenous application of Wnt3a to cell cultures expands the multipotential population of MSCs by up-regulation of cyclin D1 and c-Myc. Moreover, the overexpression of LRP5 can increase proliferation of MSCs. Dkk1 is required for the arrested hMSC to re-enter into cell cycle and subsequent proliferation. Interestingly, studies have revealed that canonical Wnt signaling stimulates hMSC proliferation at low dose while inhibits it at high dose. This dual effect of Wnt signaling suggests the intensity of Wnt signals can lead to different or even opposite biological functions.

2. Cartilage development

Cartilage development is initiated by chondrogenesis, which requires mesenchymal condensation and cartilage nodule formation. A variety of different Wnt signaling components positively or negatively regulate different stages of chondrogenesis and cartilage development (Chun et al, 2008). Chondrogenesis is inhibited by Wnt-3a via a β-catenin-dependent mechanism; Wnt-1 and -7a also inhibit chondrogenesis without significant effects on early condensation. Chondrocyte maturation and mineralization are also blocked or delayed by the forced expression of FrzB, Fzd-1, or Fzd-7. In contrast to the inhibition of chondrocyte maturation, constitutively active form of β-catenin promotes growth plate chondrocyte terminal differentiation and overexpression of Wnt-8c and -9a in chick sternal chondrocytes enhance hypertrophic maturation by upregulating type X collagen and Runx2.

3. Osteoblastogenesis and bone formation

Canonical Wnt signaling plays an important role in osteoblastogenesis and bone formation. Wnt activity in bone marrow varies throughout stages of development and has important contributions from several Wnts. Wnt7b is induced during osteoblastogenesis; Wnt10b is expressed in bone marrow; Wnt1, 4, and 14 are expressed in calvarial tissue and osteoblast cultures; Wnt1 and Wnt3a are induced by BMP2 in a mesenchymal precursor cell line. Wnt/β-catenin signaling promotes the bone formation via stimulation of the development of osteoblasts. Inhibition of GSK3 enzymatic activity with lithium chloride (LiCl) or small molecules (e.g. Chir99021 and LY603281-31-8) stimulates mesenchymal precursors to differentiate into osteoblasts. This result is supported by observations with Wnt3a, Wnt1 and Wnt10b, which activate signaling through β-catenin and stimulate osteoblastogenesis. However, Dkk1 can reduce osteoblastogenesis by inhibiting the activity of this pathway. Studies suggest that activation of Wnt/β-catenin signaling inhibits adipogenesis of

mesenchymal precursors, which may have clinical importance due to the positive correlation reported between marrow adipose content and bone fractures.

3.2.3.6 Eye development

The canonical Wnt signaling pathway has been shown to be required at multiple points in development of the eye, from specification of the eye field to differentiation of the retina and determination of retinal polarity (de Iongh et al, 2006). Here, we mainly discuss the important roles in the eye development in vertebrates.

1. Eye specification

Most of the studies on eye specification have been carried out in *Xenopus* and zebrafish embryos. During zebrafish mid-late gastrulation, canonical Wnts (Wnt1, 8b, 10b and 11) and Fzds (Fzd3, 5, 8a) are detected in the anterior neural plate (ANP). Wnt1, 8b and 10b are expressed in domains caudal to the eye field (delineated by *Rx3*, eye marker). Fzd8a expression domain overlaps the expression of several anterior neural ectoderm markers including Six3, which is expressed in the presumptive eye fields. Fzd5 expression domain appears to completely overlap the eye field delineated by Rx3. Zebrafish mutants (*masterblind* and *headless*) with mutations of *axin* and *tcf3* exhibit defects in eye formation. In addition, inhibition of GSK-3β results in eye reduction or loss. Dkk1 induces complete heads with two-well formed eyes in larger eyes. Overexpression of Wnt8b or treatment with LiCl to activate the canonical Wnt signaling, results in the loss of anterior structures (including eyes) and the loss of *six3* and *rx1* expression. These results indicate that canonical Wnt signaling inhibits eye formation. Recent inverstigations suggest that activation of Dkk2 by PITX2 can locally suppress the canonical Wnt signaling activity in eye development.

2. Lens development

Recent studies have documented the involvement of various components of the Wnt signaling pathway in lens morphogenesis and differentiation. Expression of various Wnts (Wnt2b, 7a, 7b, 8a, and 8b), Fzd, Dkk and Lef/Tcf, has been identified in the developing eye of various species. Expression of these Wnt components is restricted to the lens epithelium and down-regulated as cells exit the cell cycle and initiate differentiation into lens fiber cells. However, Wnt7b continues to be expressed in the cortical fibers of the lens undergoing terminal differentiation. The expression pattern of Wnt components in the lens placode and effects of deleting β-catenin in the ocular ectoderm indicate that Wnt signals play an important role during lens induction and early morphogenesis. Analysis of the *Tcf/Lef-LacZ* mice shows that there is transient activation of Wnt signaling in the anterior lens epithelium between E13.5 and E14.5 after closure of the lens vesicle. Several lines of evidence indicate that Wnt signals also play key roles in the differentiation of the lens fibers. The active (non-phosphorylated) form of β-catenin and inactivated GSK-3β can be found in lens fiber as well as epithelial cells.

3. Retinal development

Wnt1, -3, -5a, -5b, -7b and -13 (Wnt2b) are found in embryonic and fetal retinae, and Wnt5a, -5b, -10a and –13 in the adult retinal. In the embryonic mouse retina at E12.5, Wnt receptors Fzd3, Fzd4, Fzd6 and Fzd7 are expressed throughout the optic cup and Fzd4 is detected in the RPE (optic cup, optic stalk). The expression patterns of *sfrps* are variable during early

morphogenesis. The dynamic expression patterns of Wnt, Fzd and Sfrp genes in the developing retina suggest the involvement of canonical Wnt signaling pathway.

3.2.3.7 Liver development

Canonical Wnt pathway also is essential for liver development (Nejak-Bowen & Monga, 2008). 15 Wnts and 9 Fzs are identified in an adult mouse liver, and the Wnts expressed in various cell types of liver (Table.2). These findings suggest that distinct Wnt signals in various cell types might be associated with their different functions.

Cell type of mouse liver	Wnt ligands
Hepatocytes	1, 2, 4, 5a, 5b, 9a, 9b, 11
Biliary epithelial cells	2, 2b, 3, 4, 5a, 5b, 8b, 9a, 9b, 10a, 10b, 11
Sinusoidal endothelial cells	2, 2b, 3, 4, 5a, 5b, 8b, 9a, 9b, 10a, 11
Stellate and Kuffer cells	2, 2b, 3, 4, 5a, 5b, 6, 7a, 8b, 9a, 9b, 10a, 10b,11, 16

Table 2. Wnt genes expressed in various cell types within liver (Thompson & Monga, 2007).

In hepatocytes, Active β-catenin is detectable immediately prior to gastrulation at E5.5 in the extra- embryonic visceral endoderm and in a narrow region of cells in the epiblast at E6. Two associations with β-catenin are important in hepatocytes: One association is the connection between β-catenin and E-cadherin at the hepatocyte membrane and the other association is that of β-catenin with the hepatocyte growth factor (HGF) and receptor c-Met.

Several lines of evidence have identified that the regulation of Wnt/β-catenin signaling is a requirement for postnatal liver development. It is well known that liver derivation from the foregut endoderm occurs around somite stages 5 to 6 as a result of signaling from mesoderm in the form of FGFs and BMP4, both of which are incidentally downstream targets of the Wnt pathway. Wnt ligands such as Wnt2b, is expressed at these stages and positively regulates the induction and specification of zebrafish liver. In mouse, temporal expression of β-catenin during mouse prenatal liver development indicates its important role in hepatocyte expansion during early liver development (Lade & Monga, 2011). sFRP5 is expressed in the ventral foregut endoderm that gives rise to the liver at mouse E8.5 and can modulate Wnt activity by delineating borders between organs in the developing gut.

Canonical Wnt signaling is also important in normal liver growth and regeneration. The expression level of β-catenin is increased during postnatal development and can promote hepatic growth in mouse. In adult resting liver, the Wnt/β-catenin pathway is quiescent. When liver is not being challenged by chemical, metabolic or dietary stress, β-catenin is not required for normal physiologic function. However, if liver is injured, proliferation of the normally quiescent hepatocytes and cholangiocytes, followed by proliferation of the hepatic stellate cells and endothelial cells, quickly restores the liver to its original mass. During this regeneration process, levels of β-catenin are dramatically increased in the partial hepatectomy (PHx) model.

3.2.3.8 Kidney development

A number of Wnt family members are expressed in the mouse embryonic kidney (Merkel et al, 2007; Pulkkinen et al, 2008): Wnt-2b, -4, -5b, -6, -7b, -9b and -11 are expressed during kidney ontogeny; Wnt-6, -7b, -9b and 11 are expressed in the branching ureteric bud (UB)

during the early stages of organogenesis; while Wnt-2b and -4 are detected in the kidney mesenchymal cells. In wild type mice, UB-produced Wnt9b is necessary for tubule formation, at least in part through its activation of Wnt4 expression in the adjacent mesenchyme. However, the precise mechanism for Wnt function in tubule formation remains to be defined.

3.2.3.9 Other organgenesis

1. Epithelial stem cells

Epithelial stem cells present in many tissues, including skin, intestine, lung, kidney, and so on. The canonical Wnt signaling pathway is shown to play important roles in two leading epithelial stem cell models (Gu et al, 2010): the intestine and hair follicle. The canonical Wnt signaling is required for the normal homeostasis of epithelial stem cells. Depletion of TCF4 or overexpression of Wnt inhibitor Dkk1 in intestinal stem cells (ISCs) results in a dramatic reduction in proliferation of crypt cells. Inhibition of the Wnt pathway by conditional ablation of β-catenin or by ectopic expression of Dkk1 specifically in epithelia, blocks hair follicle formation during embryogenesis and causes a loss of the postnatal HFSC niche. Conversely, constitutive activation of Wnt pathway results in massive proliferation of epithelial stem/progenitor cells.

2. Lung development

Canonical Wnt signaling is known to regulate epithelial and mesenchymal cell biology in an autocrine and paracrine fashion (Pongracz & Stockley, 2006). Several Wnt ligands, receptors, and components of the canonical pathway are expressed in a highly cell-specific fashion in the developing lung. For instance, Wnt2 is highly expressed in the distal mesenchyme, whereas Wnt7b is expressed predominantly in the epithelium. However, transgenic deletion of Wnt2 does not result in any detectable defects of lung development and function, probably due to functional redundancy of Wnt2 proteins. β-catenin-dependent signaling is central to the formation of the peripheral airways of the lungs and responsible for conducting gas exchange, but is dispensable for the formation of the proximal airways. Apart from β-catenin and Wnts, mRNA of Fz-1, -2 and -7 and several intracellular signaling molecules including Tcf-1, -3, -4, Lef1, and secreted Fz related proteins (sFRP-1, -2 and -4) have been found to be expressed in the developing lung in specific and spatio-temporal patterns. The canonical Wnt signaling appears to be able to fulfill their roles in maintenance of adult lung structure: the components of canonical Wnt pathway such as Wnt-3, -4, -5a, -7a, -7b, -10b, and -11 as well as Fz-3, -6 and -7, Dvl, and Dkk are expressed in primary lung tissue and cell lines derived from adult lung tissue.

3. Intestinal development

Intestinal crypts constitute a niche in which epithelial progenitors replicate and prepare to differentiate in response to Wnt signals. After appearance of villi, canonical Wnt signaling was first detected. However, intervillus cells lacking signs of canonical Wnt signaling proliferate actively during villus morphogenesis. In late gestation and briefly thereafter, conspicuous Wnt activity is evident in differentiated, postmitotic villus epithelium. Further investigations indicate that neither Tcf4 nor candidate Wnt targets CD44 and cyclinD1 are expressed in late fetal villus cells with a high Wnt activity. Instead, these cells express the related factor Tcf3 and a different Wnt target, c-Myc. Premature and

downregulated β-catenin activity can cause severe villus dysmorphogenesis in transgenic mice. Lrp5 and Lrp6 are recently found to play redundant roles in intestinal epithelium development and might regulate intestinal stem/precursor cell maintenance by regulating the canonical Wnt signaling.

4. Pancreatic development

Previous studies have demonstrated the importance of canonical Wnt signaling in pancreatic development (Wells et al, 2007). The expression of Wnt1 under control of the pdx-1 promoter is associated with murine pancreatic agenesis. Other components of Wnt pathway are detectable during pancreatic organogenesis, including Wnt2, 2b, 3, 4, 5a, 5b, 7a, 7b, 14 and 15. All of ten Fzs proteins are found to express in pancreas, with the strongest expression of Fz1, 2, 4, 5, and 6 and colocalized expression of Frz 1-7 in the islets of Langerhans. Dkk 1, 3, and 4 as well as sFRP 1, 4, and 5 are expressed in the exocrine fraction, while sFRP 2 and 3 are detectable at low levels. The effects of β-catenin on mouse pancreatic development show somewhat conflicting findings (Murtaugh, 2008). The loss of β-catenin/Wnt signaling in the developing mouse results in transient pancreatitis, but exocrine pancreas has eventually recovered. In addition, a significant role of the Wnt pathway in endocrine lineage development using β-catenin knockout mice is identified. However, other studies indicate that β-catenin/Wnt signaling is essential for development of exocrine pancreas, but plays no role in endocrine development. Therefore, mechanisms underlying the regulation of pancreatic development by canonical Wnt signaling require further investigations.

5. Hair follicle and skin development

The hair follicle is an appendant miniorgan of skin. Canonical Wnt signals play an important role in hair follicle development. β-catenin inhibition or Dkk1 overexpression specifically blocks hair follicle formation during embryogenesis, and the former results in a loss of the postnatal hair follicle stem cells (HFSC) niche. Wnt10b may promote hair-follicle growth by inducing the switch from telogen to anagen. Several Wnts such as Wnt4, 10a and 10b, are expressed in the skin. Alteration of the levels and timing of LEF-1 expression during skin embryogenesis in transgenic mice disrupts the positioning and orientation of hair follicles, confirming a central role for LEF-1 in hair patterning and morphogenesis. Wnt3 or Dvl2 overexpression in transgenic mouse skin causes a short-hair phenotype owing to altered differentiation of hair shaft precursor cells and hair shaft structural defects.

3.3 Reprogramming

Reprogramming of nuclei allows the dedifferentiation of differentiated cells. Cell-cell fusion is a way to force the fate of a cell, and in the case of fusion with ESCs, this mechanism induces cellular reprogramming, that is, dedifferentiation of somatic cells. For example, ESCs treated for 24 hours with Wnt3a or with the GSK3 inhibitor, 6-bromoindirubin-30-oxime (BIO), can reprogram somatic cells after polyethylene glycol (PEG)-mediated fusion. Recent studies demonstrate that fusion-mediated reprograming of a somatic cell is greatly enhanced by dose-dependent activation of the Wnt/β-catenin signaling pathway. ESCs expressing whatever amount of β-catenin can fuse, but normally the fate of the resulting hybrids is to undergo apoptosis, unless low levels of nuclear β-catenin allow them to undergo reprogramming instead (Lluis et al, 2010). Further studies using genetic knockout

ESC models suggest that the canonical Wnt signaling pathway play important roles in reprogramming. It is known that the maintainance of mouse ESC (mESC) self-renewal requires the growth factor leukemia inhibitory factor (LIF), which stimulates two parallel pathways: Stat3/Klf4/Sox2 and PI3K/Tbx3/Nanog. In mouse ESCs, β-catenin promotes pluripotency gene expression, including Oct4, Nanog and Tbx3, depending on the regulation of Lrh-1.

4. Canonical Wnt signaling in human diseases

Abnormal expression of components in canonical Wnt signaling pathway is often associated with human diseases including almost of all human cancers (Fig.4). In addition to β-catenin, APC, GSK-3β, and Caveolin-1 are considered as key molecules in oncogenic cellular transformation, hyperplasia and metastasis owing their abilities to modulate many signaling pathways in tumor cells. Some components of this pathway such as β-catenin and Caveolin-1 are also nvolved in tumor multi-drug resistance (MDR). Additionally, the abnormal activity of canonical Wnt signaling has been shown to function in the development and progression of cardiovascular diseases, fibrosis, regeneration, wound healing, obesity, schizophrenia, osteoarthritis (OA) and diabetes.

4.1 The activity of canonical Wnt pathway in cancers and therapy

4.1.1 Tumorigenesis and cancer stem cells (CSCs)

Genetic predisposition, environmental factor, and aging are risk factors of human cancers. Dysregulation of canonical Wnt signaling always results in development of various tumors (Fig.4). Down-regulated canonical Wnt signaling inhibitors caused by epigenetic silencing and genetic alteration often cause the carcinogenesis, such as colon cancer, prostate cancer and Esophageal Squamous Cell Carcinoma.

Cancer stem cells (CSCs) are closely associated with tumorgenesis. CSCs are characterized by their tumorigenic properties, the ability to self-renew and formation of differentiated progeny. Similar to normal stem cells, CSCs express some specific surface markers (CD133, CD44 and others). CSCs contain many of active signaling pathways that are found in normal stem cells, such as Wnt, Notch, and Hedgehog (Hh). A number of studies have demonstrated that the Wnt/β-catenin pathway is crucial in the maintenance of CSCs from lung, leukemia, breast, melanoma, colon, liver and cutaneous cancers.

4.1.1.1 Colorectal cancer and intestinal cancer stem cells (ICSCs)

1. Colorectal cancer (CRC)

The majority of CRC is caused by mutations in key components of the canonical Wnt signaling pathway. In colon cancer, nearly 90% of these tumors harbor mutations that result in β-catenin mutation. Germline loss-of-function mutations in the APC gene were originally identified to be associated with familial adenomatous polyposis (FAP), about 1% of which progress to CRC. Furthermore, 85% of cases of sporadic intestinal neoplasia have mutations in APC, while activating mutations in β-catenin are found in approximately 50% of CRC tumors lacking APC mutations. Recently, investigatons suggest that β-catenin stabilization and C-terminal binding protein 1 (CtBP1) following APC inactivation contribute to

adenoma initiation as the first step, and that KRAS activation and β-catenin nuclear localization act synergistically to promote adenoma progression to carcinoma. The promoter of sFRPs is often hypermethylated in CRCs, suggesting that reintroduction of sFRP can reverse the Wnt signaling in CRCs. Moreover, when DACT3 (a member of the Dpr/Frodo family) expression is restored by the inhibition of histone methylation and deacetylation, the Wnt signaling is ihhibited and CRC cell apoptosis is induced.

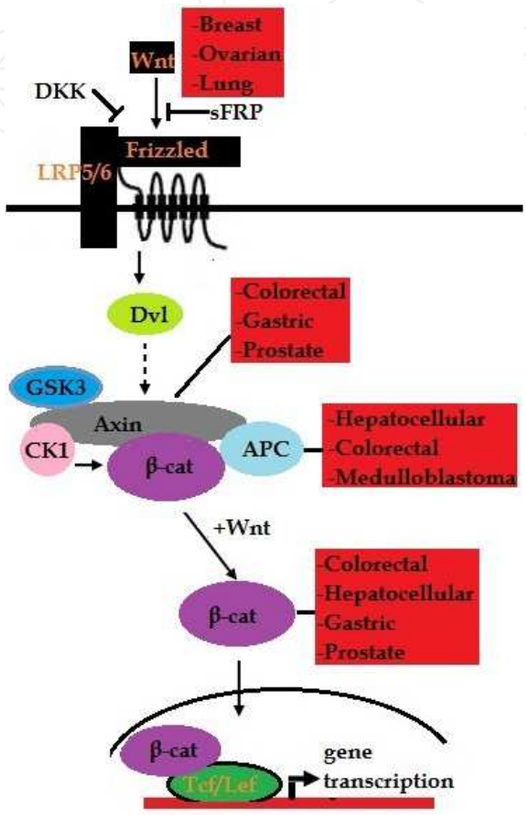


Fig. 4. Canonical Wnt signaling and dysregulation in cancers.The Wnt signaling pathway is comprised of extracellular, cytoplasmic and nuclear signaling events that are amenable to therapeutic intervention. Dysregulation at these stages are common in numerous cancers, captured in the white boxes. (Curtin & Lorenzi, 2010)

2. Intestinal cancer stem cells (ICSCs)

Extensive researches have been performed to find out which cell type is required for the cancer-initiating mutation. A critical work has shown that APC inactivation in Lgr5-positive stem cells at the crypt bottom leads to transformation within days. In contrast, APC inactivation in progenitors or differentiated cells does not cause tumor formation even after 30 weeks. These studies indicate that the cellular origin of CRC initiation might be within normal stem cells of the intestine, rather than progenitors or differentiated cells. Another study has demonstrated that severe polyposis in Apc loss-of-function mutant (Apc1322T) mice is associated with increased expression of the stem cell marker Lgr5 and other stem cell markers (Musashi1, Bmi1, and the Wnt target CD44). Furthermore, the Wnt target gene CD44 has been identified as a marker for colorectal cancer stem cells, and deletion of CD44

in APCMin/+ (heterozygous APC) mice attenuates intestinal tumorigenesis. Overall, these studies support a cancer stem cell model that Wnt signaling plays a key role in the regression of intestinal tumorigenesis.

4.1.1.2 Breast cancer and cancer stem cells (BCSCs)

1. Breast cancer

Studies in both mouse models and human breast cancers have revealed that canonical Wnt signaling is critical to mammary tumorigenesis. The mouse mammary tumor virus (MMTV) is found to integrate into the *Int-1* (*Wnt1*) locus and overexpression of Wnt1 induces mammary tumorigenesis. In human breast cancer, numerous reports have identified that the canonical Wnt pathway is dysregulated. Aberrant β-catenin expression is associated with basal and triple-negative breast cancers and poor clinical outcome. In addition, overexpression of Lrp5 correlates with basal breast cancers. Down-regulation of the sFRPs is observed in breast cancers. Although strong evidence has shown the dysregulation of Wnt pathway in human breast cancer, there are conflicting studies that fail to find an association of this pathway with metastasis or clinical outcome.

2. Breast cancer stem cells (BCSCs)

Studies in breast cancer demonstrate that stem cell populations are more resistant to radiation treatment and Wnt/β-catenin signaling mediates the resistance. These CSCs populations exhibit altered DNA repair in response to radiation and increased AKT (a serine-threonine protein kinase) and β-catenin activities. Blocking the AKT and β-catenin activation by inhibitor perifosine sensitizes the cells to radiation. These studies have underscored the importance of Wnt signaling in breast cancer and the targets for effective therapeutics.

4.1.1.3 Canonical Wnt signaling in prostate cancer and cancer stem cells (PCSCs)

1. Prostate cancer

Canonical Wnt pathway has been widely studied in prostate cancer (Kharaishvili et al, 2011). Wnt ligands are up-regulated in prostate cancer, and their expression often correlates with aggressiveness and metastasis. It is shown that elevated levels of Wnt1, Wnt5a, Wnt7b, and Wnt11 are closely associated with prostate cancer aggressiveness. In addition, Dkk1 expression increases during prostate cancer initiation but decreases during metastasis. Other Wnt pathway members such as Fz4 and Wif1 are found to be dysregulated in prostate cancer.Furthermore, in many cases of prostate cancer, APC is mutated and hypermethylated to a silent form and β-catenin is frequently mutated to an active form.

2. Prostate cancer stem cells (PCSCs)

Several lines of evidence have identified that Wnt signaling can induce prostate cancer initiation, EMT and metastasis, suggesting that canonical Wnts may play a role in the regulation of PCSCs (Bisson et al, 2009). Treatment of PCSCs with Wnt inhibitors can reduce prostasphere size and self-renewal. In contrast, the addition of Wnt3a causes increased prostasphere size and self-renewal. This process is associated with a significant increase in nuclear β-catenin, CD133 and CD44 expression. Moreover, Wnt3a treatment increases the self-renewal of putative PCSCs independent of androgen signaling.

4.1.1.4 Hepatocellular tumors and liver cancer stem cells

1. Hepatoblastoma

Hepatoblastoma is the most common malignant liver tumor. Nuclear and cytoplasmic localization of β-catenin are reported in 90% to 100% of all hepatoblastomas, familial and sporadic due to mutations in APC, CTNNB1, Axin1 and Axin2.

2. Hyperplasia and hepatic Adenoma, Hepatocellular carcinoma (HCC)

The Wnt/β-catenin pathway has been examined in several rare benign liver neoplasms and the analysis demonstrates that abnormal cytoplasmic or nuclear localization of β-catenin in 30% of hepatic adenomas from patients. A more recent analysis indicates mutation of β-catenin in only 12% of adenomas, but 46% of these adenomas progressed to HCC. This finding suggests that development of aberrant activity in the Wnt/β-catenin pathway is an important step toward progression to HCC.

The Wnt/β-catenin pathway is also an important player in the progression of hepatic adenoma and HCC. Studies have found that 20% to 90% of HCCs display activated β-catenin because of diverse mechanisms including mutation in genes encoding for *CTNNB1, Axin-1* and *Axin-2,* as well as fz7 upregulation and GSK-3β inactivation. However, the status of Wnts in HCC remains to be examined. Liver-specific deletion of APC can induce β-catenin stabilization and increased HCC. In addition, transgenic mouse models overexpressing c-myc or TGF-β result in mutation and/or nuclear translocation of β-catenin in liver tumors. Interestingly, simultaneous mutation of β-catenin and H-ras leads to 100% incidence of HCC in mice. These findings suggest that β-catenin activation is likely an initiating or contributory factor in a significant subset of HCCs. Several studies have shown that dyregulations of β-catenin in HCC are associated with hepatitis virus, such as hepatitis B virus (HBV) and hepatitis C virus (HCV). In addition, mutations in the Axin and Axin2 genes result in truncated proteins that are detected in about 10% of HCCs.

It is found that mutations in the β-catenin gene are evident only in a small subset of cholangiocarcinomas. Although the role of canonical Wnt signaling in biliary development is beginning to be understood, further investigations are needed.

3. Liver cancer stem cells

Wnt/β-catenin signaling plays an important role in the maintainance of liver CSCs. For example, elevated expression of Wnt and its downstream mediators are shown in EpCAM+ liver CSCs. It has been demonstrated that murine hepatic stem/progenitor cells transduced with mutant β-catenin can acquire excessive self-renewal capability and tumorigenicity.

4.1.1.5 Leukaemia and leukemia stem cells (LSCs)

1. Leukemia stem cells (LSCs)

Similar to HSCs, LSCs engage in complex bidirectional signals within the hematopoietic microenvironment. Two different stages of leukemia progression called “pre-LSCs" and established leukemia (LSCs) are identified in a syngeneic retroviral model of MLL-AF9 induced acute myeloid leukemia (AML). The homing and microlocalization of pre-LSCs is most similar to long-term HSCs and dependent on cell-intrinsic Wnt signaling. In contrast, the homing of established LSCs is most similar to that of committed myeloid progenitors

and distinct from HSCs. In addition, Dkk1 can impair HSC function, while does not affect pre-LSCs, LSC homing, or AML development. Moreover, cell-intrinsic Wnt activation is observed in human and murine AML samples. For example, Wnt3a can affect the self-renewal of AML and T-lymphoblastic leukemia (T-ALL) cells.

2. LSCs associated with AML, CML and MLL

Acute myelogenous leukemia (AML) is the most common acute leukemia in adults. Only small subsets of AML cells (called LSCs) are capable of extensive proliferation and self-renewal, with several markers similar to HSCs. Recent studies have demonstrated that Wnt/β-catenin signaling is required for self-renewal of LSCs derived from either HSC or more differentiated granulocyte macrophage progenitors (GMP). As discussed before, the Wnt/β-catenin pathway is normally active in HSCs, but not in GMP. In addition, β-catenin is not absolutely required for self-renewal of adult HSCs, while β-catenin is required for LSC development and maintenance in AML (Wang et al, 2010). Thus, targeting the Wnt/β-catenin pathway may represent a new therapeutic opportunity in AML.

A conditional β-catenin knockout model has identified the Wnt/β-catenin pathway as being essential for the self-renewal of normal and chronic myelogenous leukemia (CML) stem cells: the impairment of HSC self-renewal in β-catenin$^{-/-}$ mice pre-empted the subversion of this property for the generation of CML LSCs (Deshpande & Buske C, 2007).

Previous studies have found that β-catenin is activated during development of mixed lineage leukaemia (MLL) leukemic stem cells (LSCs) (Yeung et al, 2010). Suppression of β-catenin can reverse LSCs to a pre-LSC-like stage and significantly reduce the growth of human MLL leukemic cells. Conditional deletion of β-catenin can completely abolish the oncogenic potential of MLL-transformed cells. In addition, established MLL LSCs that have acquired resistance against GSK3 inhibitors are resensitized by suppression of β-catenin expression. These results unveil previously unrecognized multiple functions of β-catenin in the establishment and drug-resistant properties of MLL stem cells, highlighting it as a potential therapeutic target for an important subset of AMLs.

4.1.1.6 Other cancers

1. Lung cancer

The canonical Wnt pathway is important for the development of human lung cancer. Although increased levels of β-catenin have been reported in different types of lung cancers, mutations of APC and β-catenin are rare in lung cancers. Dysregulations of specific Wnt molecules (e.g. Wnt1, Wnt2 and Wnt7a) leading to oncogenic signaling are detected in lung cancer. Other components of canonical Wnt pathway are found to be associated with lung cancer. Overexpression of Dvl has been reported in 75% of non-small cell lung cancer samples compared with autologous matched normal lung tissue controls. Downregulation of Wnt pathway antagonists like Dkk3, Wif, Caveolin-1 and sFRP have been reported in various types of lung cancers.

2. Esophageal carcinoma

The roles of canonical Wnt signaling in esophageal carcinoma remain not well understood. Some components, including APC, β-catenin, Dkk1 and Caveolin-1, are found to express in esophageal carcinoma. The expression of Dkk1 is upregulated on both mRNA and protein

levels in esophageal carcinoma tissues compared with the adjacent normal esophageal tissues. The positive rates of APC and E-cadherin in esophageal carcinoma are lower than those in the normal group and the abnormal expression rates of β-catenin and cyclin D1 in esophageal carcinoma are higher than those in the normal group.

3. Ovarian cancer

Endometrioid ovarian carcinoma (EOC) frequently exhibit constitutive activation of canonical Wnt signaling, usually as a result of oncogenic mutations that stabilize and dysregulate the β-catenin protein. For example, the majority of low-grade endometrioid ovarian carcinomas often display nuclear immunoreactivity for β-catenin (70% of cases), and these cases often harbour mutations in the β-catenin gene at codons for residues phosphorylated by GSK-3β (54% of cases). However, high-grade endometrioid ovarian carcinomas do not display nuclear β-catenin immunoreactivity and progression is not associated with β-catenin mutations. Moreover, nuclear β-catenin in low-grade endometrioid EOC is also associated with squamous differentiation and correlates with good prognosis and lack of relapse. The EOC also shows constitutive activation of the canonical Wnt signaling pathway, usually as a result of oncogenic mutations in the APC and Axin tumor suppressor proteins. However, some studies have indicated that mutations in Axin are existed in cell lines of endometrioid EOC, while a study from other group has found that no mutations in either APC or Axin in human endometrioid EOC.

Mechanisms for activation of canonical Wnt signaling in ovarian carcinoma exhibit histotype dependence. EOC is strongly associated with active mutations in β-catenin. In contrast with endometrioid EOC, ovarian carcinomas of serous, clear-cell and mucinous histotypes have rarely been associated with activating mutations in the key proteins of the Wnt signaling pathway. Recent studies have shown that both GSK-3β and Axin2 are overexpressed in serous ovarian cancer. These findings implicate activation of Wnt/β-catenin signaling in serous EOC in the absence of activating mutations in either APC, Axin or β-catenin (Barbolina et al, 2011).

4.1.2 Potential roles in cancer therapy

As discussed before, canonical Wnt signaling is active in most of cancers and involed in the self-renewal and differentiation of cancer stem cells. Thus, inhibition of Wnt signaling activity represents a valuable strategy for cancer therapy. Several classes of drugs targeting the Wnt pathway are under development or on the market. These drugs belong to non-steroidal anti-inflammatory drugs (NSAIDs), vitamin D derivatives, antibody-based treatments, small molecule inhibitors, and so on (Table 3).

4.2 Cardiovascular diseases

4.2.1 Cardiac hypertrophy

Cardiomyocytes are terminally differentiated cells existing in heart and the abnormal increase in their individual sizes often leads cardiac hypertrophy. The canonical Wnt signaling pathway has been found to induce physiological and pathological hypertrophies. Cardiomyocyte-specific overexpression of GSK-3β in transgenic mice negatively regulates physiologic concentric hypertrophy (normal growth) of ventricular cardiomyocytes, leading to a smaller heart with depressed contractility. In addition, Fz2 expression is upregulated

during hypertrophic development in the rat heart. Using the model of mice lacking the Dvl-1 gene, an attenuated hypertrophic response upon pressure overload induced by aortic constriction, an increased GSK-3β and Akt activities and reduced β-catenin protein levels are observed in these mice. Moreover, it is interesting that β-catenin levels are found to be relatively high in the embryonic heart compared with the adult heart, whereas in hypertrophic hearts an increase in β-catenin content is observed. It is also found that β-catenin is stabilized by hypertrophic stimuli, and that overexpression of β-catenin can induce hypertrophic growth of cardiomyocyte. Cardiomyocyte-specific deletion of β-catenin is shown to attenuate the hypertrophic response upon aortic constriction *in vivo*.

Inhibitors	Subcategory	Therapeutic	Pathway target	Development stage
Small molecules	NSAIDs	Aspirin	β-catenin	Clinical
Existing drugs and natural compounds		Sulindac,	β-catenin	Clinical
		Celecoxib	TCF	Clinical
	Vitamins	retinoids	β-catenin	Clinical
		1α25,-dihydroxy-VitaminD3	β-catenin	Clinical
	Polyphenols	Querucetin	Unknown	Preclinical
		EGCG	Unknown	Preclinical
		Curcumin	Unknown	Preclinical
		Resveratrol	Unknown	Phase II
		DIF	GSK-3β	Preclinical
Molecular targeted drugs		PNU 74654	β-catenin/TCF	Discovery
		2,4-diamino-quinazoline	β-catenin/TCF	Preclinical
		ICG-001-related analogs	CBP	Phase I(2010)
		NSC668036	Dvl	Discovery
		FJ9	Dvl	Discovery
		3289-8625	Dvl	Discovery
		IWR	Axin	Discovery
		IWP	Porcupine	Discovery
		XAV939	Tankyrase1&2	Discovery
Biologics		Antibodies	Wnt proteins	Preclinical
		Recombinant proteins	WIF1 and SFRPs	Preclinical
		RNA interference	Wnt proteins	Preclinical

Table 3. A summary of various Wnt therapeutics (Curtin & Lorenzi, 2010; Takahashi-Yanaga & Kahn, 2010)

4.2.2 Heart failure

In the failing heart, the abnormal expression of sFRPs is identidfied. sFRP3 and 4 are elevated in failing ventricles compared to donor hearts, which is not the case for sFRP1 and 2, and a reduced Wnt/β-catenin signaling is observed. Although these observations have indicated the abnormal activity of canonical Wnt signaling is associated with heart failture in human, the mechanisms underlying this association remain to be further investigated.

4.2.3 Myocardial infarction (MI) and infarct healing

MI is associated with hypertrophy and directly caused by an acute occlusion of a coronary artery. Similar to that during hypertrophic development, Fz2 expression is found to be gradually upregulated in the first 10 days after myocardial infarction in the rat. The upregulation of Wnt-10b and Fzd-1, -2, -5 and -10 and the downregulation of Wnt-7b are also observed in the infarct area at 1 week after infarction in the mouse. It is worth noting that *Fz2* mRNA is detected in the border zone of the infarct area at 1 week after infarction, whereas at 2 weeks after MI, the expression is migrated into the centre of the infarct area. Dvl, directly downstream of Fz, shows high levels of expression exclusively at 4 days after MI and remains upregulated during the first week after MI. The expression patterns of both Fz2 and Dvl closely resemble the location of myofibroblasts in the infarct area. These results indicate the myofibroblasts are the Fz2 and Dvl expressing cells and may be involved in infarct healing. This conclusion is supported by overexpressing FrzA (a homologue of sFRP1) to block Wnt signaling. This intervention shows a profound effect on infarct healing: the infarct size is reduced by more than 50% at 15 days post-MI and a concomitant improvement of cardiac function is observed in the FrzA-overexpressing mice compared to wildtype controls. This effect could be attributed to reduced cardiomyocyte apoptosis, limiting the scar area. Reduced infarct size and improved function can be induced by adenoviral overexpression of β-catenin in the border zone of the infarcted rat heart, suggesting an important role of Wnt/β-catenin signaling in infarct healing.

4.2.4 Aging

Aging is the main risk factor for cardiovascular diseases, but the molecular mechanisms are poorly understood. In human mammary arteries, the expression of Fz4 and several targets of Wnt/β-catenin signaling pathway such as the Wnt-inducible secreted protein 1 (WISP1), versican, osteopontin (SPP1), insulin-like growth factor binding protein 2 (IGFBP-2), and p21, are modified with age, suggesting an activation of the Wnt /β-catenin pathway in the aging process. In aging mammary arteries, the increase in β-catenin-activating phosphorylation at position Ser675 is found. Wnt3a or Wnt1 treatment of human vascular smooth muscle cells (VSMCs) induces β-catenin phosphorylation at Ser675 and the expression of WISP1, SPP1, and IGFBP-2. These findings suggest that the activation of Wnt pathway occurs in aging human mammary artery cells, but fails to induce the proliferation of aging vascular cells (Marchand et al, 2011).

4.2.5 Other vascular diseases

Canonical Wnt pathway regulates endothelial dysfunction and vascular smooth muscle cell (VSMC) proliferation and migration and thereby intimal thickening. Moreover, the pathway

has the capacity to regulate inflammation and foam cell formation, pathological angiogenesis and calcification, which are crucial processes in plaque formation and stability. Furthermore, it is apparent that altered expression of a handful of Wnt pathway proteins occurs in or regulates atherogenesis. All of those findings indicate that canonical Wnt pathway acts as an important regulator of vascular diseases, such as atherosclerosis, coronary artery disease and hypertension (van de Schans et al, 2008; Tsaousi et al, 2011).

4.3 Nervous system diseases

4.3.1 Neural tube defects (NTD)

Various components of the Wnt signaling pathway have been implicated in NTD. For example, *Dvl2* null mutants have thoracic spina bifida, and *Dvl1/2* double mutants produce even more severe NTD. Mutations of axin, a Wnt pathway inhibitor, can result in incomplete closure of the neural tube or malformation of the head folds. Alterations (hypoactivity, hyperactivity and missense mutations) to LRP6 can cause NTD.

4.3.2 Primitive neuroectodermal tumors (PNETS)

Activation of canonical Wnt pathway can lead to PNETS. In some cerebellar and cerebral PNETS, mutations that lead to nuclear accumulation of β-catenin are present. Interestingly, stabilized β-catenin is not sufficient to cause the development of brain tumors, while stabilized β-catenin together with forced expression of c-Myc, a downstream target of the Wnt signaling pathway, can lead to increased tumors.

4.3.3 Alzheimer's disease (AD)

Alzheimer's disease (AD) is a neurodegenerative disorder associated with aging and characterized by fibrillar deposits of Aβ in subcortical brain regions. The main proteinaceous component of the amyloid deposited in AD is the amyloid-β-peptide (Aβ peptide), a 40- to 42-residue peptide that has been isolated from senile plaque cores. As discussed before, canonical Wnt signaling pathway is involved in neural induction and patterning during nervous system development. Thus, abnormal activity of this pathway is associated with neurodegenerative disorders, such as AD. Apparently, Aβ can bind with the extracellular cysteine-rich domain (CRD) of the Fz to inhibit Wnt/β-catenin signaling. Studies have indicated the exposure of rat hippocampal neurons to Aβ results in three hallmarks related with Wnt signaling: (i) destabilization of endogenous levels of β-catenin; (ii) an increase in GSK-3β activity; and (iii) a decrease in Wnt target gene transcription. In fact, relationship between Aβ-induced neurotoxicity and a decrease in the cytoplasmic levels of β-catenin has been observed. Inhibition of GSK-3β by lithium is shown to protect rat hippocampal neurons from Aβ-induced damage. Moreover, the conditioned media containing Wnt-3a and Wnt-7a are able to overcome the neurotoxic consequences induced by Aβ. In addition, LRP5/6 is also associated with AD.

4.3.4 Parkinson's disease (PD)

Parkinson's disease (PD) is caused by degeneration of the dopaminergic (DA) neurons of the substantia nigra. The canonical Wnt signaling has a role in promoting adult DA

neurogenesis (Inestrosa & Arenas, 2009). Parkin, an E3 ubiquitin ligase linked to familial PD, can directly interact with β-catenin and regulates β-catenin levels *in vivo*. The stabilization of β-catenin in differentiated primary ventral midbrain neurons results in increased levels of cyclin E and proliferation, followed by increased levels of cleaved PARP and loss of DA neurons. In addition, Wnt3a signaling also causes death of post-mitotic DA neurons in parkin null animals, suggesting that both increased stabilization and decreased degradation of β-catenin can result in DA cell death.

4.3.5 Schizophrenia

Schizophrenia is a psychiatric disorder characterized by "positive" symptoms such as delusions, hallucinations, and disorganized speech and "negative" symptoms such as lack of emotional affect and motivation. The canonical Wnt signaling pathway is a candidate for dysregulating brain development, which cause neuroanatomical defects associated with schizophrenia (Okerlund & Cheyette, 2011). Studies have shown that Wnt1 is upregulated in schizophrenic brains, and some genes associated with susceptibility to the disease are core components of the Wnt signaling pathway, such as TCF4, Dkk proteins (especially Dkk1 and Dkk3), GSK-3β, Fz3, sFRP1 and Dvl3. Taken together, the genetic and pharmacological data suggest a potential connection between canonical Wnt signaling and the pathogenesis and therapeutics of schizophrenia.

4.4 Polycystic kidney disease

Cystic kidney disease is the most common genetic cause of end-stage renal failure. These diseases are characterized by the progressive development of cysts in the nephron and collecting ducts, and patients often require dialysis and kidney transplantation. One class cystic kidney disease is polycystic kidney disease (PKD), which contains two types of autosomal dominant (ADPKD) and autosomal recessive PKD (ARPKD). ADPKD typically arises during adulthood, whereas ARPKD arises during childhood and is much more severe. A second class of cystic nephropathy is characterized as glomerulocystic kidney disease, including medullary cystic kidney disease (MCKD) and nephronophthisis (NPHP). NPHP is inherited in a recessive fashion and like ARPKD. Two genes have been identified for the development of ADPKD: polycystic kidney disease gene 1 (*PKD1)* and *PKD2* that encode polycystin-1 (PC1) and polycystin-2 (PC2), respectively. However, 11 candidate genes are identified in the NPHP and NPHP-like disorders.

The canonical Wnt signaling is suggested to affect the cystogenesis. For example, the C-terminus of PC1 can facilitate the nuclear accumulation of β-catenin and the downstream transcription is decreased by overexpression of the C-terminus *in vitro*. In kidney, PC1 expression is increased in cystic epithelium of patients with PKD. Cysts arise in animal models of PKD with complete or partial loss of protein (as in, *Pkd1-/-*, *Pkd1+/-* and hypomorphic mice) and with overexpression of PC1 protein, suggesting the kidney is especially sensitive to gene dosage. These studies indicate that PC1 expression seems to be tightly regulated in the kidney, and canonical Wnt signaling might also require similar fine tuning. Other canonical Wnt components are also associated with cystic renal disease. For example, APC inactivation leads to cystic renal phenotypes in mice. Further evidence for a role of canonical Wnt signaling in renal cyst disease comes from work with NPHP animal

models. Nphp2 and Nphp3 both negatively regulate the canonical Wnt cascade through regulation of Dvl, both *in vitro* and *in vivo* in *Xenopus laevis* embryos. Canonical Wnt activity is also affected in a mammalian model of NPHP: knockout mice for *Ahi1*, a gene mutated in the ciliopathy Joubert syndrome (JS), have decreased the canonical Wnt signaling in the kidney compared to wild-type mice.

4.5 Fibrosis

4.5.1 Kidney fibrosis

The Wnt/β-catenin signaling is involved in the pathogenesis of renal fibrosis (He et al, 2009). It has been identified that the majority of 19 different Wnts and 10 frizzled receptor genes are expressed at various levels in the normal mouse kidney. All members of the Wnt family except Wnt5b, Wnt8b, and Wnt9b are upregulated in the fibrotic kidney with distinct dynamics after unilateral ureteral obstruction. In addition, the expression of most Fzd receptors and Wnt antagonists is also induced. Furthermore, obstructive injury leads to a dramatic accumulation of β-catenin in the cytoplasm and nuclei of renal tubular epithelial cells, indicating activation of the canonical Wnt signaling. Numerous Wnt/β-catenin target genes (c-Myc, Twist, lymphoid enhancer-binding factor 1, and fibronectin) are also induced and their expression is closely correlated with renal β-catenin abundance. Wnt antagonist Dkk1 can inhibit myofibroblast activation, suppress expression of fibroblast-specific protein 1, type I collagen, and fibronectin, and reduce total collagen content in the model of obstructive nephropathy. In summary, these results suggest that Wnt/β-catenin signaling is involved in the promotion of renal fibrosis.

4.5.2 Lung fibrosis

The canonical Wnt signaling pathway is associated with pulmonary fibrosis based on studies in animal models and human diseases. Idiopathic pulmonary fibrosis (IPF) is the most common form of lung fibrosis. Studies have revealed the overexpression of Wnt genes, including Wnt-2 and -5a, the receptors Fz7 and -10, and Wnt regulators, such as sFRP1 and -2, in IPF lungs compared with normal lungs or those with other interstitial lung diseases. In addition, several Wnt target genes, such as matrix metalloproteinase 7, osteopontin, or Wnt1-inducible signaling protein (WISP) 1, are recently identified in experimental and idiopathic lung fibrosis. Furthermore, the nuclear localization of β-catenin is found in alveolar epithelial type II (ATII) cells and interstitial fibroblasts in IPF lungs. These results suggest the existence of an activated canonical Wnt signaling in IPF. Morover, abnormal activities of other canonical Wnt signaling components, such as GSK-3β, are observed in ATII cells. The increased activity of the Wnt pathway in IPF is confirmed by increased phosphorylation of LRP6 and GSK-3.

4.5.3 Liver fibrosis

Liver fibrosis represents chronic wound repair and is causally associated with persistent hepatic stellate cell (HSC) activation. Expression of Wnt and Fz genes is induced in HSC isolated from experimental cholestatic liver fibrosis, and Dkk-1 expression ameliorates this form of liver fibrosis in mice. These results suggest canonical Wnt signaling plays a role in liver fibrosis through activating HSC (Cheng et al, 2008). Moreover, both Wnt1 and Wnt10b

potently suppress adipocyte differentiation via its inhibition of the adipogenic transcription factors CCAAT/enhanced binding protein family (C/EBP) and peroxisome proliferator-activated receptor-γ (PPARγ). The activated rat HSCs are shown to express higher levels of Wnt4, Wnt5, and Fz2 in culture and experimental liver fibrosis model .

4.6 Other diseases

4.6.1 Diabetes

It is known that the canonical Wnt pathway is involved in the development and genesis of mouse pancreatic islets, pancreatic beta cell growth. The canonnial Wnt signaling is also associated with diabetes (Jin, 2008). Several components of this pathway play important roles in normal cholesterol metabolism and glucose-induced insulin secretion and the production of the incretin hormone glucagon-like peptide-1 (GLP-1). For example, polymorphisms in *TCF7L2,* also known as TCF-4, have by far the biggest effect on the risk of type 2 diabetes. The human LRP5 gene is mapped to within the IDDM4 region, which is linked to type 1 diabetes on chromosome 11q13, and polymorphisms in LRP5 have shown to be associated with obesity phenotypes, and missense mutations in LRP6 are shown to be associated with the risk of bone loss, early coronary disease and the metabolic syndrome. In addition, forkhead box transcription factor subgroup O (FOXO) and TCF proteins are able to compete for the limited pool of β-catenin, and ageing will lead to increased FOXO-mediated gene transcription and reduce TCF-mediated gene transcription. Thus, these findings indicate that type 2 diabetes is an age-dependent disease.

4.6.2 Obesity

Obesity is linked to major adverse health outcomes such as insulin resistance and type 2 diabetes. With obesity, adipose tissue mass expands and adipocyte (fat cell) size increases. As previously discussed, canonical Wnt signaling pathway is essential for adipogenesis and type 2 diabetes. Thus, canonical Wnt signaling plays an important role in the genesis of obesity.

4.6.3 Osteoporosis and Osteoarthritis (OA)

Osteoporosis and osteoarthritis (OA) cause significant morbidity in the middle-age and elderly population. Bone tissue and chondrogenesis are involved in the pathogenesis of OA, which is characterized with subchondral sclerosis and the formation of osteophytic new bone. It is well known that the canonical Wnt pathway has emerged as an important regulator of chondrogenesis, bone development and remodeling. Thus, it is indicated that this Wnt pathway may be involved in the pathogenesis of OA (Velasco et al., 2010). For example, loss-of-function mutations of the LRP5 gene can result in osteoporosis, whereas activating mutations are associated with increased bone mass. It is reported that targeted deletion of the FrzB gene can increase the injury-associated loss of articular cartilage in mice, in association with increased cortical bone thickness and density. Recent work has found that expression of seven genes (BCL9, Fz5, Dvl2, EP300, FrzB, LRP5, and TCF7L1) is consistently upregulated both in tissue samples and in cell cultures from patients with knee osteoarthritis. These studies also demonstrate that three SNPs of the LRP5 gene and one in the LRP6 gene show marginally significant differences in allelic frequencies across the patient groups.

5. Conclusion and prospection

The canonical Wnt signaling is widely known as one of the key pathways that are essential for the embryogenesis of vertebrates. In recent years, increasing lines of evidence have demonstrated that abnormal activivation of this pathway is closely associated with the development and progression of human diseases such as various tumors. Although investigations have provided new insights into the molecular mechanism underlying the roles and regulation of canonical Wnt signaling in animal models and patients, many critical questions remain to be answered. For example, it is largely unclear how the expression of components in this pathway is spatiotemporally controlled and secreted during embryonic development, what mechanisms are involved in the control of Wnt/β-catenin activity by newly identified proteins, and what factors control the nuclear import of β-catenin that is necessary for the expression of downstream genes. Since the functions of canonical Wnt signaling in the formation of animal organs such as liver and the development of some of human diseases are not well understood, it will be of great interest to investigate its effects on activity and regulation of key signaling molecules in developmental and pathological contexts. We firmly believe that a better understanding of canonical Wnt signaling regulation will have a broad impact on biology and medicine.

6. Acknowledgements

In this chaper, we have cited many findings in previous publications and the origins of many results in the text are not mentioned due to the limitiation of reference numbers. Therefore, we would like to take this opportunity to express our sincere gratitude to all authors whose works are cited in this chapter.

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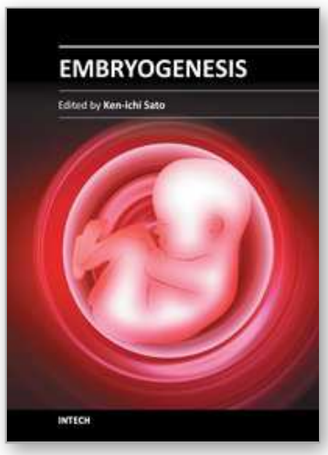
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University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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