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The Wnt Signaling Pathway in Cancer

Yann Duchartre[1], **Yong-Mi Kim**[1], and **Michael Kahn**[2,3,*]

[1]Children's Hospital Los Angeles, Department of Pediatrics and Pathology, Division of Hematology, Oncology and Bone Marrow Transplantation, Keck School of Medicine, University of Southern California, Los Angeles, California, US

[2]Department of Biochemistry and Molecular Biology, Keck School of Medicine of University of Southern California, Los Angeles, California, US

[3]Norris Comprehensive Cancer Research Center, University of Southern California, Los Angeles, California, US

Abstract

The Wnt signaling pathway is critically involved in both the development and homeostasis of tissues *via* regulation of their endogenous stem cells. Aberrant Wnt signaling has been described as a key player in the initiation of and/or maintenance and development of many cancers, via affecting the behavior of Cancer Stem Cells (CSCs). CSCs are considered by most to be responsible for establishment of the tumor and also for disease relapse, as they possess inherent drug-resistance properties. The development of new therapeutic compounds targeting the Wnt signaling pathway promises new hope to eliminate CSCs and achieve cancer eradication. However, a major challenge resides in developing a strategy efficient enough to target the dysregulated Wnt pathway in CSCs, while being safe enough to not damage the normal somatic stem cell population required for tissue homeostasis and repair. Here we review recent therapeutic approaches to target the Wnt pathway and their clinical applications.

Keywords

Wnt-signaling; Cancer Stem Cells; Microenvironment

1) Introduction

Despite significant progress in cancer treatment and remission rates, numerous hurdles in the management of cancer persist. Resistance to treatment associated with disease relapse and metastasis still represent major critical problems that need to be addressed. A subset of cancer cells: the cancer stem cell (CSC) or cancer-initiating cell (CIC)[1] populations are the

Corresponding author: ymkim@chla.usc.edu.

Conflict of interest

None

key players associated with these problems. CSCs, by definition, share the same properties of self-renewal and pluripotency as normal somatic stem cells (SSCs). Self-renewal constitutes the ability to produce at least one daughter cell identical to the mother cell, thereby retaining its stem cell properties, while the pluripotency of stem cells allows them to differentiate into multiple divergent committed and specialized cell types. CSC may emerge from normal somatic stem cells in the affected tissue or organ system after genetic alterations acquired during DNA replication, via various insults and/or from microenvironmental factors and are believed to be responsible in cancer initiation[1]. Over the past few years, a major effort in cancer research has been to better characterize the CSC population and most importantly to efficiently and safely target these cells. One way to achieve this goal is the identification of major pathways involved in the stemness of CSCs and how to target them without affecting the normal somatic stem cells. As in SSC, CSC properties are governed by the evolutionarily conserved signaling pathways Notch[2], Hedgehog[3] and Wnt/β-catenin[4,5]. Here, we will review recent findings on Wnt signaling and its role in cancer initiation, maintenance and drug resistance and the promise of new compounds targeting this pathway.

2) Cancer Stem Cells and Their Role in Tumorigenesis

Over the past decades, the concept of cancer stem cells has re-emerged and led to impressive efforts in this field of research. Recent advances in the field have decreased doubt about CSC existence[6-9] and their function in many cancers, however, the question of their origin remains highly controversial with two alternative theories. On the one hand, CSCs could result from a genetic alteration in a cancer cell from the tumor bulk, leading to the activation of one or more of the major signaling pathways previously cited, thereby acquiring self-renewal properties[10,11]. On the other hand, CSC could originate from acquired mutations of normal somatic stem cells transforming them into CSCs[1,12]. Both of these hypotheses may potentially apply according to the type of cancer and tissue affected. CSCs, represent a rare population of cells amongst the tumor bulk that are able to maintain the tumor via proliferation and self-renewal[13] capabilities and telomerase expression[13]. They are also known to be more resistant to conventional treatment (chemotherapy and radiotherapy) and responsible for cancer relapse and metastasis[14,15]. Their quiescent state and specific interactions with their microenvironment play a significant role in their drug resistance properties[16]. All these characteristics aid in the explanation of why CSC are thought to be responsible for cancer establishment, progression, drug resistance and relapse[17,18] and are strongly correlated to poor outcome in clinical reports[19,20]. Evidence of CSC existence and tumor initiating properties was first provided by Dick and colleagues in leukemia showing that only a very small proportion of primary acute myeloid leukemia (AML) cells, defined by the $CD34^+$ 38^- markers, was able to initiate disease in immunodeficient mice[21]. These Leukemic Stem Cells (LSC) possessed the self-renewal property of CSC and the capability of pluripotency leading to leukemia[22] but could also give rise to non-LSC populations. Subsequently, over the past decade, a large number of studies have identified CSCs in multiple tumor types, including brain tumors[23], melanoma[24], breast[25], liver[26], pancreatic[27] and colon cancer [28].

3) Wnt signaling in Embryonic Development and Homeostasis

Wnt signaling is involved in numerous fundamental processes essential for embryonic development and normal adult homeostasis. The first member of the Wnt family, initially discovered as the proto-oncogene "Int-1" in mice[29], was found five years later to be the homolog of the "wingless" gene, one of the main regulators of *Drosophila melanogaster* segment polarity[30]. The term "wnt" was created by the fusion of these two gene names. The Wnt family is a highly evolutionarily conserved family of proteins as shown by ectopic expression of Wnt1 from *Drosophila* in other organisms, causing serious developmental issues[31,32]. The human Wnt family is composed of nineteen different cysteine-rich glycoproteins acting as ligands for more than 15 receptors or co-receptors[33]. This signaling pathway has already been shown to be involved in many cellular functions essential for normal organ development including cell proliferation, survival, self-renewal/differentiation etc[34,35]. Very rapidly after discovery of the pathway, multiple dysfunctions and mutation of these pathways were shown to be related to several diseases, including metabolic (e.g. type II diabetes[36], degenerative (e.g. Parkinson's[37], Alzheimer's[38]) and particularly cancers (hepatocarcinoma[39,40], colon cancer[41], leukemias[42].

The Wnt signaling pathway has been extensively studied and reviewed[4,43-44]. The pathway is generally dissected into three sub-pathways: canonical, non-canonical planar cell polarity (PCP) pathway and non-canonical Wnt/calcium pathway. The canonical pathway requires Wnt ligand binding to Frizzled receptors as well as LRP5/6 co-receptors (low density lipoprotein receptor-related protein 5/6) to initiate intracellular signaling via β-catenin nuclear translocation. β-catenin is a highly unstable protein with a tightly controlled cytoplasmic presence. In the absence of Wnt ligands, cytoplasmic β-catenin is targeted by a so-termed degradation complex. This complex is composed of the tumor suppressor Adenomatous Polyposis Coli (APC), the scaffolding protein AXIN and two kinases CK1α (casein kinase 1α) and GSK-3β (glycogen synthase kinase 3 β)[45] (Figure 1A). These last two components are able to phosphorylate β-catenin on several serine and threonine residues in its N-terminus. Phosphorylated β-catenin is then recognized by β-Transducin, which is part of an ubiquitin ligase complex, leading to poly-ubiquitination and proteasomal degradation of β-catenin[46]. Wnt ligand binding to Frizzled receptors in association with LRP5/6 induces Dishevelled (DVL) phosphorylation, which subsequently recruits Axin thereby deconstructing the degradation complex and achieving β-catenin stabilization and subsequent nuclear translocation. In the nucleus, β-catenin can bind members of the TCF/LEF (T-cell Factor/Lymphoid Enhancer Factor) family of transcription factors and recruit the transcriptional Kat3 co-activators p300 and/or CBP (CREB-binding protein) to transcribe Wnt target genes and engender chromatin modifications [47-50] (Figure 1B).

Two different Wnt pathways, qualified as "β-catenin-independent pathways" also co-exist with the canonical Wnt pathway and are more generally associated with differentiation, cell polarity and migration. In the non-canonical Planar Cell Polarity pathway (PCP), Wnt ligands can bind Frizzled receptors and activate small GTPases such as RhoA (Ras homolog gene family member A), RAC (Ras-related C3 botulinum toxin substrate) and Cdc42 (cell division control protein 42), via recruitment and activation of Dishvelled[51] (Figure 2A). The PCP pathway affects the cytoskeleton and triggers the transcriptional activation of target genes responsible for cell adhesion and migration[52]. In the Calcium-dependent pathway, Wnt ligands utilize Frizzled receptors and RYK or ROR (alternative receptors) enhancing cell migration and Wnt canonical pathway inhibition through the management of intracellular calcium flux and activation of calmodulin kinase II (CaMK2), Jun kinase (JNK) and PKC[53] (Figure 2B).

Although dissection into 3 different pathways facilitates our understanding of this highly complex signaling system, it has already been shown that in reality Wnt signaling involves the integration of these three pathways and they all need to be considered to derive a complete vision of the effects of Wnt signaling modulation[54,55,56]. These Wnt pathways are critical in major functions at the embryonic stage of development, including stem cell pool regulation, cell migration and specialization as well as at the adult stage in wound healing, and tissue homeostasis via SSC maintenance (including hair, skin[57] and intestine[58]).

4) The Role of Wnt Signaling in Cancer Stem Cells

Dysfunctional Wnt signaling has been related to the evolution of and maintenance of leukemic stem cells as well as many other different cancers. This is not surprising given the importance of the Wnt pathway in stem cell homeostasis[59]. Examples of aberrant Wnt signaling in cancer stem cell development include the progression of chronic phase CML toward blastic crisis phase due to GSK3β mutations and β-catenin stabilization in GMP cells (granulocyte-macrophage progenitor cells)[60]. A recent study showed that despite the inhibitory effect of tyrosine kinase inhibitor (TKI) on Wnt signaling pathway in CML stem cells, relapses occur in patients at least in part by reactivation of the Wnt pathway[61]. TKI treatment induces a down-regulation of miR29 involved in CD70 promoter methylation. The overexpression of CD70 enhances the transcription of CD27 which is a known activator of the Wnt signaling pathway[62]. Wang *et al*. also showed also that constitutive activation of the canonical Wnt pathway, via expression of a stabilized β-catenin is necessary to generate AML leukemic stem cells from MLL-AF9-transduced progenitors cells[11]. This study suggests that aberrant Wnt pathway activation could give rise to leukemic stem cells (LSC) not only from hematopoietic stem cells (HSC) but additionally from more committed progenitors. Recently, Giambra and colleagues showed, using a Wnt reporter construct expressing GFP under the TCF promoter, that minor subpopulations of bulk T-cell acute lymphoblastic leukemia (T-ALL) had highly activated Wnt/β-catenin pathway signaling and that these cells were able to transplant the disease in a limiting dilution assay[63]. Leukemic stem cells were highly enriched in the GFP^+ Wnt expressing population compared to the GFP^- (ratio of over 200 fold) suggesting that Wnt signaling is also required for T-ALL stem cell self-renewal. In this model, the transcription of the β-catenin seems to be triggered by the transcription factor HIF1-alpha (Hypoxia-Induced Factor 1-alpha) and deletion of HIF1-alpha leads to LSC targeting[63]. Our group recently demonstrated the implication of the Wnt pathway in the self-renewal of B-cell acute lymphoblastic leukemia (B-ALL). The treatment of B-ALL cells with a small molecule that specifically binds to the N-terminal of CBP, ICG-001, inhibits the interaction between β-catenin and CBP leading to differentiation and loss of self-renewal[64]. iCRT14, a novel β-catenin-TCF interaction inhibitor, leads to a decrease in Wnt target gene expression, decreases viability of ALL cell lines in combination with chemotherapy and sensitizes chemoresistant patient samples-derived ALL cells responsible of relapse[65].

In order to efficiently target CSCs, researchers initially focused on ways to identify them. Even if the normal SSCs and CSCs usually express the same cell surface markers[66], some reports have successfully characterized cancer stem-like cells in breast cancer based upon specific marker sets (expression of $CD44^{high}CD24^{low}$)[25]. Both CD44 and CD24 are direct Wnt target genes[67-70]. CD44 acts like a positive regulator of the Wnt pathway by playing on LRP6 localization and activity [68,70,71]. The Wnt signaling pathway also appears to play an important role in another hallmark of cancer stem cells and metastasis, i.e. the epithelial-to-mesenchymal transition (EMT)[72,73,74]. The down-regulation of E-Cadherin (usually tightly associated with β-catenin in normal epithelium) triggers the nuclear translocation of β-catenin and activation of canonical Wnt signaling[75]. The gene slug, a marker gene of EMT, also induces nuclear translocation of β-catenin[76,76]. Moreover, twist and slug, strong activators of EMT are both putative β-catenin targets[77]. Furthermore, a number of Wnt/β-catenin targets genes have been associated with invasion, migration and metastasis (including *S100A4, fibronectin, L1CAM, CD44, MMP7, uPAR*, etc.)[78]. Wnt signaling may also play an important role in the resistance of cancer stem cells to chemotherapy. The promoter sequence of the multidrug resistance gene ABCB1/MDR-1 contains several TCF binding elements triggering its transcription in colorectal cancer[79]. Inhibition of the β-catenin/CBP interaction using the small molecule ICG-001 decreases the expression of Survivin/BIRC5, which is an inhibitor of apoptosis and a target of CBP, leading to eradication of drug resistant ALL cells *in vitro* and prolonged survival of ALL engrafted mice[64]. Similar results have been obtained using ICG-001 with CML LSC (Zhao et al., 2015 Oncogene in the press). Wnt signaling has also been linked to hematopoietic CSC which seem to be dependent on this pathway[11,80]. In CML, Wnt pathway deregulation favors the progression of disease to more advanced phases[81]. The deregulation of Wnt signaling can also occur at the epigenetic level. For example, the promoters of several Wnt pathway inhibitors (i.e. SFRP, DKK and WIF-1) were found to be hypermethylated in ALL and AML, correlating negatively with the survival of these patients[82,83].

5) Wnt signaling and the hematopoietic stem cell niche

Hematopoietic Stem Cell fate is tightly controlled by both internal and external signals, the latter coming mostly from the very specific HSC microenvironment, termed, the hematopoietic niche[84-86]. This niche is composed of various cell types (osteoblasts, osteoclasts, endothelial cells, mesenchymal stem cells, etc.) and communicates actively with HSC via direct contacts (integrins, N-Cadherin, etc.) and soluble factors including Wnt ligands[87-89,90,91]. Even after an extensive research, the role of the microenvironment and Wnt ligands on both HSC and LSC is highly controversial[92,93]. Under physiologic conditions, some studies show the critical role of the canonical Wnt signaling pathway for HSC quiescence and self-renewal maintenance: the expression of the Wnt pathway inhibitor Dickkopf1 (Dkk1), specifically in osteoblasts, leads to a decrease of Wnt signaling in HSC and loss of their stem cells properties, via uncontrolled proliferation and division[94] while activation of Wnt signaling in the stroma induces Notch ligand secretion which activates self-renewal programs in HSC[95,96]. However, other researchers demonstrated that the canonical Wnt pathway was dispensable for the adult hematopoiesis in mouse models[97-99]. On the contrary, the overexpression of β-catenin in the hematopoietic system leads to failure in the maintenance of the HSC pool and to a leukemic-like differentiation block in both the myeloid and lymphoid compartments[100]. Under pathologic conditions, Wnt signaling seems to have different effects according to the leukemia studied. In the chronic myeloid leukemia (CML), Zhang *et al* showed that the microenvironment (mimicked by mesenchymal stem cells (MSC)) reduces apoptosis and improves the engraftment of CML LSC/progenitors treated with imatinib. This protective effect is mediated by direct interaction between leukemic cells and MSC through N-Cadherin and induces activation of the Wnt signaling pathway via stabilization of β-catenin[101]. To corroborate these findings, Heidel *et al.* showed that inhibition of β-catenin allows the targeting of CML LSC that are resistant to imatinib[80]. However, inhibition of extrinsic-Wnt signaling by Dkk1 does not impair homing and/or leukemogenesis of AML LSC or pre-LSC *in vivo* as the translocation t(9;11) induces sufficient cell-intrinsic Wnt signaling to promote leukemia development[93]. The microenvironment may be implicated in many ways as well, either in the initiation of the disease or by favoring LSC proliferation and drug resistance[102,103], thus making it a potentially attractive new target for treatment. However, the role of the niche is still not completely understood. Kode *et al* showed the essential role of osteoblasts in acute myeloid leukemia initiation via Wnt and FoxO1 expression[104]. Recently, Bowers *et al* demonstrated the importance of the bone marrow microenvironment in HSC homeostasis and in leukemia development showing that osteoblast depletion impairs the quiescence and self-renewal of normal HSC and also leads to accelerated leukemia development in a mouse model of CML[105]. Taken together, these data suggest that the microenvironment may represent an attractive alternative to target LSC, notably via modulation of the Wnt signaling pathway. However, further studies are required to fully understand its fundamental role in leukemia establishment and maintenance.

6) Wnt Inhibiting Molecules: Biologics and clinics

After decades of research and discovery on the Wnt signaling pathway, few molecules are now considered to be relatively specific for targeting the Wnt pathway, and to date none has been approved by the US Food and Drug Administration (FDA). Some other FDA-approved molecules, like Non-Steroidal Anti-Inflammatory Drugs (NSAIDS, used for treatment of pain, fever) or vitamin derivatives, demonstrated interesting anti-cancer effects[106,107] and particularly in Wnt-dependent cancers e.g colorectal cancer[108,109]. Cyclooxygenases (COX1 and 2) metabolize arachidonic acid into prostaglandins (PG) that via their G-protein Coupled Receptors can lead to β-catenin stabilization and activation of canonical Wnt signaling[110,110-112]. The inhibition of COX by NSAIDS (aspirin, sulindac or specific COX2 inhibitors like celecoxib) suppresses the synthesis of prostaglandins and thereby inhibits Wnt signaling. These compounds, especially celecoxib, also showed COX-independent anticancer effects notably in a xenograft model of COX-2-deficient tumors[113-117]. NSAIDS have the capacity to decrease the number of polyps in a mouse model of Familial Adenomatous Polyposis (FAP) mouse, where the APC gene is truncated and Wnt/β-catenin signaling constitutively actived[118,119]. FAP patients treated for 6 months with the NSAID sulindac showed a reduction in nuclear β-catenin in polyps and a reduction in polyp formation[120-123]. The aspirin derivative NO-ASA (NO-releasing aspirin) showed even better efficacy in reduction of polyp formation *in vitro* and *in vivo* possibly via disruption of the β-catenin/TCF complex without any observable toxicity to the normal intestine[124-126].

Retinoids, produced from vitamin A metabolism, demonstrated anti-cancer effects at least in part via Wnt signaling pathway inhibition[127]. 1α,25-dihydroxy-vitamin D3, the active form of vitamin D, demonstrated tumor suppressor activity, notably by formation of a transcriptional complex able to bind β-catenin and thereby enhancing the expression of E-cadherin. These effects lead to retention of β-catenin in the cytoplasm, resulting in inhibition of the Wnt pathway in breast and colon cancers[128]. A novel humanized antibody (UC-961, cirmtuzumab) targeting the Receptor tyrosine kinase-like Orphan Receptor 1 (ROR1), expressed by the chronic lymphocytic leukemia cells (CLL) but not on normal tissues, showed anticancer effects in a CLL animal model[129]. This antibody recently entered a Phase I clinical trial to determine the safety and the effects of this antibody and is currently recruiting (NCT02222688).

Besides these FDA-approved non-specific Wnt inhibitors, several molecularly targeted agents have been developed and have entered pre-clinical or clinical trials. Dvl, being one of the key regulators of the Wnt canonical pathway, is a focus of numerous studies and has engendered the development of several inhibitors. The PDZ domain of Dvl plays an essential role in Dvl-Frizzled receptor interactions and the intracellular transduction of the Wnt signal. Some inhibitors of this PDZ domain (NSC 668036, FJ9, 3289-8625 – Figure 3), discovered by *in silico* screening, showed the ability to inhibit the Wnt pathway *in vivo*[130-132]. Other compounds, designed to inhibit key steps in the Wnt pathway have also been designed. LGK974 is a porcupine (PORCN) inhibitor, which entered into a phase I clinical trial in 2011 (Novartis, NCT01351103, recruitment phase). Porcupine is a member of the membrane-bound O-acetyltransferase (MBOAT) family and is responsible for lipid modification of Wnt and secretion[133,134]. The trial will investigate the effects of LGK974 on the Wnt signaling pathway in patients affected with Wnt-dependent cancers (pancreatic adenocarcinoma, BRAF mutant colorectal cancer) (clinicaltrials.gov). Recently, another PORCN inhibitor, ETC-1922159 (ETC-159), developed in a collaboration between the Agency for Science, Technology and Research (A*STAR) and Duke-National University of Singapore Graduate Medical School (Duke-NUS) entered into a phase I clinical trial in Singapore. The first patient was dosed on Jun 18, 2015. ETC-159 inhibits Wnt secretion and activity and is highly efficient preclinically in different cancers driven by Wnt signaling and notably in R-spondin translocation colorectal cancers[135]. The tankyrase inhibitors (XAV-939 and IWR-1) stabilize axin and induce the degradation of the β-catenin[136] and may act as anti-tumor drugs also by participating in telomere shortening[137].

Among the few agents already in clinical trials, two were developed by Oncomed Pharmaceuticals Inc. OMP-18R5 (Vantictumab) is a fully humanized antibody directed against minimally five different Frizzled receptors. In pre-clinical studies, OMP-18R5 demonstrated anti-proliferative effects in various human tumors model (lung, pancreas, breast and colon) and had synergistic effects with conventional chemotherapy[138]. The results of the first Phase Ia showed a decrease in Wnt pathway gene expression and increased expression of differentiation genes associated with some adverse events including fatigue, vomiting, diarrhea, constipation, nausea and abdominal pain (ASCO, 2013). This compound is now in Phase Ib trials in combination with standard chemotherapy for solid tumors (breast, lung and pancreas cancers). OMP-54F28, another agent developed by Oncomed Pharmaceuticals, is a recombinant fusion protein containing the extracellular ligand binding domain of human Frizzled 8 receptor fused to a human IgG1 Fc fragment[139]. OMP-54F28 can bind native Fzd8 receptor's ligands and thereby inhibit Wnt signaling. Preclinical studies demonstrated the anti-tumor efficacy of OMP-54F28: reduction of tumor growth and decrease of CSC frequency as a single agent and in combination with other chemotherapeutic agents. A phase I trial (NCT01608867) is currently ongoing. It is a dose escalation study in patients with advanced solid tumors. Subjects will be assessed for safety, immunogenicity, pharmacokinetics, biomarkers, and efficacy (NCT01608867). It appears that the most common adverse events are fatigue, muscle spasms, alopecia, nausea, decreased appetite and dysgeusia (http://www.eurekalert.org/pub_releases/2014-05/uocd-rip053014.php). Additionally, patients are followed for bone density evolution, as bone fracture was observed in one patient at the highest tested dose (20mg/kg every three weeks after 6 cycles). Three Phase 1b studies have been started to check the dose escalation of OMP-54F28 in ovarian (NCT02092363), pancreatic (NCT02050178) and hepatocellular (NCT02069145) cancers in combination with respective conventional chemotherapy.

Wnt signaling can also be modulated very late in the pathway. Our group used a secondary structure-templated chemical library to identify ICG-001 which can efficiently modulate the Wnt pathway[140]. Despite the huge homology between the two Kat3 co-activator proteins CBP and p300, ICG-001 was shown to bind specifically to the cyclic AMP response element-binding protein (CBP) and not to the related transcriptional coactivator p300[140,141]. This molecule disrupts the ß-catenin/CBP complex and increases the proportion of ß-catenin/p300 leading to down-regulation of survivin/BIRC5 mRNA and specific apoptosis in colon cancer cells *in vitro* and *in vivo*. Recently, Prism Pharmaceuticals developed a second generation ß-catenin/CBP inhibitor PRI-724. In a Phase Ia safety study in colon cancer, this compound was able to decrease in a dose-dependent manner the expression of survivin/BIRC5 in circulating tumor cells, with an acceptable toxicity profile (ASCO, June 2013 and NCT01302405[142]). Three patients had stable disease for 8, 10 and 12 weeks. Three Phase I/II trials are ongoing in patients with AML/CML (NCT01606579, alone or in combination with AraC or dasatinib), with advanced or metastatic pancreatic adenorcarcinoma (NCT01764477, in combination with Gemcitabine) and in patients with newly diagnosed metastatic colorectal cancer (NCT02413853, in combination with bevacizumab, leucovorin calcium, oxaliplatin, and fluorouracil). A Phase I dose escalation trial in patients with HCV-induced cirrhosis is also on going (NCT02195440).

7) Concluding remarks

After more than 30 years of discovery and investigation, the complexity of the Wnt signaling pathway is clear. Many of its components have been revealed and its implications in a broad range of diseases have been described. However, to date no therapeutic agent is available on the market that specifically and efficiently targets this pathway. In the past 5 years, some Wnt signaling modulating agents, targeting different key steps in the pathway (Wnt secretion, signal transduction or ß-catenin transcriptional activity) entered the clinic to determine inhibitory efficacy and also critically safety. Indeed, Wnt signaling is a highly evolutionarily conserved pathway involved in multiple crucial homeostatic functions, suggesting that targeting this pathway may induce serious adverse events (e.g. OMP-54F28 trials, where all patients were monitored for bone mineral density modification (BMD) and turnover and received zoledronic acid when their BMD declines (NCT01608867)). Moreover, many of the potential targets like ß-catenin are also implicated in others critical functions (cell-cell adhesion, development, self-renewal…)[143,144]. Clearly, precise modulation of the Wnt pathway will be necessary to balance anti-tumor efficacy with adverse events and will be a challenge for ongoing and future clinical trials. Despite these concerns, new regulators of the Wnt signaling cascade offer the opportunity for us to increase our comprehension of this exceedingly complex pathway and potentially for the treatment of Wnt-related diseases including cancer.

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Biographies

Michael Kahn

USC Norris Comprehensive Cancer Center, USC Center for Molecular Pathways and Drug Discovery, University of Southern California, Los Angeles, California 90033, USA.

Michael Kahn is the Provost Professor of Medicine and Pharmacy at the University of Southern California (USC), USA. Prior to joining USC, he was at the University of

Washington, Seattle, USA, as well as the scientific director at the Institute for Chemical Genomics. His laboratory utilizes forward chemical genomics strategies to dissect complex signalling pathways. His laboratory has been working extensively in the area of WNT signalling for the past 15 years.

Yong-Mi Kim

Yong-mi Kim is an Associate Professor of Pediatrics and Pathology at the University of Southern California, Children’s hospital Los Angeles. Her laboratory has been working in the area of drug resistance and the microenvironment of leukemia.

Yann Duchartre

Yann Duchartre graduated with a Ph.D. in Cell Biology and Physiology from University of Bordeaux (France) in December 2012. After a first postdoctoral position looking for new therapeutic approaches in CML at the Beckman research Institute of City of Hope, he joined the Children’s Hospital of Los Angeles to further study the pre B-ALL cells mechanisms of resistance to therapy.

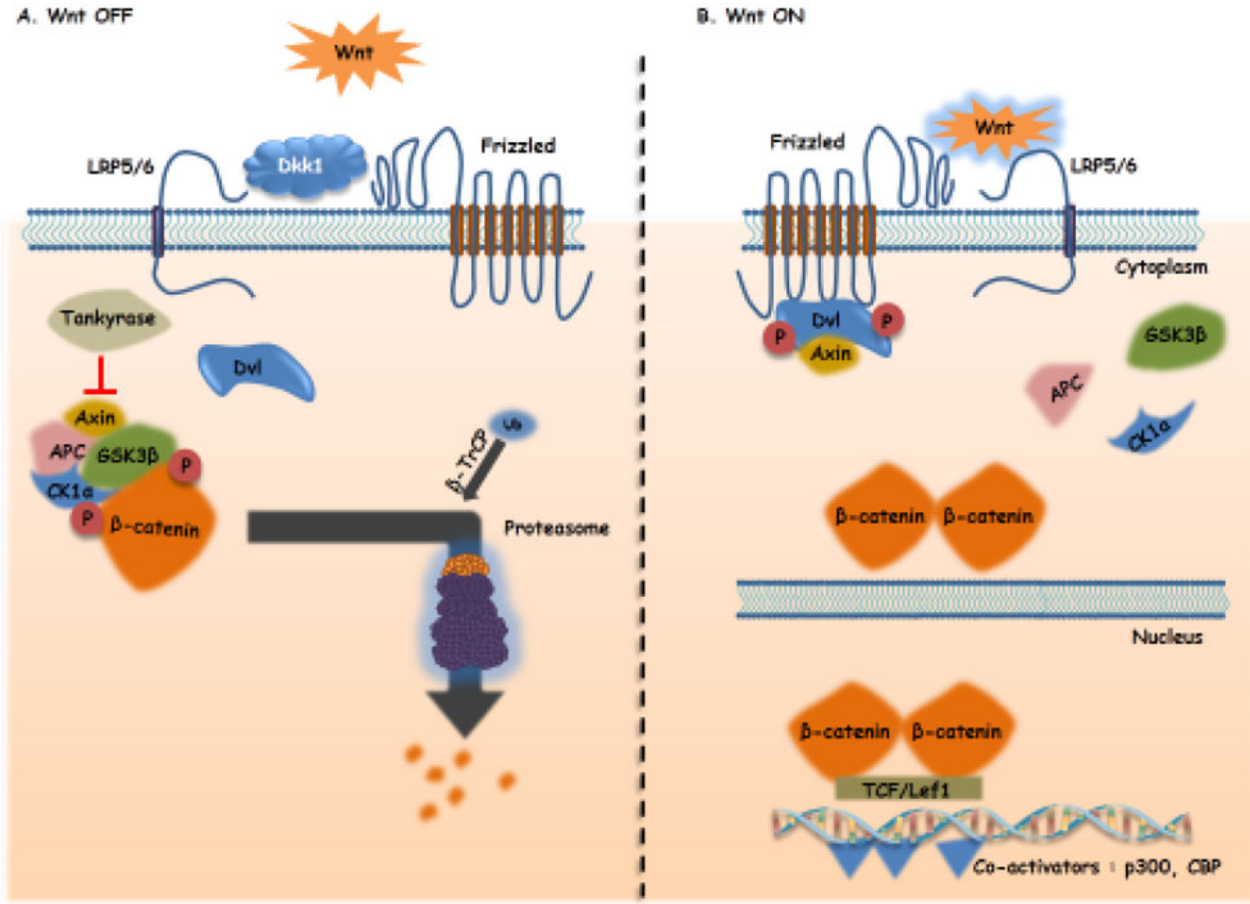

Figure 1.

A: "Wnt Off". In the absence of Wnt ligands, a destruction complex composed of Axin-1 and its tumor suppressor partners Adenomtous Polyposis Coli (APC), Glycogen synthase kinase 3 beta (GSK3B) and Casein kinase 1 (CK1α) is formed. The destruction complex phosphorylates ß-catenin and targets it for proteasomal degradation regulating the cytoplasmic level of ß-catenin.

B: "Wnt On". Wnt ligands bind to the Frizzled/ Lrp5/6 (Low density lipoprotein receptor-related proteins 5 or 6) receptors leading to the phosphorylation of a negative regulator of the destruction complex, Dishevelled (Dvl). Dvl recruits Axin, inhibiting its interaction with other components of the destruction complex. ß-catenin is then free to accumulate in the cytoplasm and translocates to the nucleus, where it activates the trancscription of Wnt target genes after association with transcription factors of the TCF/Lef family and co-activators such as CBP (cyclic AMP response element-binding protein) and p300. Arrows indicate activation/induction, blunt ended lines indicate inhibition/blockade.

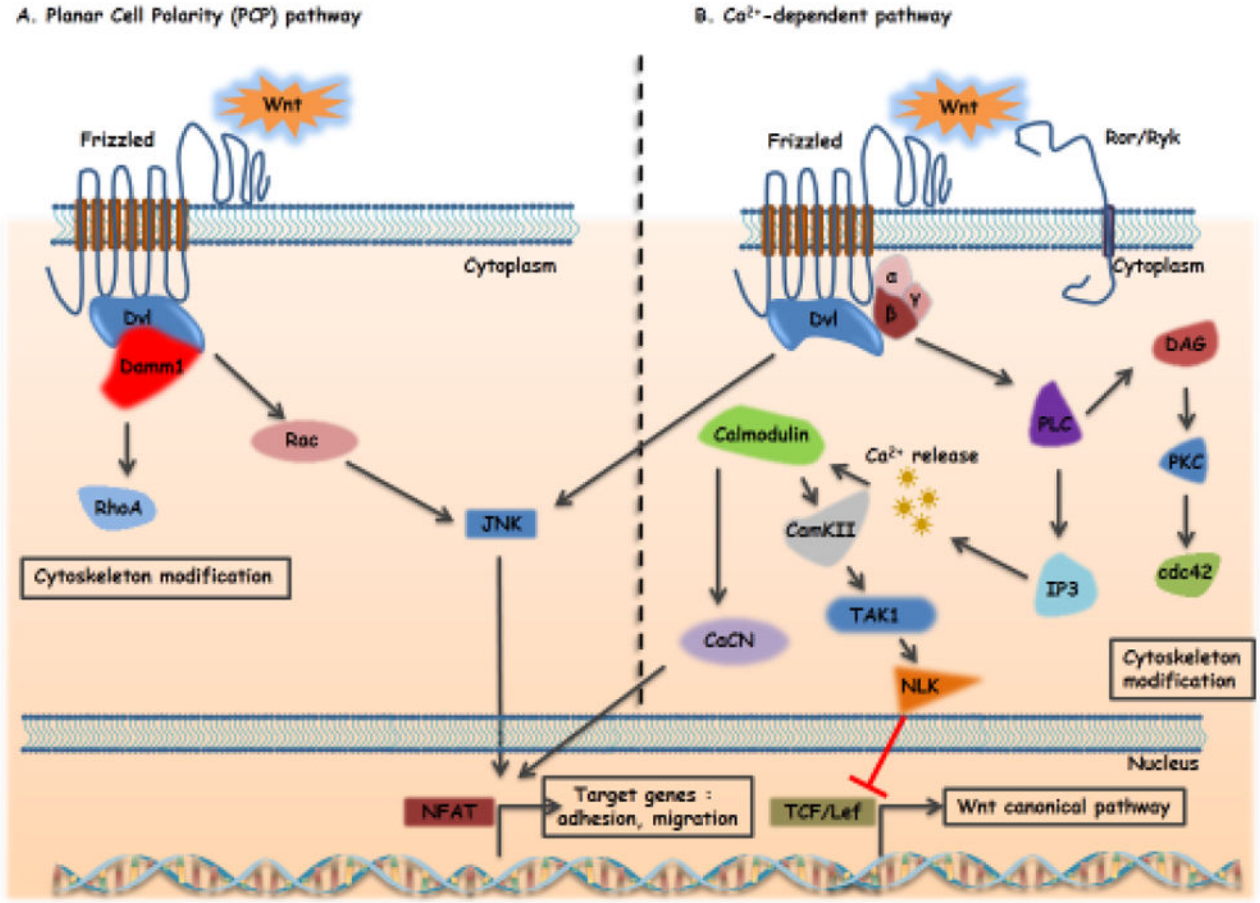

Figure 2.

A: Non-Canonical Wnt-signaling: Non-canonical Wnt/PCP (planar cell polarity) pathway. Wnt ligand binding to frizzled receptors leads to activation of Dishevelled (Dvl) which recruits DAAM1 (Dishevelled associated activator of morphogenesis 1) enhancing the stimulation of GTPases Rac (Ras-related C3 botulinum toxin substrate) and RHOA (Ras homolog gene family member A) leading to actin cytoskeleton rearrangement. In addition, Dvl activates Rac and finally JNK (c-Jun-N-terminal-kinase) thereby modulating cell migration.

B: Non-canonical Wnt/calcium pathway. Wnt ligands bind to frizzled receptors and Ror/Ryk co-receptors, activating Dvl and trimeric G-proteins (Gα,β,γ). This leads to the generation of IP3 (inositol 1,4,5-triphosphate) and DAG2 (diacylglycerol) through PLC (Phospholipase C) activation. IP3 triggers the release of calcium ions (Ca^{2+}) from the endoplasmic reticulum activating calmodulin and subsequently CAMKII (calcium/ calmodulin- dependent kinase II), TAK-1 (TGF-β activated kinase 1) and NLK (Nemo-like kinase) thereby inhibiting the canonical Wnt pathway. Moreover, calmodulin activation stimulates calcineurin and NFAT (Nuclear Factor of Activated T-cells) involved in adhesion and migration processes. This pathway activates also PKC (Protein Kinase C) and Cdc42 (cell division control protein 42) rearranging the actin cytoskeleton. Arrows indicate activation, blunt ended lines indicate inhibition/blockade.

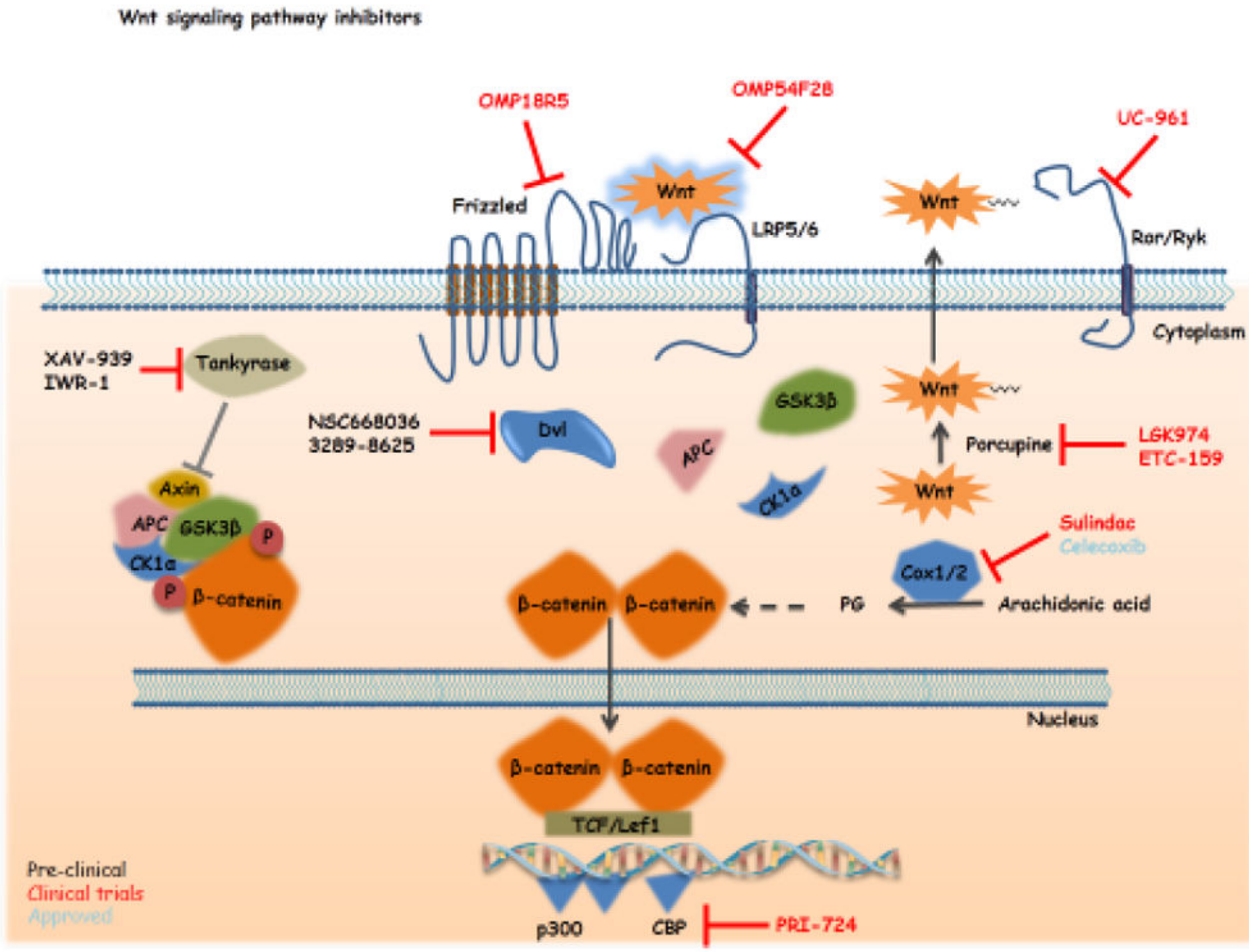

Figure 3. Schematic of Wnt inhibitors currently in clinical trials

This figure summarizes the different Wnt pathway modulators with variable specificities and at different stages of development (fully described in the main text). The current agents in clinical trials are the porcupine inhibitor LGK974, which inhibits Wnt posttranslational palmitoylation and secretion. OMP18R5, is a fully humanized monoclonal antibody specifically binding to multiple Frizzled (Fzd) receptors and OMP-54F28 is a Fc fusion protein with Fzd8, which binds all Wnt ligands. Both inhibit the intracellular transduction of the Wnt signal. PRI-724 specifically targets the β-catenin transcriptional co-activator CBP thereby blocking their interaction. UC-961 (cirmtuzumab) is a humanized antibody targeting the targeting specifically the Wnt receptor ROR1. Arrows indicate activation/induction, blunt ended lines indicate inhibition/blockade.