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Review

Wnt signaling: the good and the bad

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Since the first Wnt gene was identified in 1982, the functions and mechanisms of Wnt signaling have been extensively studied. Wnt signaling is conserved from invertebrates to vertebrates and regulates early embryonic development as well as the homeostasis of adult tissues. In addition, both embryonic stem cells and adult stem cells are regulated by Wnt signaling. Deregulation of Wnt signaling is associated with many human diseases, particularly cancers. In this review, we will discuss in detail the functions of many components involved in the Wnt signal transduction pathway. Then, we will explore what is known about the role of Wnt signaling in stem cells and cancers.

Keywords Wnt signaling; β-catenin; cancer; stem cell

The orchestration of proliferation and differentiation of each cell in a specific spatial and temporal manner is critical in the development of any multicellular organism. To this effect, multiple signaling pathways have evolved to help coordinate these events and facilitate between cells. The Wnt signaling pathway is exploited in a wide variety of contexts to achieve these aims. Wnt signaling controls many events in early development including axis determination, patterning of organs, and cell fate. In the adult organism, Wnt signaling is critically involved in the homeostasis of many tissues, including the intestine, skin, bone and hematopoietic system [1,2]. In addition, recent research suggests that Wnt signaling is also essential in stem cell self-renewal [1,2]. Moreover, the Wnt pathway as a whole and the majority of its components are preserved in many organisms used in biological research, including *Drosophila melanogaster*, *Caenorhabditis elegans*, *Xenopus laevis*, and *Mus musculus*.

The Wnt-1 gene was first identified as a preferential insertion site for the murine mammary tumor virus, resulting in overexpression of the Wnt-1 ligand and the formation of mammary tumors [3]. Wnt-1 was originally called int-1. Its *Drosophila* homolog, Wingless (Wg), controls segment polarity in early development [4], whereas injection of Wnt-1 mRNA into ventral side of a *Xenopus* embryo induces body axis duplication [5]. Wnt proteins activate three different downstream pathways: the canonical pathway, the planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway. This review will focus on the canonical pathway, which regulates cellular responses through β-catenin. Readers interested in the PCP pathway and Ca^{2+} pathway are directed to several excellent reviews already written on the subject [6–9].

Deregulated Wnt signaling has been implicated in many hereditary diseases and cancers. Constitutive activation of Wnt signaling is the initiating event in both colorectal cancer and hepatocellular carcinoma, whereas mutations that result in decreased or absent Wnt signaling have been found in several disorders as well. For example, osteoporosis-pseudoglioma syndrome, which causes defects in bone density, and Familial Exudative Vitreoretinopathy, which results in defective vasculogenesis of the retina [1,2]. A comprehensive resource of information about Wnt signaling can be found on the web at http://www.stanford.edu/~rnusse/wntwindow.html.

Major Components of the Wnt Pathway

Wnt genes have been identified in many organisms, including insects, nematodes, *Cnidaria* and vertebrates [10]. In both human and mouse genomes, 19 Wnt genes have been found. Wnt proteins can activate canonical pathway, PCP pathway, and/or Ca^{2+} pathway. The latter two pathways are collectively referred to as the "non-canonical" pathways. Thus, Wnt species are generally classified according to which particular pathways they activate [1,2].

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In the canonical Wnt pathway, a large number of components work together to transduce an external signal into changes in gene expression within the target cell (**Figs. 1** and **2**). Wnt is a secreted ligand that binds to its receptor at the cell membrane. The major effect of Wnt binding its ligand is the stabilization of cytoplasmic β-catenin through inhibition of the β-catenin degradation complex. β-catenin is then free to enter the nucleus and activate Wnt-regulated genes through its interaction with TCF (T-cell factor) family transcription factors and concomitant recruitment of co-activators such as p300/CBP, Pygopus, and BCL9/Legless. This section will explore the many different components of the Wnt pathway in more detail.

Wnt is a secreted ligand

Despite the different effects individual Wnts can have on target cells, all Wnt proteins are similar in that they have an N-terminal signal peptide, one or more N-linked glycosylation sites, and 23 conserved cysteine residues [2]. In addition, most Wnt proteins are lipid-modified and are thus hydrophobic in nature, explaining the difficulty many have had in their purification [11]. The acyl-transferase protein Porcupine regulates lipid modification of Wnt proteins in the endoplasmic reticulum and is critical in the transport and secretion of Wnt (**Fig. 1**) [11,12]. However, the secretion of *Drosophila* WntD does not require lipid modification [13].

Wntless (Wls, also known as Evenness Interrupted, EVI, or Sprinter, SRT) is a multi-trans-membrane protein localized in the Golgi apparatus and in the cell membrane that also regulates the secretion of Wnt [14–16]. Moreover, Wntless genes have been found in both worms and mammals, suggesting that it is a conserved member of the Wnt pathway. Wntless directly interacts with Wnt and may act as a receptor for transporting Wnt from the trans-Golgi network to endosomes. In both the Golgi and endosomes, Wntless co-localizes with retromer, a highly conserved multi-protein complex involved in cell sorting. Furthermore, in *C. elegans*, RNA interference (RNAi)-mediated knockdown of a key component of the retromer complex, Vps35, hampers the formation of Wnt gradients [17]. Additional studies in *Drosophila* and *C. elegans* suggest that retromer regulates the retrieval of Wntless (mig14 in *C. elegans*). For example, when Vps35 is inhibited, Wntless is targeted to the lysosome for degradation, compromising the secretion of Wnt [18,19].

The Wnt receptors—Frizzled, and others

After Wnt is secreted from a cell, it diffuses to nearby cells and binds to its receptor, Frizzled (Fz) (**Fig. 2**). Fz was identified as the Wnt receptor in *Drosophila* through biochemical and genetic means [20]. Many vertebrate homologs of Fz were identified soon after [21]. Fz receptors are seven trans-membrane repeat proteins that belong to a

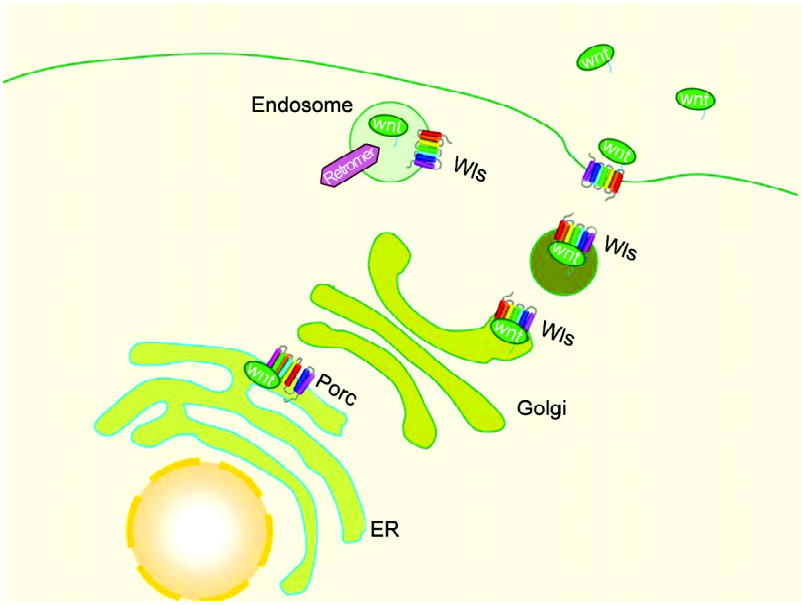

Fig. 1 Secretion of Wnt proteins Wnt is synthesized by ribosomes and acylated by Porcupine (Porc) in the endoplasmic reticulum (ER) Wnt is then transferred to the Golgi. Wntless (Wls) helps transfer Wnt to endosomes, which then fuse with the cell membrane and Wnt is released outside of the cell. However, the secretion of *Drosophila* WntD does not require lipid modification.

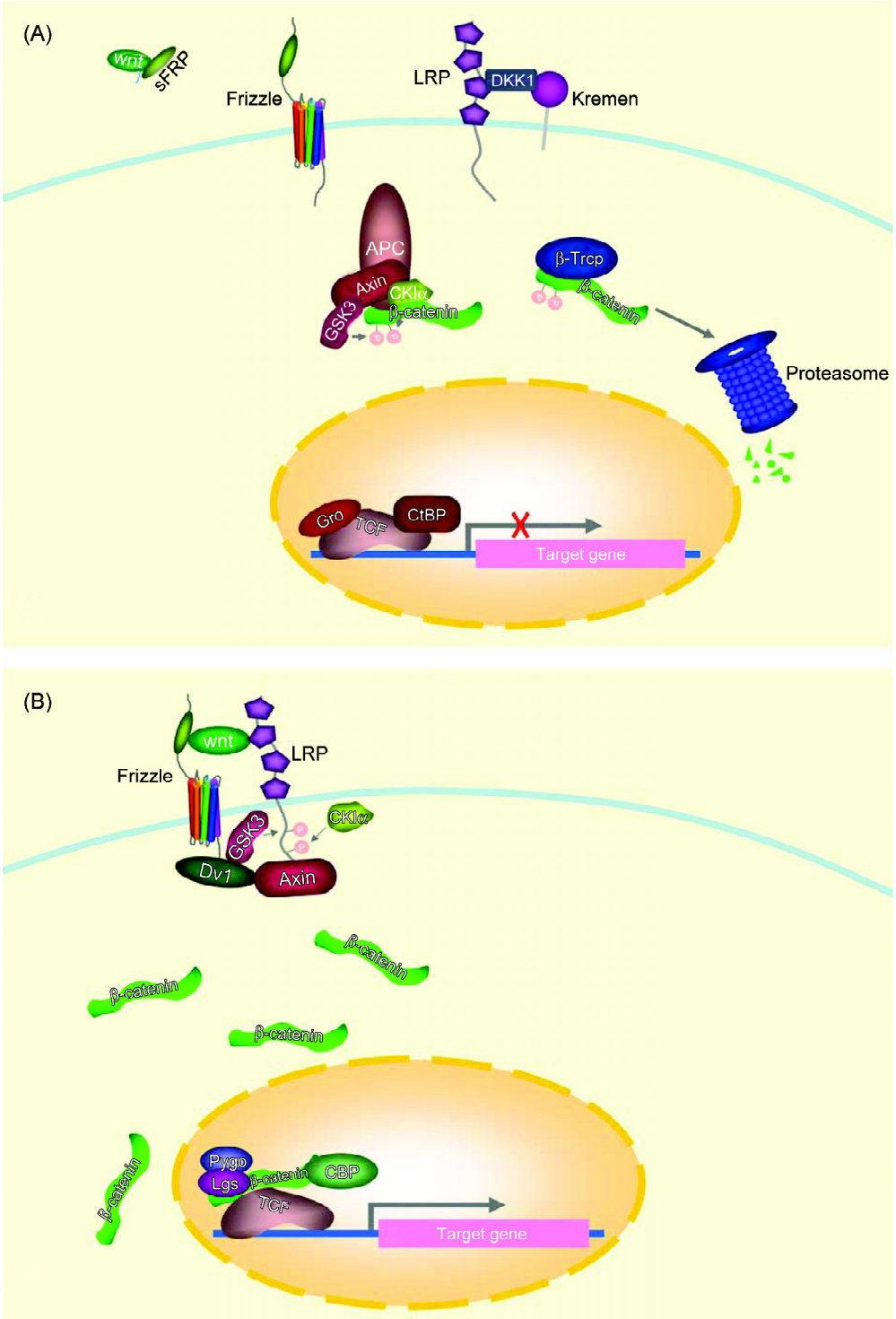

Fig. 2 Wnt signaling transduction pathway (A) When Wnt signaling is "turned off", cytoplasmic β-catenin is degraded by the Axin complex consisting of adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3) and casein kinase I α (CKIα). Within this complex, CKIα and GSK3 phosphorylate β-catenin, phosphorylated β-catenin is recognized by β-Trcp and degraded via the ubiquitin-proteasome pathway. Wnt protein can be inhibited by secreted Frizzled-related proteins (sFRPs), and low-density-lipoprotein receptor-related proteins 5 and 6 (LRP5/6) can be inhibited by Dickkopf-1 (DKK1). (B) When Wnt signaling is "turned on", Wnt protein binds its receptor Frizzled and co-receptor LRP5/6 and stimulates LRP5/6 phosphorylation with the help of Dishevelled (Dv1). Phosphorylated LRP5/6 recruits Axin to the membrane and disrupts the Axin complex. β-catenin accumulates in the cytoplasm and enters nucleus, where it binds T-cell factor (TCF)/LEF and co-activators, such as Pygopus (Pygo), legless (Lgs) and p300/CBP, and activates gene expression. Gro, Groucho.

family of G-protein coupled receptors. Interestingly, this family also includes Smoothened, a key component in Hedgehog signaling [22]. The extra-cellular N-terminus of Fz contains a cysteine-rich domain (CRD) that directly interacts with Wnt. *In vitro*, each Wnt can bind to many different Fz receptors [23,24]. *In vivo*, specificity may be achieved by additional factors or by the restricted temporal expression of both Wnt and Fz. The cytoplasmic tail of Fz has a conserved KTxxxW motif that interacts with a downstream mediator of Wnt signaling, Dishevelled (Dvl), through its PDZ domain [25,26]. In addition, some Fz receptors have an S/TxV motif that may also bind to the PDZ domain of Dvl [27]. In addition to signaling through Dvl, it has also been suggested that Fz may activate signaling through heterotrimeric G protein [28,29].

Low-density-lipoprotein receptor-related proteins 5 and 6 (LRP5/6) are co-receptors of Fz, whereas arrow is the *Drosophila* homolog [30–32]. Genetic deletion of LRP5/6 in mice results in a phenotype that resembles a Wnt null mutation, suggesting that LRP5/6 is a critical component of the Wnt pathway [30,32,33]. LRP5/6 is a single transmembrane protein. The extracellular domain contains a YWTD β-propeller and an EGF-like domain, and is required for binding Wnt as well as signal transduction [31,34]. On the cytoplasmic side, the C-terminus of LRP5/6 contains five conserved PPP(S/T)P motifs which are equally indispensable for the transduction of Wnt signaling (see below). The proper expression and localization of LRP5/6 is itself subject to regulation. Mesd, an ER chaperone protein, is involved in the maturation of the LRP5/6 receptor and its transport to the cell surface. Boca is its *Drosophila* homolog [35,36].

Additional receptors for Wnt have also been reported. For example, the atypical receptor tyrosine kinase Ryk binds Wnt and regulates neurite outgrowth. Derailed, its *Drosophila* homolog, similarly mediates axon guidance [37, 38]. Wnt5a can also signal through Ror2, a receptor tyrosine kinase, to regulate convergent extension through the activation of PI3K and Cdc42 [39–41].

In addition to Wnt, several other ligands can bind Fz receptors and activate the canonical pathway. For example, Norrin binds Fz4 and this interaction appears to be required for vascular development in eye and ear [42]. In addition, mutations in either Norrin or Fz4 result in the retinal vascular defects found in both Norrie disease and Familial Exudative Vitreoretinopathy (FEVR) [42].

Similarly, R-spondin family members bind Fz8 and LRP6 and activate expression of Wnt target genes [43–45]. In vertebrates, the R-spondin family contains four members. R-Spondin2 activates Wnt signaling in *Xenopus* embryo [46], whereas the intestinal epithelium of mice overexpressing R-spondin1 has hyperplasia and elevated β-catenin levels [46]. Additional roles of R-spondin family members have been reported in limb and lung development as well as sex determination [47–49].

Extracellular Wnt antagonists

Many extracellular inhibitors of Wnt signaling have been reported. For example, both secreted Frizzled-related protein (sFRP) and Wnt-inhibitory factor (WIF) antagonize Wnt signaling by sequestering the Wnt protein in the extracellular matrix. sFRP binds Wnt through its CRD domain, whereas [50,51] WIF protein binds Wnt through its WIF domain [52].

Using an entirely different mechanism Wise [53], SOST [54,55], and Dickkopf (Dkk) [56] antagonize Wnt through interactions with LRP. In addition, Dkk1 bridges LRP6 and another transmembrane protein, Kremen, inducing endocytosis of LRP6. Without LRP6 available at the cell surface, Wnt signaling is effectively inhibited [57].

Crossing the membrane

The extracellular binding of Wnt to Fz and LRP5/6 modulates intracellular components of the Wnt pathway through at least two mechanisms (**Fig. 2**).

First, the binding of Wnt induces structural changes in the receptor that result in recruitment of Dvl to the cytoplasmic tail of Fz through its intracellular KTxxxW motif [25,26,58,59]. Dvl is an important cytoplasmic component of the Wnt pathway that is conserved in both flies and vertebrates [60–62], and genetic epistasis studies place Dishevelled downstream of Fz, but upstream of GSK3 (glycogen synthase kinase 3) and β-catenin [63]. Wnt also induces phosphorylation of Dvl [64], although the role of the phosphorylation of Dvl is still unclear. However, several kinases phosphorylate Dvl, including casein kinase I (CKI), CKII, and Par-1 [65–67].

Second, the C-terminus of LRP5/6 interacts with Axin, which is an inhibitory downstream component of the Wnt pathway. Recruitment of Axin to the cell membrane inhibits its function, resulting in the stabilization of β-catenin [34]. The binding of Wnt also induces phosphorylation of the co-receptor LRP5/6 at its PPPSP motif, creating a docking site for Axin [68]. Phosphorylation of the PPPSP motif is mediated by membrane-bound GSK3 and CKI [69]. In response to Wnt stimulation, LRP5/6 is phosphorylated at an additional site, N-terminal to PPPSP motif by the membrane-bound protein casein kinase Iγ (CKIγ). Moreover, phosphorylation at this site is indispensable for Wnt signaling [70].

Although it is thought that the physical proximity of Fz and LRP5/6 induced by Wnt binding is crucial in activating downstream components, surprisingly, Arrow mutant flies were only inefficiently rescued by a Frizzled-arrow fusion protein. To explain this, a two-step signaling mechanism was recently proposed. The initiation step requires both Fz and arrow, whereas the amplification step depends only on arrow [71]. In support of this, both Fz and Dishevelled are required for phosphorylation of LRP6 [72–74]. Thus, the PPPSP motif of LRP5/6 may function as an amplifier of Wnt signaling. Using live imaging of vertebrate cells, one study found that Wnt induces the organization of phosphorylated LRP6 into aggregates known as signalosomes, in a Dvl-dependent manner [74]. These findings suggest that Fz, LRP5/6, Dvl and Axin must organize into macromolecular complex in order to efficiently mediate Wnt signaling.

In the cytoplasm

β-catenin was originally identified as E-cadherin binding partner and important in cell-cell adhesion, prior to the discovery of its involvement in the Wnt pathway [75]. However, it was found that mutations of the *Drosophila* homolog of β-catenin, Armadillo (Arm) [76,77], gave a similar phenotype as the Wg mutant [4], suggesting that Arm might be part of the Wg signaling pathway. Further studies found that injection of β-catenin mRNA into the ventral side of *Xenopus* embryos induced a secondary axis, a hallmark of Wnt signaling [78]. Thus, it became clear that β-catenin/Arm functions downstream of Wnt/Wg [79], in addition to its previously characterized role in cell-cell adhesion.

Many of the other components of the Wnt pathway were soon found by a variety of means. In mice, Axin is encoded by the *fused* locus, and Axis duplication was observed in *fused* homozygous mutant embryo [80]. Similar results were found in *Xenopus* embryos, implicating Axin as a negative regulator of Wnt signaling [80]. The serine/threonine kinase GSK-3β was initially found as a key regulator of glycogen metabolism, as it can phosphorylate and inactivate glycogen-synthase. However, it was later found to be essential in several signaling pathways as well [81]. Zeste-White 3, the *Drosophila* homologue of GSK-3β, was found to be a negative regulator of segment polarity downstream of Wg [82]. In the *Xenopus* embryo, GSK-3β suppresses axis formation induced by Wnt [83].

Adenomatous polyposis coli (APC) is a tumor suppressor protein frequently mutated in colorectal cancer [84,85]. In early attempts to identify the function of APC, it was found that APC could directly interact with β-catenin and decrease levels of cytoplasmic β-catenin [86–88], whereas mutations of APC found in human colorectal cancer lead to accumulation of β-catenin [89,90]. In addition, APC also binds and is phosphorylated by GSK-3β [89].

Thus, it is now clear that the central task of canonical Wnt signaling is to regulate β-catenin stability. The level of cytoplasmic β-catenin is tightly controlled by the cytoplasmic degradation complex (**Fig. 2**), which contains the scaffold protein Axin, as well as β-catenin, CKI, GSK3 and APC [91–95]. In unstimulated cells, this complex mediates the degradation of cytoplasmic β-catenin through a multi-step process. First, β-catenin is phosphorylated at the N-terminus by CKIα [95,96] and GSK-3β [97]. Phosphorylated β-catenin is then ubiquitinated by β-Trcp, a component of an E3 ubiquitin ligase complex [98–102]. Ubiquitinated β-catenin is then rapidly degraded by proteasome.

The structure of part of this destruction complex has been solved [103–112]. The structures of central armadillo repeats as well as the full-length β-catenin have also been solved [109,113]. These studies suggest that β-catenin degradation is regulated by a dynamic protein complex. APC may regulate the assembly of Axin complex. When β-catenin is phosphorylated by CKI and GSK-3β within this complex, APC is also phosphorylated. Phosphorylated APC binds β-catenin with a significantly higher affinity, displacing β-catenin from the Axin complex [108,114,115]. Axin is the least abundant protein among the destruction complex proteins and appears to be the rate-limiting factor [116]. β-catenin can be phosphorylated in colon cancer cell line SW480, which contains truncated APC. However, β-catenin ubiquitination cannot be detected in SW480 cells, suggesting that separate domains of APC are required for phosphorylation and ubiquitination. In addition, overexpression of a functional APC fragment can restore β-catenin ubiquitination and degradation, further suggesting that APC regulates β-catenin phosphorylation and degradation by distinct domains and steps.

As mentioned earlier, Wnt proteins bind Fz and LRP5/6, resulting in phosphorylation of cytoplasmic tail of LRP5/6 and recruitment of Dvl [73,74]. Additionally, phosphorylated LRP5/6 relocates Axin to the cell membrane, inhibiting the cytoplasmic degradation complex through a mechanism that is not completely understood. β-catenin is then free to accumulate and translocate into the nucleus. Moreover, Axin degradation upon Wnt stimulation provides another way to stabilize β-catenin [34,117–119].

Since phosphorylation plays important roles in Wnt signaling, the many associated kinases and phosphatases

have been extensively studied. For example, Axin binds the catalytic domain of protein phosphatase 2A (PP2A) [120]. However, both positive and negative roles for PP2A in Wnt signaling have been reported [121–128]. The exact role of PP2A in Wnt signaling may depend on the composition of different PP2A regulatory subunits and needs further examination. Protein phosphatase 1 (PPI) has a clear positive role in Wnt signaling [129]. PP1 binds and de-phosphorylates Axin, decreasing its affinity for GSK-3β, therefore leading to stabilization of β-catenin [129].

By tandem-affinity purification, WTX (Wilms tumor suppressor X chromosome), was found to interact with β-catenin, Axin, APC and β-Trcp. Moreover, WTX promotes β-catenin degradation and ubiquitination in mammalian cells, as well as zebrafish and *Xenopus* [130]. As WTX is inactivated in one third of Wilms tumors [131], it will be interesting to investigate its role in other types of tumors.

Into the nucleus

β-catenin does not contain a nuclear localization sequence. It has been suggested that β-catenin can directly interact with nuclear pore components, bypassing the importin/karyopherin proteins, in order to enter the nucleus [132, 133]. Recently, it was discovered that JNK2 phosphorylates β-catenin at Ser191 and Ser605 in response to Rac1 activation, and that phosphorylation at these two serine controls nuclear translocation of β-catenin [134].

β-catenin contains a nuclear export sequence, which is consistent with its ability to shuttle in and out of the nucleus in response to changes in Wnt signaling; however, how other factors regulate its export is still a contentious issue. One model suggests that Axin [135] or APC [136–138] actively export β-catenin from the nucleus, in addition to their more fully characterized role in β-catenin degradation. An alternate model suggests that these factors do not actively participate in shuttling, but rather as an "anchor" to retain β-catenin within their respective compartments [139]. In this model TCF-4, Pygopus, and BCL9 function as nuclear "anchors" [140], and Axin functions as a cytoplasmic "anchor" [141].

In the nucleus β-catenin interacts with the TCF family of transcription factors [142,143]. The TCF family includes TCF-1, LEF-1 (lymphoid enhancer factor-1), TCF-3, and TCF-4. Among them, TCF-4 is the primary member of the TCF family that is regulated by β-catenin in response to Wnt signaling in the intestine. In the unbound state, TCF/LEF family members actively recruit co-repressors such as CtBP [144], HDAC1 [145,146], and Groucho/TLE [147–149] to inhibit transcription. Groucho/TLE, in turn, interacts with hypo-acetylated histone H3, presumably to help maintain a repressive chromatin environment [150]. However, once β-catenin enters the nucleus, it binds TCF-4 through its central armadillo repeats, displaces Groucho/TLE-1 from TCF/LEF [151] and recruits co-activators through its N- and C-terminal transactivation domains (**Fig. 2**).

The N-terminal transactivation domain of β-catenin, extends from the region just C-terminal to the regulatory region involved in its stability, to the first four Armadillo repeats [152]. This transactivation domain directly associates with BCL9/Legless, which in turn recruits the transcriptional co-activator Pygopus [153–156]. Pygopus contains a plant homeodomain (PHD). PHD domain can interact with tri-methylated histone H3, and is thought to regulate epigenetic modifications on target genes [157]. In addition, Pygopus can dimerize through this domain *in vitro* [110].

The C-terminus of β-catenin contains a strong transactivation domain [142,158,159]. This transactivation domain recruits p300/CBP, which is required for Wnt signaling [158,160]. p300 and CBP are paralogous transcriptional co-activators; they acetylate nearby histones, loosening chromatin in order to facilitate binding of other transcription factors [161,162]. In addition, the C-terminal transactivation domain associates with Parafibromin, a component of PAF1 complex, and is recruited after Pygopus. PAF1 is important for the initiation and elongation steps of transcription through its interaction with RNA polymerase II. The association of β-catenin with the PAF1 complex is required for transactivation. Overexpression of Parafibromin compensated for loss of Legless *in vivo* [163].

Other data suggest that β-catenin can interact with the co-activator FHL2 [164], the basal transcription factor TBP [165], the ATP-dependent chromatin remodeling factors Brg-1/Brahma [166] and the ATP-dependent helicase TIP49a/Pontin52 [167,168]. However, these interactions have not been fully characterized.

Wnt Signaling in Stem Cells

The cells of mammalian organisms are highly dynamic. Every day, millions of cells are replaced due to physical, chemical and immunologic injuries. Stem cells are required to maintain the architecture and function of organisms. These cells reside in a special micro-environment called a niche and they maintain the proliferative potential of tissues throughout the life of an organism. Key features of stem

cells are self-renewal and their ability to give rise to different cell lineages. Wnt signaling is critical in the self-renewal of stem cells in many different tissues, including the skin, intestine, brain and blood. This section will further explore the role of Wnt signaling in this context.

Intestinal stem cells

The gut is a tube-like organ that originates from all three germ layers: the endoderm, mesoderm and ectoderm. The luminal surface of the gut is covered by a continuous sheet of epithelial cells derived from endoderm. In the epithelium of the small of intestine, this sheet folds into finger-like protrusions that extend into the lumen, called villi. In between each villus, the epithelial sheet additionally invaginates inward to form the crypts of Lieberkühn [169] (**Fig. 3**). Notably, no villi are present in the colon and instead the colonic epithelium consists entirely of crypts. Stem cells that replenish the intestinal epithelium are located at the bottom of crypts. Crypt stem cells produce transit-amplifying cells that ultimately differentiate into enterocytes, goblet cells, and enteroendocrine cells (**Fig. 3**). In the small intestine, transit-amplifying cells additionally differentiate into Paneth cells [169,170]. Enterocytes are the most abundant cell type of the intestine, and carry out its primary absorptive function. Goblet cells secrete mucin that protects the luminal surface. Enteroendocrine cells are located throughout the crypt-villus axis and secrete intestinal hormones. Paneth cells are found at the bottom of crypts and release lysozyme as well as other anti-microbial molecules. With the exception of Paneth cells, terminally differentiated cells migrate along the crypt-villi axis and are shed into lumen after 5–7 days. In each crypt, stem cells have to generate around 300 cells per day in order to replenish those lost [170].

Wnt signaling is critical in the regulation of intestinal homeostasis. TCF-4, encoded by *Tcf7l2* gene, is a downstream target of Wnt signaling and is highly expressed in the intestinal epithelium. TCF-4 knockout mice lack crypts, suggesting that TCF-4 is essential for maintenance of epithelial stem cell compartment [171]. Overexpression of Dkk1, a Wnt inhibitor, in intestine causes loss of crypt and secretory cell lineages [172]. These data suggest that Wnt is essential for the homeostasis of intestine epithelium. A similar phenotype was observed when Dkk1 was overexpressed using adenoviruses [173]. As mentioned earlier, APC is a negative regulator of β-catenin and a tumor suppressor in colorectal cancer. Targeted deletion of APC in the mouse intestine activates Wnt signaling and results in expansion of the crypts [174]. In addition, Goblet cells are lost, and Paneth cells are mis-positioned throughout the crypts-villus axis [174], resembling loss of the cell-sorting receptor EphB3 [175]. Expression of the Wnt agonist R-spondin1 in mice induces crypt cell proliferation [46]. Crypt epithelial cells consistently produce Wnt3,

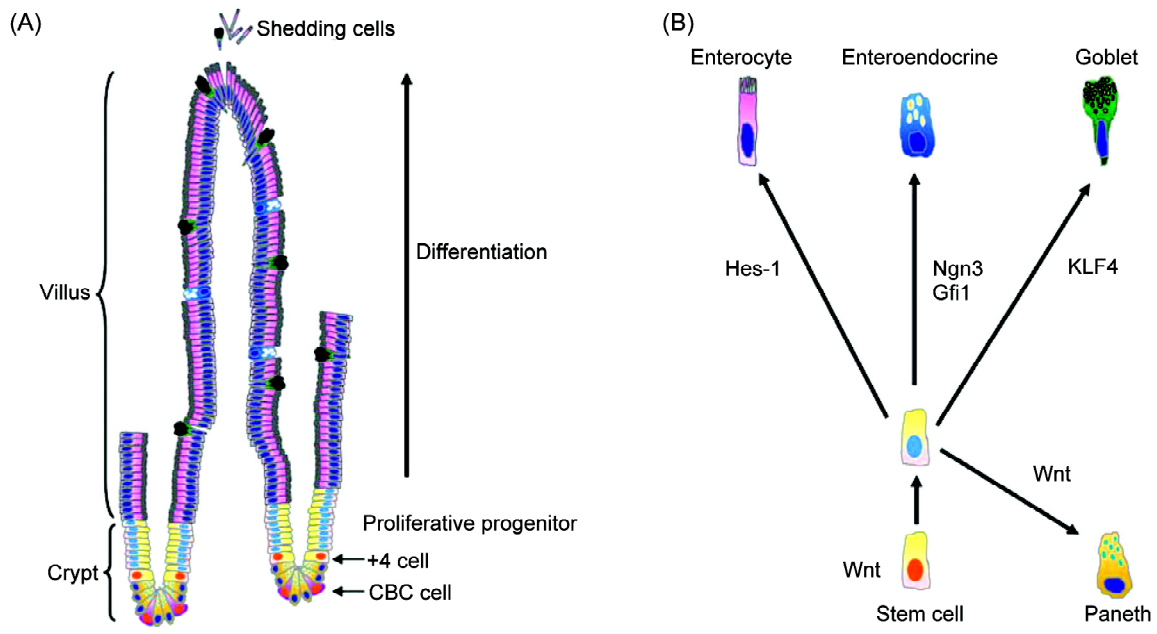

Fig. 3 Structure of the intestine (A) and differentiation of intestinal stem cell (B)

Wnt6, and Wnt9b [176], suggesting that Wnt might function in a paracrine or autocrine manner. *MYC* is a well-established Wnt target gene [177]. Interestingly, deletion of *MYC* in *APC*$^{-/-}$ intestine rescues the defects in proliferation and migration found in *APC*$^{-/-}$ mice [178]. These data suggest that Wnt is an essential mitogen in the crypt.

Because no specific marker for intestinal stem cells has been found, the exact position of these cells within the crypts is still unclear. However, experiments following the retention of labeled DNA suggest that the stem cell might be found at the +4 position, just above the crypt base columnar, and Paneth cells at the base of the crypt [170]. In addition crypt base columnar cells have been suggested to have stem cell activity [179]. LGR5 is an orphan G-protein coupled receptor first identified as Wnt target gene in micro-array studies [180]. *In situ* hybridization and reporter knock-in studies suggest that LGR5 is an intestinal stem cell marker and that the crypt columnar base cells are the stem cells of the intestine [181]. However, the relationship between +4 cells and the crypt columnar base cells remain to be determined.

Recent studies suggest that a small subset of cells in tumors have stem cell-like characteristics. Two groups independently reported the identification of a colon cancer initiating cell based on the surface marker CD133 [182, 183]. Since various Wnt signaling pathway components, such as APC, Axin or β-catenin, are mutated in more than 90% of colorectal cancer patients, it is of great interest to know the role of Wnt signaling in colorectal cancer stem cells.

Hematopoietic stem cells

Hematopoietic stem cells (HSCs) are multi-potent cells that are able to give rise to all blood cell lineages. The fates of HSCs progeny are determined in a stepwise, hierarchical fashion. HSCs give rise to common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) cells. Red blood cells, macrophages, granulocytes and platelets derive from CMP cells, whereas T cells, B cells, dendritic cells and natural killer cells derive from the CLP cells. In the early embryo, HSCs derive from the mesoderm. Hematopoiesis begins in the yolk sac but then quickly shifts to the liver. Later, fetal HSCs home to the bone marrow, where they reside throughout the life of the adult [184].

HSCs are the best characterized type of stem cell because of the ability to purify them to homogeneity based on cell surface markers. For example, mouse HSCs express cell surface proteins C-kit and Sca-1 but are negative for lineage markers (Lin-Sca-1^{+}c-Kit^{+}, or LSK cells) [185].

Recent studies have begun to uncover the role of Wnt in hematopoiesis. TCF-1 and LEF-1, transcription factors targeted by the Wnt signaling pathway, and are expressed in a specific pattern, suggests Wnt signaling might be important in hematopoiesis, both in self-renewal and differentiation [186,187]. For example, knockout TCF-1 or LEF-1 in HSCs blocks T-cell differentiation [188], whereas *in vitro* purified Wnt3a stimulates self-renewal of HSCs [11]. Overexpression of activated β-catenin promotes the growth of HSCs and maintains an immature phenotype in long term culture. In addition, these expanded HSCs reconstitute the blood system more efficiently in lethally irradiated mice [189]. Inhibition of Wnt signaling by overexpressing Axin leads to slow growth of HSCs and a reduced percentage of reconstituted stem cells [189].

However, other reports have found that knocking down expression of β-catenin had no effect on the ability of HSC to reconstitute hematopoiesis in an irradiated host mouse [190]. Other studies found that simultaneous knockout of β-catenin and γ-catenin did not impair reconstitution [191,192]. Using a synthetic reporter, however, another report found that canonical Wnt signaling was still active in these double knockout cells [192]. Enhancing Wnt signaling by treating lethally irradiated mice with a GSK3 inhibitor increases the likelihood of hematopoietic repopulation [193]. On the other hand, overexpression of β-catenin in transgenic mice blocks lineage differentiation and results in an inability to repopulate irradiated hosts [194,195]. Although complete resolution of these discrepancies will require additional carefully designed experiments, it is likely that supra-physiologic levels of β-catenin enforced cell cycling of HSCs and exhausted the long-term stem cell pool [195]. Thus, it appears that maintaining a critical level of β-catenin might be important for the normal function of HSCs.

Additional data suggest that non-canonical Wnt signaling also has role in hematopoiesis. For example, treating HSC cells with Wnt5a enhances their ability to reconstitute hematopoiesis [196]. In addition, Wnt5a antagonizes canonical Wnt signaling, keeping HSCs in the quiescent, G_0 state, and increases the ability of HSCs to repopulate the irradiated hosts [197].

In addition, the bone marrow itself provides cues for HSCs in deciding between self-renewal and differentiation. For example, both osteoblasts and vascular cells have been implicated in maintaining the stem cell niche. Osteoblasts may function to retain HSCs in the bone marrow and regulate their stemness [184]. For example, using a transgene driven by a collagen 1a promoter, targeted overexpression of Dkk1 in osteoblasts results in HSCs that

are unable to reconstitute bone marrow in lethally irradiated mice. Since Dkk1 is a Wnt antagonist, this implies that Wnt signaling is required for self-renewal, although interestingly, the transgenic donor mice themselves have a relatively normal hematopoietic cell population. However, analysis of these HSCs by flow cytometry suggests they are not quiescent, suggesting that Wnt signals from neighboring osteoblast cells may keep HSCs in the quiescent phase and able to maintain their stemness [198].

Skin stem cells

Skin is the largest organ of the human body. It separates an organism from the outside world, is the first barrier to fight against microbes, and protects the body from chemical and physical injury. To protect itself from permanent injury, the epithelium of the skin rapidly turns over, replacing the entire barrier every 4 weeks.

Similar to the intestinal epithelium, the skin relies on stem cells to replenish lost cells. Some evidence suggests that the epidermal stem cells are found within the basal cell compartment. Stem cell progeny migrate upward towards the surface as they deposit cytokeratins. Terminally differentiated keratinocytes become enucleated and contain cross-linked cytokeratins. Eventually, these cells are sloughed off at the surface [199].

In addition to the replenishing keratinocytes, the skin must also repopulate the cells that constitute a hair follicle. Hair follicle stem cells reside in the bulge region, located in the middle of hair follicles. Bulge cells are quiescent and retain BrdU labeling after administration. Activated bulge stem cells move out of this niche and proliferate to supply the hair regeneration at the beginning of a new hair cycle [199].

The importance of Wnt signaling in the skin homeostasis has long been observed and several Wnt genes are expressed in skin. In addition, LEF-1 deficient mice lacked body hair and whiskers [200]. Conditional ablation of β-catenin in the skin blocks placode formation and hair follicle growth [201]. Blocking Wnt signaling by expressing Dkk1 in the skin results in a similar phenotype [202]. Transient or continuous overexpression of a stabilized β-catenin mutant in skin causes excess follicle formation [203–206].

Wnt10a and Wnt10b are specifically expressed in placode [207]. Using a galactosidase reporter in transgenic mice, Wnt signaling appears to be active in the cortical cells of the hair shaft, whereas the bulge region is largely inactive [208]. LEF-1 may mediate Wnt activity in cortex by the fact the co-staining of nuclear β-catenin and galactosidase reporter in the precotex [209,210]. TCF-3 is expressed in the bulge region and keeps the follicle stem cell in a quiescent state by inhibiting Wnt signaling [209, 210]. In studying hair follicle regeneration after wounding the skin, it was found that the regeneration process was enhanced by overexpression of Wnt7a, whereas it was blocked by express Dkk1 in skin [211]. These data highlight the critical role of Wnt in hair follicle formation.

Neural stem cells

Adult neural stem cells are present in the subventricular zone of the lateral ventricles and in the subgranular zone of the hippocampus. Neurons from the subventricular zone migrate towards the olfactory bulb, while neurons from the subgranular zone integrate into the existing circuitry [212].

In situ hybridization studies suggest that Wnt3 is expressed close to the subgranular zone [213]. Staining patterns in a transgenic mouse with Wnt signaling reporter demonstrate that Wnt signaling is active in the subgranular zone and dentate the granule cell layer [213]. Overexpressing Wnt3 in purified hippocampal stem cells increases neuronal production [213], whereas blocking Wnt signaling with the dominant negative Wnt1 blocks neurogenesis at the subgranular zone [213]. In addition, expressing Wnt3 in the subgranular zone region by injecting lentiviruses enhances neurogenesis in the hippocampus [213]. Details of the role of Wnt signaling in the adult neural stem cell will be uncovered by further analysis, using tissue-specific knockout and transgenic animals.

Embryonic stem cells

Embryonic stem (ES) cells derive from the inner cell mass (ICM) of mouse blastocysts. In contrast to tissue stem cells, embryonic stem cells are pluripotent. When injected into an embryo, an ES cell is able to give rise to all cell lineages in the adult. Pluripotency of the ES cell is controlled by an intricate signaling network [214].

Individual APC mutations have marked differences in their ability to regulate the level of β-catenin in ES cells. In addition, these ES cells show different levels of Wnt signaling, as demonstrated by activity of the TOPFlash reporter. Differentiation patterns of teratomas generated by the $APC^{1638T/1638T}$ ES cell are indistinguishable from wild type teratomas, whereas $APC^{1638N/1638N}$ ES cells, which have low levels of APC expression, have differentiation defects in the neuroectodermal, dorsal mesodermal and endodermal lineages. ES cells from $APC^{Min/Min}$ mice on the other hand could not form teratomas at all. Interestingly, ES cells with a stabilizing mutation in β-catenin are able to form teratomas with only limited differentiation capabilities [215].

Activation of Wnt signaling by a GSK3 inhibitor

maintains the undifferentiated state of ES cells [216]. The small molecule IQ-1 targets the PR72/130 subunit of PP2A. It increases β-catenin/CBP mediated expression at the expense of β-catenin/p300 mediated expression. Through this mechanism it helps maintain the pluripotency of embryonic stem cells [217]. Oct4 and Nanog are two important transcriptional factors that control ES cell pluripotency. In ES cells, TCF-3, Oct4, and Nanog co-occupy the promoters of many genes throughout the genome [218]. Knockdown TCF-3 expression or treatment with Wnt-conditioned medium stimulates Oct4 and Nanog expression and facilitates the maintenance of pluripotency [218]. Thus, the function of TCF-3 may be to balance self-renewal and differentiation in ES cells. These data suggest that the Wnt signaling levels are critical in regulation of ES cell differentiation and self-renewal.

Wnt Signaling in Cancers

As a central pathway in both development and homeostasis, the Wnt pathway regulates cell growth, survival and movement, and uncontrolled activation of this pathway can result in neoplasia and cancer. Mutations of β-catenin, APC, and Axin have been found in many cancers, including colon, liver, ovary, brain, prostate, uterus, and skin cancer [219].

Colorectal cancer (CRC)

Aberrant Wnt signaling was first linked to cancer by the observation that familial adenomatous polyposis (FAP) patients had a mutation in the APC gene [220–222]. In addition, aberrations in Wnt signaling have been identified in 90% of sporadic CRCs [223]. The absence of functional APC protein results in chronic activation of Wnt signaling, resulting in the formation of adenomas that ultimately progress to adeno-carcinomas. Genetic studies using the $APC^{min/+}$ mouse model clearly demonstrate the role of mutant APC in initiating the formation of tumors in the intestine [224,225]. These mice are heterozygous for a C-terminal truncated form of the APC gene. Loss-of-heterozygosity of the wild-type allele leaves only mutant APC, which is deficient to participate in the cytoplasmic degradation complex. This allows β-catenin to accumulate and results in constitutively active Wnt signaling [90,226]. Moreover, conditional deletion of the APC gene in the mouse adult intestine results in a "crypt progenitor-like" phenotype with altered patterns of proliferation and differentiation [174,227], and eventually leads to the formation of tumors [228].

In sporadic colorectal tumors that retain wild-type APC, mutations are frequently found in the β-catenin gene (CTNNB1) [226,229] or Axin2 [230]. Moreover, targeted deletion of the N-terminus of β-catenin in the intestinal epithelium of mice produces thousands of adenomatous polyps within weeks [231]. Finally, in a mouse model of colitis-associated colorectal carcinoma, using 1,2-dimethyl-hydrazine and dextran sulfate sodium, mice develop dysplastic lesions and invasive colorectal cancer that strongly stains for β-catenin in the nuclei [232].

Although APC and β-catenin mutations are the initiating step of colonic tumorigenesis [85], downregulation of other tumor suppressor genes may also contribute to the development of colon cancer. For example, Krüppel like factor 4 (KLF4), interacts with β-catenin, repressing Wnt signaling and inhibiting tumor growth [233]. $KLF^{+/-}/APC^{Min/+}$ mice developed, on average, 59% more intestinal adenomas than $APC^{Min/+}$ mice [234]. It is important to further analyze the cross-talk between Wnt signaling and other signaling pathways, such as PTEN/Akt, Notch, bone morphogenetic protein (BMP) and Hedgehog in the tumorigenesis of colon cancer as well as other cancers.

Prostate cancer

Prostate cancer is the most commonly diagnosed malignancy in American males. The prostate gland is an organ dependent on androgen. Androgen, via the androgen receptor (AR), controls the initial growth of prostatic tumor. Androgen ablation therapy causes tumor regression in the early stages of prostate cancer [235,236], clearly highlighting the dependence of tumor growth on androgens. In prostate cells, the binding of androgen hormones to AR allows AR to interact with β-catenin and stimulate AR-mediated transcriptional activity [237–244]. In prostate cancer, β-catenin similarly binds AR and activates AR target gene expression [245]. On the other hand, AR can promote β-catenin nuclear translocation in prostate cells [238]. Mutations in components of the Wnt pathway have also been found in prostate cancer. In stark contrast to colorectal cancer, mutations in APC are rarely detected [246], and instead N-terminal stabilizing mutations of β-catenin are much more frequent [245,247]. Mutant β-catenin induces hyperplasia, squamous cell trans-differentiation and prostate intraepithelial neoplasia (PIN) in mice, suggesting that β-catenin can induce neoplastic transformation in the prostate [248,249]. β-catenin activity is also regulated by other molecules in prostate cancer. For example, growth factors such as IGF and HGF activate β-catenin [250,251]. The tumor suppressor PTEN, which is frequently mutated in prostate cancer, inhibits β-catenin signaling [250]. This suggests some degree of cross-talk

between the Wnt and PI3K pathways in the context of prostate cancer.

Liver cancer

Wnt signaling also plays a central role in regulating liver cell proliferation during development [252–254] and in governing essential functions of the adult liver [255–257]. Moreover, aberrant reactivation of Wnt signaling due to accumulation of β-catenin is evident in many different tumors of the liver [258]. Mutations in the β-catenin and Axin genes that lead to constitutive activation of β-catenin have been found in hepatocellular carcinoma (HCC) and hepatoblastoma. In addition, frequent overexpression of the Wnt receptor Fz7 is a common early event in hepatocarcinogenesis [259,260]. Genotype-phenotype correlation analysis in hepatocellular adenoma showed that mutation of β-catenin occurs in only 12% of adenomas but in 46% of these adenomas progressed to HCC [261], suggesting a role for β-catenin in the progression of pre-cancerous lesions to HCC. Furthermore, simultaneous mutation of β-catenin and H-ras leads to 100% incidence of HCC in mice [262]. These findings suggest that the aberrant Wnt signaling is important in the progression of HCC.

Skin cancer

Wnt signaling regulates hair morphogenesis. Mice expressing a truncated form of β-catenin have abnormal hair follicle morphogenesis [203]. Pilomatricoma is a common benign skin adnexal tumor showing differentiation towards the matrix cells of the hair follicle. About 75% of pilomatricomas have an activating mutation in β-catenin at the N-terminal phosphorylation site, which results in cytoplasmic accumulation and nuclear translocation of β-catenin, resulting in transcriptional activation of many target genes, such as c-Myc, cyclin D_1 [263].

Isolation of $CD34^+/K14^+$ cells from early mouse epidermal tumors results in a population of cells that are more than 100-fold more potent in initiating secondary tumors than the original heterogeneous mixture of cells isolated from the tumor. These cells express many markers of bulge skin stem cells, suggesting that the $CD34^+/K14^+$ cells might be a type of cancer stem cell. Nuclear β-catenin and high expression level of Axin2, both hallmarks of Wnt signaling, are also evident in these skin tumors. Moreover, their tumor-initiating ability depends on β-catenin signaling as loss of β-catenin results in tumor regression [264].

Research from the last two decades strongly emphasizes the importance of Wnt/β-catenin signaling in stem cell and cancers. Whether Wnt signaling has a general role in cancer stem cells is not yet known. However, clearly a deeper understanding of the molecular mechanisms of Wnt signaling in human cancers will lead to translational research regarding novel methods in cancer diagnosis and treatment.

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