Minireview

The physiological role of Wnt pathway in normal development and cancer

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Impact statement

Wnt pathway does not only play a critical role in mammalian development but has been hijacked by cancer cells and the tumor microenvironment to promote tumor progression and metastasis. Recent evidence supports further interrogation of the Wnt pathway for bench-to-bedside translation into cancer therapy. This review highlights the role of the Wnt pathway in normal development and tumorigenesis, along with an overview of new therapies currently undergoing clinical trials.

Abstract

Over the decades, many studies have illustrated the critical roles of Wnt signaling pathways in both developmental processes as well as tumorigenesis. Due to the complexity of Wnt signaling regulation, there are still questions to be addressed about ways cells are able to manipulate different types of Wnt pathways in order to fulfill the requirements for normal or cancer development. In this review, we will describe different types of Wnt signaling pathways and their roles in both normal developmental processes and their role in cancer development and progression. Additionally, we will briefly introduce new strategies currently in clinical trials targeting Wnt signaling pathway components for cancer therapy.

Introduction

The Wnt proteins were first identified as Wingless (Wg) protein in *Drosophila* through genetic screenings and phenotypic analysis.[1] Segmentation pattern defects in larva with Wg mutations indicated that these proteins played an important role in early embryonic development.[2] Work in mice first identified the oncogenic properties of Wnt proteins through analysis of genomic integration of the mouse mammary tumor virus (MMTV); this study showed that integration of MMTV near the *Int1* loci (now known as *Wnt1*) induced tumorigenesis.[3] Further studies determined the mouse proto-oncogene *Int1* and the Wg gene in *Drosophila* to be homologous.[4] These studies laid out the significance of *Wnt* genes in developmental and oncogenic processes. Since, Wnt signaling pathways have been shown to play a role in many other processes such as tissue homeostasis, apoptosis, cell motility, and cancer progression.

Overview of Wnt signaling pathways

Wnt proteins and Frizzled (Fzd) receptors

Wnt proteins. Wnt proteins are cysteine rich molecules ∼40 kDa in size expressed by all metazoan species containing several conserved features, with 19 independent Wnt genes identified in mice and humans.[5] Following synthesis and translocation to the endoplasmic reticulum (ER), Wnt proteins go through multiple post-translational modifications (PTMs), which are important for secretion.[6,7] The most prominent PTMs in Wnt proteins that occur in the ER are glycosylation and acylation. Variable glycosylation processing allows for differentiation between basolateral and apical secretion.[8] It used to be thought that acylation occurred at both cysteine and serine residue of Wnt molecules; however, recent studies showed that monopalmitoylation occurs only at a conserved serine residue.[7,9] An ER locating O-acyltransferase Porcupine (PORCN) is

responsible for transferring the palmitoleic acid onto the Wnt molecules.[10] PORCN mutations abolish Wnt palmitoylation and lead to embryonically lethal phenotypes in mice knockouts.[11]

After PTMs in the ER, Wnt molecules are ready for secretion through vesicular trafficking in cells.[6] In the ER, palmitoylated Wnt molecules bind to a sorting receptor Wnt ligand secretion (WLS) modulator, which requires palmitoylation on the conserved serine residue of Wnt.[12,13] WLS binds to Wnt molecules and accompanies them to the Golgi, where cargo p24 family proteins aid in promoting the exit and re-localization of WLS–Wnt complexes to the cell surface.[14,15] V-ATPase-mediated vacuolar acidification allows for release of Wnt molecules from WLS and p24 proteins allowing for subsequent release into the extracellular environment where they bind to and activate transmembrane receptors, initiating a variety of Wnt signaling pathways.[16]

Frizzled receptors. Fzd proteins are seven-pass transmembrane ligand-activated receptors with a conserved extracellular cysteine rich domain (CRD) at the N-terminal and an intracellular C-terminal domain. This protein family consists of 10 members where each Fzd receptor has a preferred Wnt ligand.[17,18] Structural studies identified the general hand-like structure of Wnt proteins with thumb and index fingers pinching the extracellular CRD of Fzd receptors (Figure 1).[20] The N-terminal CRD of Fzd proteins recognizes the unsaturated palmitoleic acid on the Wnt molecule. The palmitoleic acid moiety on Wnts is suggested

to determine the Wnt–Fzd specificity, as it inserts into a hydrophobic crevice in the CRD of the Fzd receptor.[20] Binding to Wnt bridges two CRD monomers on Fzd, which leads to the dimerization of the Fzd proteins.[21] Additionally, Wnt binding to Fzd induces the formation of dimeric/multimeric structure with Wnts and other co-receptors, such as low density lipoprotein receptor related protein 5/6 (LRP 5/6).[22–24] Once activated, the C-terminal domain of Fzd proteins interacts with downstream cellular proteins like the Dishevelled (DVL) protein or non-receptor tyrosine kinases for signal transduction.[25,26] Additionally, Wnt binding to Fzd receptors has been shown to be coupled with heterotrimeric G proteins, triggering G protein-mediated signal transduction.[27,28] To date, the signaling pathways initiated by Wnt–Fzd binding include the canonical Wnt/β-catenin pathway and other non-canonical pathways.
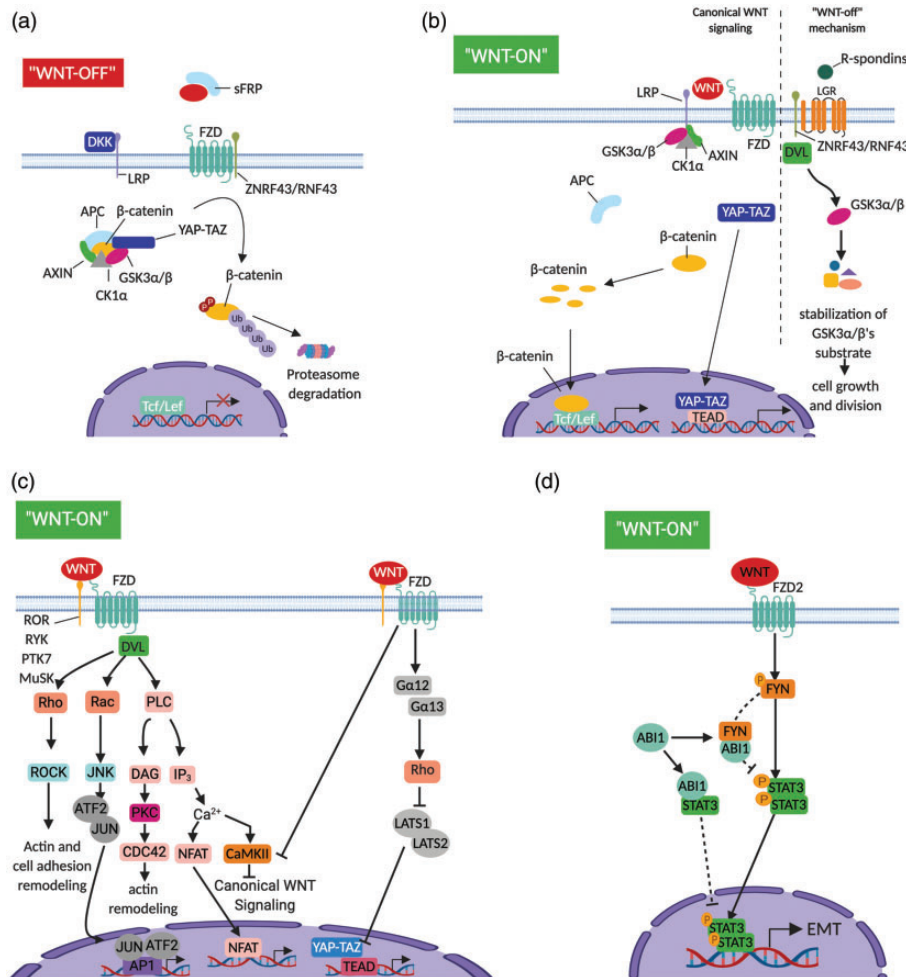
Wnt pathways

Wnt pathways are generally classified as either canonical (β-catenin dependent) or non-canonical (β-catenin independent); however, the boundaries between these pathways vary in different cellular context (Figure 2).[19] Despite the controversy surrounding Wnt pathway classification, the initiation of Wnt signaling events requires a Wnt molecule binding to a Fzd receptor and/or other co-receptors to initiate signal transduction.

The canonical Wnt/β-catenin pathway. The Wnt/β-catenin pathway is the most studied Wnt-mediated pathway and has been shown to play critical roles in developmental and oncogenic processes. The hallmark of this pathway is the stabilization and nuclear translocation of β-catenin in the cell (Figure 2(a) and (b)). In the absence of Wnts, β-catenin levels remain low in the cell due to the presence of a ubiquitin-dependent proteasome degradation complex, initially thought to be composed of scaffold proteins Axin and APC with kinases CK1α and GSK3α/β.[31] However, a study in 2010 illustrated the involvement of the Hippo signaling pathway components, the Yes-associated protein (YAP) and the transcription co-activator with PDZ-binding motif (TAZ) in stabilizing the destruction complex through binding to Axin.[32] Kinase components of the destruction complex phosphorylate conserved N-terminal serine/threonine residues on β-catenin, which leads to recruitment of E3-ubiquitin ligase SCF[β-TRCP] for ubiquitination and subsequent proteasome-mediated degradation of β-catenin.[33,34]

Wnt binding to Fzd-LRP5/6 complex triggers recruitment of the cytoplasmic protein DVL to the intracellular C-terminal domain of Fzd.[35] DVL recruits the Axin–GSK3 complex to the membrane by interacting with Axin. After that, the PPPSP motif on the LRP5/6 intracellular tail is phosphorylated by GSK3 and another kinase complex CDK14–Cyclin Y and eventually, the LRP5/6 intracellular tail is further phosphorylated by the membrane-anchored kinase casein kinase 1 γ.[36–38] Earlier studies showed that the phosphorylated tail of LRP5/6 has high affinity for Axin, thereby leading to destabilization of the destruction
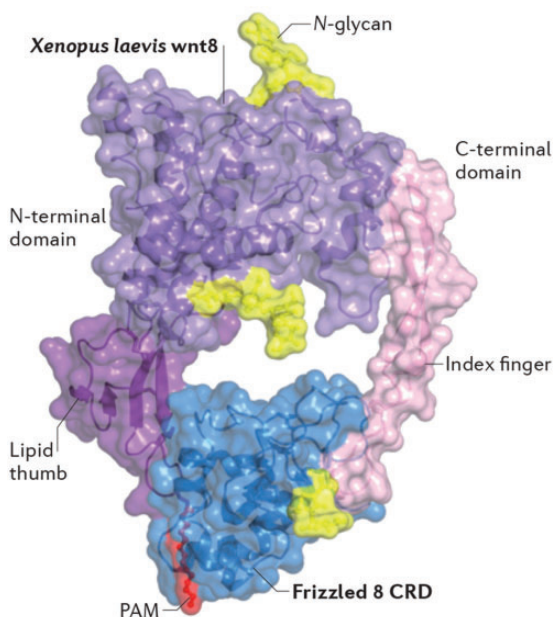
Figure 1. Crystal structure of the Wnt–Frizzled binding complex. Overall cryo-EM structure of the interaction between *X. laevis* Wnt8 and the CRD of Frizzled 8 receptor, shown in a 'face on' presentation. Purple: the core of the *X. laevis* Wnt8. Deep purple: lipid thumb domain on the amino-terminal (N-terminal) of the *X. laevis* Wnt8. Light pink: The index finger domain on the carboxy-terminal of the *X. laevis* Wnt8. Red: the palmitoleic acid motif on the *X. laevis* Wnt8. Yellow: *N*-glycans motifs on the *X. laevis* Wnt8. Blue: The CRD of the Frizzle 8 receptor. Source: Figure cited from Niehrs.[19]
CRD: cysteine rich domain; PAM: palmitoleic acid motif.

Figure 2. Wnt signaling pathways. (a) In the absence of Wnt molecules, the canonical Wnt/β-catenin pathway is inactivated due to the existence of the β-catenin destruction complex, which is made of scaffold protein APC, Axin, Hippo pathway proteins YAP–TAZ, and kinases CK1a, GSK3a/b. The destruction complex mediates β-catenin phosphorylation for its following proteasome degradation. The canonical Wnt pathway can be negatively regulated by extracellular Wnt-binding protein like sFRPs, or by proteins like Dkk1 that downregulate membrane co-receptors (LRP5/6) of the canonical pathway. (b) In the presence of canonical Wnt molecules, Wnt binding to Fzd receptors promotes dimerization of the Fzd receptor to its co-receptor LRP5/6. Then, LRP5/6 recruits components of the β-catenin destruction complex to its cellular carboxy-terminal tails, which dissociates the destruction complex and leads to β-catenin stabilization and accumulation in cells to allow β-catenin nuclear translocation for its mediated downstream transcriptional responses. Also, the dissociation of the destruction complex prevents GSK3a/b's substrates phosphory-lation and degradation, which is called Wnt–STOP mechanism. (c) In the presence of non-canonical Wnt molecules, Wnt binding to Fzd receptors promotes dimer-ization of the Fzd receptor to variable types of co-receptors for recruitment of the adaptor protein DVL. The recruitment of DVL triggers multiple β-catenin independent non-canonical downstream events to mediate cellular actin remodeling processes, variable types of transcriptional responses, or canonical Wnt pathway down-regulations. (d) For multiple types of cancers, a novel Wnt5a-mediated non-canonical pathway is found to mediate the EMT. In this pathway, Wnt5A binding to Frizzled-2 receptors activates Src family kinases Fyn by promoting its phosphorylation. Then, Fyn activates the STAT3 to trigger STAT3-mediated EMT processes. This non-canonical pathway is negatively regulated by Abl interactor 1 (ABI1). ABI1 can directly interact with either Fyn or STAT3, although roles of these interactions in regulating the Wnt5a–STAT3 pathway remain unknown. (A color version of this figure is available in the online journal.) Source: Figure modified from Wang *et al.*[29] and Murillo-Garzon and Kypta.[30]

DVL: Dishevelled; EMT: epithelial–mesenchymal-transition; JNK: JUN-N-terminal kinase; LATS1: larger tumor suppressor 1; LATS2: larger tumor suppressor 2; LGR: leucine-rich repeat-containing G-protein coupled receptor; LRP: lipoprotein receptor related protein; RNF43: ring finger protein 43; ROCK: Rho-associated kinase; sFRP: secreted frizzled related protein; ZNRF3: zinc and ring finger 3.

complex and the stabilization of β-catenin.[39] More recent studies showed that the phosphorylated tail of LRP5/6 directly blocks GSK3 activity to prevent β-catenin degrada-tion.[40] Both of these studies highlight the importance of LRP5/6-mediated β-catenin stabilization but through dif-ferent mechanisms. More studies need to be conducted to determine if LRP5/6-mediated mechanisms can act simul-taneously, or if different cellular states favor one over the other.

Once stabilized and accumulated in the cell, β-catenin is translocated to the nucleus where it serves as a

transcriptional co-activator by interacting and modulating the activity of T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) DNA binding proteins.[41,42] TCF/LEF proteins are repressors of Wnt responsive genes and their binding to β-catenin allows for transcription of Wnt respon-sive genes, promoting cell differentiation and proliferation during developmental and oncogenic processes.[43,44]

Non-canonical Wnt pathways. Non-canonical Wnt path-ways or β-catenin independent pathways are those that do not depend on β-catenin/Tcf or β-catenin/Lef binding for

modulating downstream signaling. Some non-canonical Wnt pathways elicit transcriptional responses, while others directly regulate cellular actin dynamics and modulate actin-related events such as polarity, migration, and adhesion. Currently, five non-canonical Wnt pathways have been identified: Wnt/PCP pathway, Wnt/Ca^{2+} pathway, Wnt/ROR pathway, Wnt/YAP–TAZ pathway, and Wnt–FYN–STAT3 pathway (Figure 2(c) and (d)).

Wnt/PCP signaling pathway. Planar cell polarity (PCP) refers to the polarization of epithelial cells along the plane of a tissue, a process highly controlled by Wnt/PCP signaling. PCP formation involves reorganization of actin dynamics as well as transcriptional responses of several downstream genes. During development, Wnt/PCP signaling is maintained by the asymmetric distribution of highly conserved core Wnt/PCP components, which leads to asymmetric actin rearrangements.[45–47] In the dorsal sites of cells, Wnt binding to the Fzd receptor recruits DVL to the C-terminal tail of the Fzd receptors, which triggers activation of Rho family GTPases and JUN-N-terminal kinase (JNK).[48,49] The downstream output of this pathway is Rho-associated kinase (ROCK) mediated cellular actin remodeling and JNK-mediated transcriptional responses (Figure 2(c)). Negative regulators of Wnt/PCP signaling tetraspanin-like transmembrane scaffold protein Van Gogh (Vangl in mammals) and intracellular scaffold Prickle (Pk) form a complex to antagonize the signal transduction of the Fzd/DVL complex in the proximal sites of the cell.[50] Vangl and Pk recruit other proteins to negatively regulate the Wnt/PCP pathway either by mediating DVL ubiquitination and proteasome degradation, or directly altering Rho GTPase-mediated actin cytoskeleton remodeling and JNK-mediated transcriptional responses.[51–54] Negative regulatory mechanisms of the Wnt/PCP pathway are still not well understood and require further investigation.

Wnt/Ca^{2+} pathway. The Wnt/Ca^{2+} pathway (Figure 2(c)) was first noticed with the finding that in some instances Wnt–Fzd binding activated heterotrimeric G proteins and triggered Ca^{2+} release from the ER through phosphatidylinositol signaling.[55] Further work indicated that the binding of Wnt to Fzd receptors recruits DVL to activate heterotrimeric G proteins by GEFs through direct protein–protein interactions. The activated G proteins trigger phosphatidylinositol signaling to release Ca^{2+} from the ER to the cytosol, which further promotes actin rearrangement in cells, or triggers downstream transcriptional responses.[56–58] In addition, the Wnt/Ca^{2+} was suggested to inhibit the canonical β-catenin signaling, suggesting negative feedback inhibition exerted by activation of non-canonical Wnt pathways over canonical Wnt pathways.[59] However, there are few primary studies addressing the function of this pathway and its existence remains controversial, and believed to only play a role under some extreme circumstance.

Wnt/ROR pathway. Receptor tyrosine kinase-like orphan receptors (RORs) are transmembrane receptor tyrosine kinases that contain an extracellular CRD for Wnt recognition and an intracellular tyrosine kinase domain.[60] There are two well-known RORs: ROR1 and ROR2. RORs, especially ROR2, can serve as co-receptors for Fzd receptors to trigger non-canonical Wnt signaling.[61] Similar to the activation mechanism of canonical Wnt pathway, ROR2 binding to Fzd promotes serine/threonine phosphorylation of the ROR2 intracellular tail for the recruitment of downstream scaffold proteins, thereby initiating signal transduction.[61] Meanwhile, ROR itself can also serve as an individual receptor for Wnt molecules to trigger multiple non-canonical signal events, like actin remodeling and JNK-mediated transcriptional responses (Figure 2(c)).[62–64]

Wnt/Yap–TAZ pathway. Through major Hippo pathway modulators larger tumor suppressor 1 and 2 (LATS1/2) mediated phosphorylation, the YAP–TAZ complex is ubiquitinated and degraded.[65] A recent study indicated that the YAP–TAZ can be activated through small GTPase Rho-mediated LATS1/2 inhibition.[66] Based on that, a following study showed the novel activation mechanism of the YAP–TAZ pathway through Wnt signaling.[67] The Wnt5a/b or Wnt3a binding to the Fzd receptors promotes formation of the Wnt–Fzd–ROR complex, which activates G protein G$_{\alpha 12/13}$. The activated G$_{\alpha 12/13}$ turns on Rho's activity, which inhibits LATS1/2 and stabilizes the YAP–TAZ complex, allowing for activation of downstream transcriptional responses (Figure 2(c)).

Wnt–FYN–STAT3 pathway. The epithelial–mesenchymal-transition (EMT) is a reversible process in which epithelial cells adopt mesenchymal characteristics through alterations in morphology, cellular architecture, adhesion, and migratory capacity.[26] During tumor progression, cells undergoing EMT obtain enhanced invasiveness, which allows them to invade surrounding tissue. Through screening multiple cancer cell lines and high-grade tumors, Gujral *et al.*[26] identified overexpression of Wnt5A/B and their receptor Fzd2, and that Wnt5A/B binding to Fzd2 initiates an unrecognized non-canonical Wnt pathway which promotes EMT phenotypes. In this study, Gujral *et al.* illustrated that Wnt5A/B binding to Fzd2 promotes tyrosine phosphorylation on the C-terminal tail of the Fzd2 receptor, which recruits the Src family kinase FYN through protein–protein interactions. Binding with Fzd2 activates FYN, which then can promote tyrosine phosphorylation of its substrate signal transducer and activator of transcription 3 (STAT3). Through this novel non-canonical Wnt–FYN–STAT3 pathway, STAT3, as a key EMT activator transcriptional factor, is activated to promote the EMT process in several cancer types (Figure 2(c)).[26] A recent study identified ABI1, an integral component of WAVE complex, as novel regulator of Wnt–FYN–STAT3 pathway and suppressor of EMT in prostate cancer (PCa) (see also 'PCa' section) (Figure 2(c)).[68] Further studies are needed to determine whether the ABI1-mediated regulation

of Wnt pathway is pertinent to tumors originating from other tissues.

Modulation of Wnt pathways

Due to their important role in development and oncogenic processes, Wnt signaling pathways are tightly regulated. A Wnt ligand has a preference for specific types of Fzd and co-receptors, which provides the first level of regulation based on receptor and ligand availability.[19] Direct modulation of Wnt molecules provides further regulation of downstream signal transduction. A typical regulation of Wnt molecules is through a transmembrane protease Tiki-mediated N-terminal cleavage, which oxidizes Wnt molecules and abrogate their ligand activity without inhibiting Wnt secretion.[69] Additionally, Wnt could be modified extracellularly by the secreted Wnt deacylase Notum, which removes the palmitoleic acid on Wnt molecules. This leads to oxidized Wnt oligomer formation and removal of its ligand activity.[70] Furthermore, Wnt molecules interact with other secreted proteins that contain CRD like Wnt inhibitory factor and secreted Frizzled related proteins (sFRPs), which prevent them from binding to Fzd receptors (Figure 2(a)).[71–73]

Another important signal modulating mechanism in Wnt signaling is endocytosis. For downstream signal transduction, studies have shown that the signalosome complex of ligand, receptors, and downstream signal adaptors need to be internalized into the cell.[74–76] During endocytosis, elements of the β-catenin destruction complex, like Axin and GSK3, are sequestered to prolong the half-life of many GSK3 substrates like β-catenin.[19,77] For the Wnt/PCP pathway or other non-canonical pathways, the mechanism of how endocytosis promotes signal transduction in cells is currently unclear, but clathrin-mediated endocytosis of Wnt-receptor complex is suggested to be important for Wnt/PCP signaling transduction.[74] Several negative-feedback mechanisms exist to terminate or dampen Wnt signaling at the receptor level, which also involves endocytosis. A famous Wnt antagonist, Dickkopf-1 (Dkk1), is upregulated through the β-catenin pathway, and Dkk1 forms a ternary complex with its transmembrane receptor Kremen and LRP5/6.[78] This ternary complex is rapidly endocytosed to stop Wnt signaling cascades (Figure 2(a)).[79] Negative-feedback mechanisms have also been reported as β-catenin signaling induces upregulation of transmembrane E3-ubiquitin-ligase ring finger protein 43 (RNF43) and zinc and ring finger 3 (ZNRF3).[80,81] RNF43/ZNRF3 promote ubiquitination of Fzd receptors at the plasma membrane, leading to Fzd receptor endocytosis followed by proteasome degradation (Figure 2(a) and (b)).

Recently, an important positive-feedback mechanism has been revealed to promote cellular responses under low ligand availability. R-spondins are secreted proteins that were first identified as Wnt agonist for Wnt/β-catenin pathways.[82] R-spondins do not possess intrinsic signaling activity, but they form a ternary complex with RNF43/ZNRF43 and membrane receptor protein leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5).[83] This ternary complex is endocytosed rapidly to decrease RNF43/ZNRF3 levels in the plasma membrane, thereby preserving Fzd receptors to amplify both Wnt/β-catenin and Wnt/PCP pathway activity (Figure 2(b)). Interestingly, LGR5, a well-established stem cell marker, is also upregulated by the β-catenin pathway (Figure 2 (b)).[84,85] This positive-feedback mechanism has been shown to amplify Wnt signaling in early embryonic development, stem cell renewal, and tissue homeostasis. This mechanism has been exploited by cancer cells and is the basis for some Wnt-targeting drugs that will be discussed later in this review.

Wnt signaling pathways in development and tissue homeostasis

After fertilization, the fertilized egg undergoes several rounds of cleavage. On day 3.5 a blastocyst is formed, which contains an inner cell mass (ICM) surrounded by a trophoblast epithelium (trophectoderm). During implantation, the trophoblast attaches and invades through the maternal uterine epithelium and further differentiates into nutrient providing tissues like the placenta. Meanwhile, the embryonic stem cells (ESCs) in the ICM rapidly proliferate to form the epiblast from where the entire embryo will originate. Then, through the process of gastrulation the blastocyst further differentiates into the three-layers of the embryo (endoderm, mesoderm, and ectoderm). Through these processes, the embryo forms major body axis, the primitive streak and somites and stem cells differentiate into tissues and organs. The canonical Wnt/β-catenin and non-canonical Wnt/PCP pathways are major regulators of these developmental processes, which will be reviewed in detailed below.[43,86]

ESCs' pluripotency

ESCs are pluripotent and have the potential to further differentiate into all adult cell types; the pluripotency preservation process in proliferating ESCs is called self-renewal which is lost during gastrulation. Mechanisms for ESCs' self-renewal and differentiation are important for tissue/organ formation in early embryonic development. Maintenance of pluripotency requires the functional interplay between Wnt/β-catenin pathway activation, leukemia inhibitor factor (LIF) signaling pathway activation, and MAPK pathway inhibition.[43] The Wnt/β-catenin pathway was initially found to not be required for ESCs' self-renewal as β-catenin$^{-/-}$ mouse ESCs (mESCs) can fully differentiate into cell types from all three germ layers when injected into a mouse blastocyst.[87] However, as LIF signaling is required for the maintenance of pluripotency of mESCs *in vitro*, amplifying Wnt/β-catenin signaling by using GSK inhibitors or recombinant Wnt3a can bypass this requirement.[88] Once injected into a permissive site of a host, normal mESCs form teratoma tumors containing a variety of cell types derived from all three germ layers.[89] However, genetically engineered mESCs with homozygous deletion of β-catenin destruction complex scaffold APC fail to form teratomas or form tumors deficient in neuroectoderm differentiation.[89,90] Results from these studies suggest that upregulation of

Wnt/β-catenin pathway directs the mESCs to the self-renewal track by inhibiting their differentiation.

## The primary dorsoventral and anteroposterior axis formation

In vertebrates, the *Wnt* gene is first shown to regulate primary body axis formation in *Xenopus laevis* embryos.[91] Before gastrulation, the primary body axis or dorsoventral axis coincides with the formation of early embryonic signaling center known as Spemann organizer in amphibians, the shield in zebrafish, and the node in mice.[92] During gastrulation, the single-layered blastula is reorganized into a multilayer structure known as the gastrula. In triploblastic organisms, the well-known three germ layer of the gastrula (ectoderm, mesoderm, and endoderm) will further differentiate into specific tissues and organs. The dorsoventral and anteroposterior body axis determines the spatial distribution of tissues and organs.

Dorsoventral body axis formation. During fertilization, entry of the sperm triggers microtubule-dependent rearrangement of the cortical plasma from the vegetal pole to the future dorsal side in the egg, a process known as cortical rotation.[92] An important outcome of cortical rotation is the accumulation of the β-catenin in dorsal nuclei of early blastulae, which can be detected as early as in the 2–4 cell stage.[93] The nuclear β-catenin accumulation activates the transcription of TCF genes, many of which are specific for dorsoventral body axis formation.[94] Mutations that reduce β-catenin dorsal accumulation cause defects in zebrafish embryo dorsal development.[95] Cortical rotation also leads to the dorsal accumulation of downstream Wnt/β-catenin components to stabilize β-catenin for constitutive activation of the β-catenin pathway at the dorsal site[96] (Figure 3).

Following initiation of β-catenin pathway activation at the early stage of body axis development, maternal β-catenin target genes activate secondary developmental pathways that crosstalk with the canonical Wnt pathway to regulate embryonic development.[97,98] The activation of the bone morphogenetic protein signaling pathway triggers zygotic Wnt8 signaling at the ventral and posterior side to initiate ventral/posterior development in the blastula.[97] Activation of the canonical Wnt pathway upregulates their antagonists at the dorsal and anterior sides of the embryo, inhibiting their activity.[92]

Anteroposterior body axis formation. The anterior inhibition of the canonical pathway triggers anteroposterior body axis formation, which is marked by the initiation of head structure development.[43] Another maternal β-catenin targets the FGF signaling pathway, which is suggested to be important for dorsal and posterior development.[99] The FGF signaling pathway triggers Wnt8 expression at the dorsal and posterior site. The zygotic Wnt8, together with other secreted Wnt molecules, preferably triggers the canonical pathway at the dorsal and posterior sites, which initiates tail development at the posterior end.[100]

Interestingly, Wnt/β-catenin pathway shows variable activation patterns at different developmental stages of
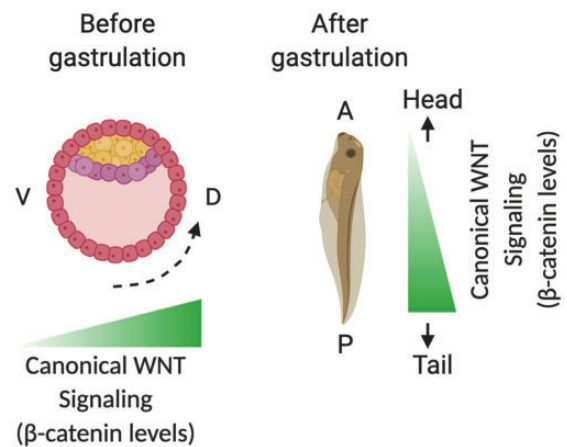
Figure 3. Roles of Wnt/β-catenin pathways in early *X. laevis* embryo body axis formation. The post-fertilization cortical rotation (black dotted arrow) leads to early accumulation of maternal β-catenin in the dorsal equatorial region, which activates canonical Wnt pathway genes to generate the Spemann organizer. At the same time, several Wnt antagonists are upregulated at the dorsal site and translocated to the anterior site to inhibit anterior activation of canonical Wnt pathway for anterior site development. During gastrulation, the zygotic activation of the canonical Wnt8 molecule leads to asymmetric β-catenin accumulation, specifically in the ventral and posterior sites of the embryo. The ventral and posterior activation of the canonical Wnt pathway upregulates genes important for ventroposterior mesodermal fate determination and subsequent tail formation. (A color version of this figure is available in the online journal.) Source: Figure modified from McMahon and Moon[91] and Hikasa and Sokol.[92]

both dorsoventral and anteroposterior axis formation, suggesting a complicated regulatory mechanism of the canonical pathway during early embryonic development. As described, in early body axis formation, Wnt/β-catenin pathway is regulated through secreted antagonists, and signaling integration with other developmental pathways.[72,92,101] An important role of β-catenin is to modulate histone methylation through recruitment of arginine methyltransferase to the promoter of target genes, an important epigenetic change involved in regulation of gene transcription at various developmental stages.[92,102]

Primitive streak formation, somitogenesis, and left–right determination

Early mice embryonic developmental processes are tightly regulated by the Wnt/β-catenin pathway.[86] During early embryologic development, fertilized mice oocytes undergo complicated division and differentiation processes before and after gastrulation for primary body axis formation. At the same time, embryos develop the primitive streak at the blastula stage to establish bilateral symmetry. After that, somitogenesis and left–right determination processes further establish the embryos' spatial morphology for tissue and organ development.

*Primitive streak formation.* In the early blastocyst developmental stage, an ICM region is formed surrounded by a layer of trophoblast epithelium. The ICM rapidly proliferates to form the epiblast, from where the entire embryo originates. The Wnt/β-catenin signaling pathway triggers the proximal-posterior epiblast axis formation during early body axis development.[103] The proximal-posterior epiblast

axis is the place where the primitive streak forms and along this axis, the expression of Wnt molecules extends distally in the epiblast, marking the formation of the primitive streak.[104]

Formation of the primitive streak involves rearrangement of the epiblast polarity. During early body axis formation, the initiation of the Wnt/β-catenin pathway upregulates several Wnt molecules, which trigger the non-canonical PCP pathway to regulate epiblast polarity by modulating actin-dynamics and JNK-mediated transcriptional responses.[19] Deletion of negative PCP pathway regulator Prickle (Pk) (mice homolog *mpk1*) leads to early embryonic lethality with failure of primitive streak formation due to abnormal epiblast polarity formation.[105]

Somitogenesis. During gastrulation, the Wnt3a secretion along the primitive streak creates a gradient for its target Brachyury (T), a transcription factor. In response to T-mediated gene transcription, epiblast cells enter through the primitive streak to give rise to the mesoderm and definitive endoderm.[106] After gastrulation, the primitive streak continues to produce mesoderm that further differentiates into the paraxial presomitic mesoderm (PSM) and lateral plate mesoderm for somitogenesis. In mammals, somites eventually develop into the trunk of the body. In mice embryos, the uneven Wnt3a secretion forms a Wnt3a gradient along the primitive streak, which activates the canonical Wnt pathway in the PSM to form a somite every 2 h.[107] In this process, the activated Wnt3a pathway leads to expression of Notch pathway ligand protein Delta-like1 (Dll1) to trigger Notch pathway in the PSM for somite formation.[108–110] Gradual reduction of Wnt activity in the anterior PSM arrests Notch activity, which sets boundaries for the somite and activates genes for somite determination (Figure 4).[111]

Left–right determination. After gastrulation, a transient midline structure, called 'node,' is formed at the rostral end of the primitive streak.[112] On the ventral surface of nodal cells, there is a ciliated pit, where the rotational movements of the node cilia generate a leftward fluid flow that eventually leads the left–right axis determination in the embryo. The node cilia is initially positioned centrally and gradually posteriorly tilted, which decides its specific rotation pattern.[113] The posteriorly tilted pattern of the node cilia is inhibited in mice embryos with defective Wnt/PCP signaling.[114] This illustrates that Wnt/PCP pathway is involved in left–right axis determination by regulating posterior tilt of the node cilia. After that, the canonical Wnt3a pathway finalizes the left–right body axis. Once the position of nodal cilia is determined, the tilted cilia-mediated leftward flow enhances Nodal signaling on the left side. Nodal is a product of previously described Wnt3a–Notch cascade and it activates Smad2/3-mediated transcriptional responses to promote development.[115] Nodal is initially symmetrically expressed along both the left and right periphery of the node. The final asymmetrical activation of Nodal signaling pathway breaks the left–right symmetry to finalize the left–right body axis (Figure 4).[116]
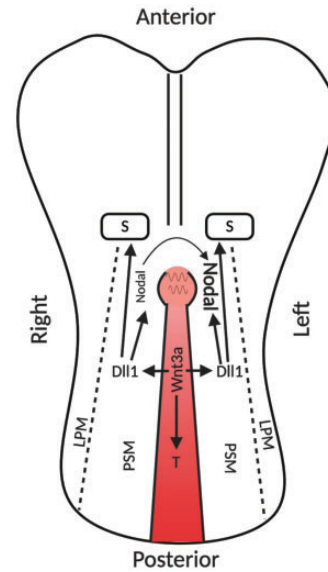
Figure 4. Roles of Wnt/β-catenin pathways in mice embryo somitogenesis and left–right axis determination. The expression of canonical Wnt3a molecules along the primitive streak upregulates the transcription factor T which sustains mesoderm production. In addition, the canonical Wnt3a pathway initiates the expression of the Notch pathway ligand Dll1 to regulate somitogenesis in the adjacent PSM. The expression of Dll1 in the PSM also mediates Nodal expression at the periphery of the node by activating the Notch signaling pathway. The leftward flow generated by rotating nodal cilia leads to left side Nodal accumulation to eventually enhance Nodal signaling on the left side for left–right body axis determination. (A color version of this figure is available in the online journal.) Source: Figure modified from Barker *et al.*[85] and Wang *et al.*[86]
LPM: lateral plate mesoderm; PSM: paraxial presomitic mesoderm.

Tissue development and homeostasis

During gastrulation, three germ layers are formed. This process requires inhibition of Wnt/β-catenin pathway to allow ESCs' differentiation. During specific tissue/organ developmental processes, Wnt/PCP pathway promotes the original germ layer cells to align themselves with certain polarity to ensure proper cell organization for tissue morphogenesis. After development, specific stem cells mediate tissue/organ turn over processes routinely, to maintain their homeostasis. Thus, the canonical and non-canonical Wnt pathways work together to regulate the development and homeostasis of tissues and organs.

Tissue development. In early embryologic development, the asymmetric activation of Wnt/β-catenin pathways along the body axis leads to organ/tissue initiation at the β-catenin pathway inactive sites, where ESCs differentiate. After that, the PCP pathways are important to regulate tissue morphological development. Mice with dysfunctional Wnt/PCP inhibitor Vangl show randomized orientation of the stereocilia, which are the mechanosensing organelles of the inner ear hair cells that regulate hearing and balance.[117] Moreover, disruption of PCP signaling in *Xenopus*, zebrafish, and mice embryos manifests abnormalities in the shapes of many organs and tissues like the cochlea tube, stomach, and intestines.[118,119] *Xenopus* and mice embryos with PCP mutations also show defects in neural tube closure, which is essential for central nervous system formation.[120–122] Similar neural tube closure defects

are also observed in patients carrying Vangl1/2 mutations.[123,124] Some patients with congenital skeletal disorders have been shown to carry Wnt5a or ROR2 mutations, supporting the important role of Wnt5a-mediated non-canonical Wnt pathways in normal limb development.[125–127]

Tissue homeostasis. Aside from development, the balance between proliferation and differentiation of tissue-specific stem cells maintains adult tissue homeostasis in the body, which, as described, is tightly regulated by the Wnt/β-catenin pathway. For instance, adult intestinal epithelia turn over fully every 4–5 days, which requires rapid intestinal stem cell proliferation and differentiation. Genetic disruption of the Wnt/β-catenin pathway abolishes intestinal epithelium proliferation and turnover, which disrupts intestinal homeostasis.[128–130] Similar regulatory mechanisms are found in bone where genetical modulation of Wnt/β-catenin pathway co-receptor LRP5/6 leads to abnormal mesenchymal stem cell differentiation. This process breaks the balance between osteoblast and osteoclast and leads to tremendous decreases or increases in bone mass density.[131] In hair follicle development, autocrine β-catenin pathway is required for hair follicle stem cell cycling for hair follicle renewal.[132] All these examples illustrate the important role of the Wnt/β-catenin pathway in maintaining adult tissue homeostasis.

Wnt signaling pathways in cancer

During oncogenesis, benign cells undergo de-differentiation processes and obtain enhanced proliferation and anti-apoptotic abilities allowing them to sustain uncontrolled growth. The identification of mice proto-oncogene *Int1*, now known as *Wnt1* gene, suggested that the Wnt signaling pathway may be a key regulator of carcinogenic processes.[5] As discussed before, activation of Wnt pathways promotes proliferation, survival, migration, polarity, and specification of cell fate, all of which are important for tumor initiation, progression, and metastasis. Genetic studies and pathological analysis of patients show that Wnt pathways are frequently dysregulated in different cancers, which makes Wnt signaling pathways a potential therapeutic target for cancer therapies. Our current understanding of the role of Wnt pathways in oncogenesis will be reviewed in detail below, along with current anti-Wnt pathway treatments undergoing clinical trials for cancer treatment.

Role of the canonical Wnt/β-catenin signaling pathway in cancer

Wnt/β-catenin pathway promotes cell proliferation by upregulating the transcription of many oncogenes. Meanwhile, activation of canonical Wnt signaling initiates a β-catenin-independent Wnt–STOP mechanism (Figure 2(b)). Wnt binding to Fzd inhibits GSK3β-mediated poly-phosphorylation and poly-ubiquitination of its target proteins which comprise 20% of the proteome.[77,133] These target proteins include prominent oncogenes, like c-Myc.[134] Mutations in this pathway are found in a variety of different cancers.[44] Here, we will review the Wnt/β-catenin pathway in hormone independent gastrointestinal cancers, hormone dependent prostate cancer, and leukemia to illustrate how these pathways have been shown to be dysregulated in various types of cancers.

Gastrointestinal cancers. Abnormal β-catenin stabilization and accumulation by mutations drive gastrointestinal cancer development. Loss of function mutations on APC is commonly found in patients with colorectal carcinoma (CRC) (80% of studied cases).[135] APC loss of function mutations found in CRC lead to constant upregulation of Wnt/β-catenin pathway. This carcinogenic process can be model in APC CRISPR knockout human intestinal organoids.[136] A study using reversible APC shRNA knock down in mice of CRC models indicates that APC restoration reverses the adenoma phenotypes.[137] APC mutations upregulate Wnt–STOP signaling which promotes microtubule dynamics, causing abnormal mitotic processes and triggering chromosomal instability, leading to CRC development.[138] Furthermore, upregulation of Fzd receptors is often observed in CRC, pancreatic ductal adenocarcinoma (PDAC), and cholangiocarcinoma, with enhanced R-spondin/Lgr5/RNF43 modulating activity.[139] R-spondins' overexpression or RNF43 loss of function mutations reduce RNF43 activities to preserve Fzd receptors. Thus, tumor cells with dysfunctional RNF43 rely heavily on Wnt secretion to sustain the β-catenin signaling, rendering them highly susceptible to Wnt secretion targeted therapy.

Prostate Cancer (PCa). In its initial hormone driven state, PCa is highly reliant on androgen receptor (AR) signaling for survival and growth. Unlike in CRC, APC mutations are rarely found in PCa.[140] It seems like β-catenin is not a major driver for PCa since in normal mice prostate tissue, upregulation of β-catenin pathway only results in high-grade prostate intraepithelial neoplasia, a non-invasive benign tumor. A secondary event, like *Pten* loss or upregulated AR signaling, is required for tumorigenesis.[141,142] In these mice, overexpression of AR accelerates tumor development and invasion with reduced survival, while castration shrinks tumors.[142] In untreated patient tumors, β-catenin is shown to work with AR through direct protein–protein binding to promote oncogenic transcriptional responses.[143] AR and β-catenin are enriched in early-onset (diagnosed at ≤ 50 years old) high-grade tumors with enhanced progression potential.[144] In fact, AR has been shown to compete with transcriptional regulator Tcf/Lef for β-catenin binding.[145] In the presence of Wnt, AR overexpression inhibits β-catenin targeted canonical Wnt pathway antagonists expression, while the Wnt–STOP is still activated to preserve several oncogenic proteins in the cell, thereby promoting oncogene tumor progression.[142] In castration-resistant prostate cancer (CRPC) patient samples, β-catenin signaling upregulation is frequently found.[146] Also, in human PCa cell line LNCaP, AR antagonist enzalutamide redirects β-catenin from AR to Tcf/Lef, thereby activating Wnt/β-catenin signaling.[147] These studies suggest that

without AR, the β-catenin signaling pathway is switched on to promote tumor progression in CRPC patients.

Leukemia. Wnt/β-catenin signaling is important for proliferation and self-renewal of stem cells, including hematopoietic stem cells. Wnt activity is increased in most leukemias, which maintains cancer cells in an undifferentiated state and promotes their growth.[148] In acute myelogenous leukemia (AML), frequent chromosomal translocations upregulate Wnt/β-catenin signaling by enhancing key components of transcription.[149] In AML, leukemia initiating cells (LICs) arise from a pre-LIC stage, which is promoted by Wnt/β-catenin signaling through transcriptional modifications.[149,150] LICs for T-cell acute lymphoblastic leukemia (T-cell ALL) usually harbor active Notch signaling mutations.[151] However, β-catenin pathway activation in T-cell ALL mouse models inhibits Notch signaling in T-cells.[152] Those T-cells still exhibit enhanced proliferation and resistance to apoptosis, suggesting a novel Notch independent T-cell ALL subtype. A subset of chronic lymphocytic leukemia (CLL) has been shown to be highly reliant on canonical Wnt signaling for survival.[153] In CLL cases, epigenetic silencing of the Wnt/β-catenin pathway negative regulators is frequently found.[154] Meanwhile, 14% of CLL studied cases carry somatic gain-of-function mutations on Wnt/β-catenin pathway components, while knockdown or mutated Wnt/β-catenin pathway components decrease the viability of CLL cells.[29]

Roles of non-canonical Wnt pathways in cancer

Robust evidence suggests that non-canonical pathways are important for tumor cell growth, survival, invasion, and angiogenesis.[30] The non-canonical Wnt5a is highly expressed in 30% of 237 high-grade gastric cancer cases, promoting its invasiveness and migration.[49] Similar regulations are also observed in other types of cancer.[155] However, Wnt5a has also been reported to sometimes serve as a tumor suppressor in low-grade tumors.[156] Here, we will review the role of non-canonical Wnt pathways in hormone independent gastrointestinal cancers, prostate cancer, and leukemia to describe how non-canonical Wnt pathways are dysregulated in a variety of different malignancies.

Gastrointestinal cancers. Interestingly, studies have shown that in non-metastatic HCT 116 cells derived mice CRC xenografts, Wnt5a overexpression impairs tumor growth.[157] This suggests Wnt5a is a potential tumor suppressor for CRC initiation, potentially through suppression of the canonical Wnt/β-catenin pathway. However, paradoxical results from another study suggest that Wnt5a promotes high-grade metastatic tumor invasiveness and metastasis through accelerating focal adhesion assembly.[158] Also, receptors and co-receptors of Wnt5a pathways have been correlated with a poor prognosis in patients with CRC as they promote tumor metastasis.[155,159,160] Taken together, the role of non-canonical Wnt5a pathway might differ depending on the disease stage in CRC. Similar regulations have also been illustrated in other types of gastrointestinal cancers. As described, Wnt5a has been shown to also enhance tumor aggressiveness by upregulating focal adhesion kinase and Rac activity in gastric cancer.[49] Also, a recent study illustrates that targeting Wnt5a receptor Fzd5 with anti-Fzd5 antibodies decreases proliferation of PDAC cells and reduces tumor burden in PDAC derived mice xenografts.[161]

Prostate Cancer (PCa). A study showed Wnt5a expression is upregulated in 27 out of 98 cases (28%) of PCa and in those cases, Wnt5a overexpression is more frequently found in high-grade invasive tumors with Gleason score 8.[162] Wnt5a initiates JNK-mediated transcriptional response for MMP-1 to enhance tumor invasiveness. After androgen deprivation therapy, non-canonical Wnt genes and their receptors are expressed to promote CRPC growth and/or neuroendocrine differentiation.[30] Enhanced Wnt5a is associated with AR-dependent tumor growth in mice xenografts studies.[163] Another potential role of Wnt5a is promoting EMT through activation of Fzd2-mediated FYN–STAT3 pathway in high grade PCa.[68,164] Activation of FYN–STAT3 is inhibited by ABI1, which sequesters FYN and thus controls STAT3 activation and its nuclear localization; as a result EMT is suppressed. Although these studies have shown that ABI1 interacts with key members of this pathway, FYN and STAT3, the mechanism of how ABI1 is able to regulate this pathway is still not completely understood.[68]

Wnt5a can also serve as tumor suppressor by inhibiting the canonical Wnt pathway in localized low-grade PCa tumors, as 503 patients with localized low-grade tumors show Wnt5a overexpression with higher biochemical recurrence free survival rates and lower bone metastasis.[165–167] As in CRC, the role of Wnt5a signaling in PCa also appears to be dependent on disease stage.

Leukemia. In leukemia, non-canonical Wnt pathway components are regularly found to be upregulated, promoting cell migration, invasion, survival, and resistance to chemotherapy.[155] In AML, Wnt5a co-receptor PTK7 is often found to be upregulated. PTK7 is an orphan tyrosine kinase and in AML cells, PTK7 promotes cell migration by promoting focal adhesion assembly and vascular endothelial growth factor signaling via phosphorylation.[168] PTK7 overexpression reduces chemotherapy-induced apoptosis in AML patients. Furthermore, patients with PTK7 overexpression show a poor disease-free survival rate, suggesting that PTK7 can be used as a biomarker to predict poor clinical outcomes and could also be a potential therapeutic target. In addition, PTK7 is abnormally upregulated in the bone marrow of T-ALL patients with unknown function in promoting T-ALL cell growth.[169] For CLL, multiple PCP pathway components are upregulated with some of them accumulating as the disease progresses, enhancing cell migration in response to Wnt5a through activation of Rho family small GTPases.[170] It has been shown that targeting PCP component Ror1 in CLL decreases organ localization in immunodeficient mice models.

Targeting Wnt pathways for cancer therapies

Because of their prominent role in promoting oncogenesis and tumor progression, Wnt pathways have been suggested to be promising therapeutic targets for cancer therapy. In the past decades, many compounds have been used to target Wnt pathways both *in vitro* and *in vivo*, some of which are currently undergoing clinical trials[44,155,156] (Figure 5 and Table 1). As mentioned, Wnt molecules require Porcupine-mediated palmitoylation for secretion and ligand recognition by its cognate receptors. Small molecules LGK974 and ETC-159 have been shown to significantly inhibit Porcupine's activity, which impairs tumor growth in variable cancers (Table 1).[171,172] Currently, they have been used in clinical trials against metastatic cancers that highly rely on Wnt secretion.[173,174] Wnt ligands and their (co)receptors are also a potential target for cancer
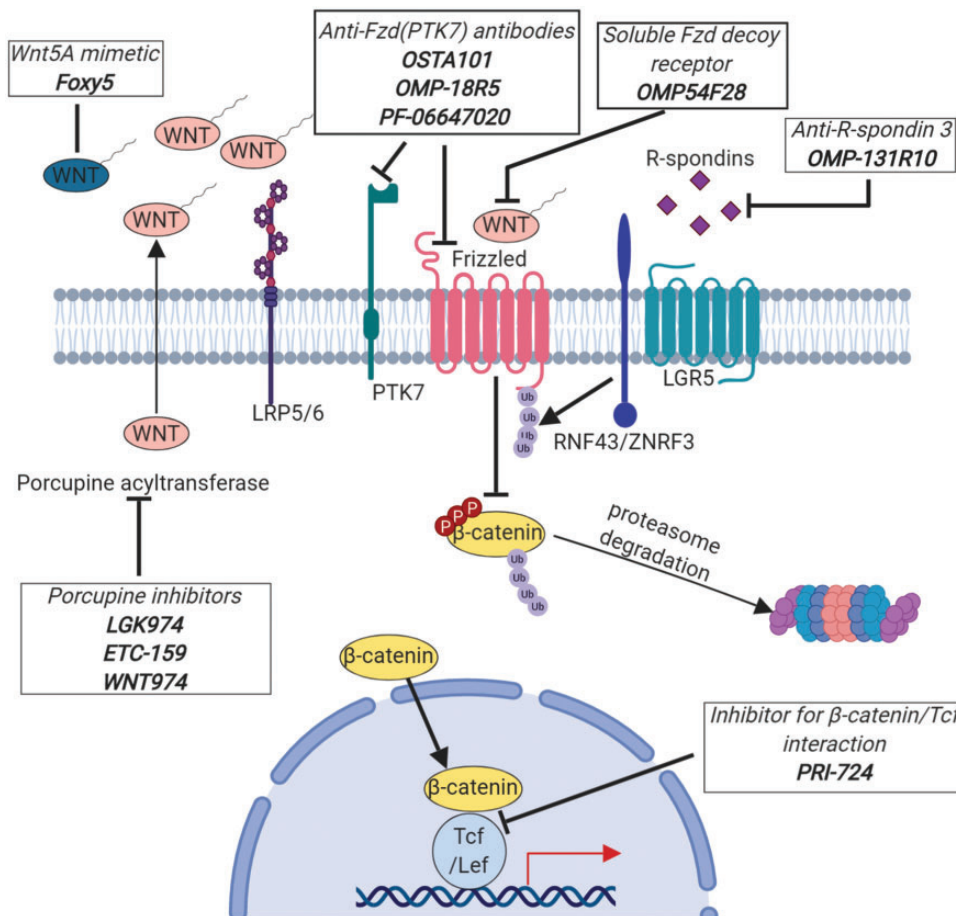
Figure 5. Drugs targeting Wnt pathways in cancer treatments. All depicted drugs are currently undergoing phase1/2 clinical trials against various types of cancers. (A color version of this figure is available in the online journal.)
LRP 5/6: lipoprotein receptor related protein 5/6.

Table 1. Drugs targeting Wnt pathways. All depicted drugs are currently undergoing phase1/2 clinical trials against various types of cancers.

Target gene	Compound	Cancer
Wnt-5a	Foxy5	BCa, CRC, PCa
Porcupine	LGK974, ETC-159	Metastatic CRC, HNSCC, other solid tumors
PTK7	PF-06647020	Advanced solid tumors
R-Spondin 3	OMP131R10	Metastatic CRC
FRIZZLED-10	SYNFRIZZ, OTSA101	Advanced synovial sarcoma
FRIZZLED-7	OMPI8R5 (vantictumab)	NSCLC, metastatic BCa, PDAC
FIRZZLED-8	OMP54F28	Ovarian cancer, PDAC, HCC
TCF/β-catenin interaction	PRI-724	AML, CML, CRC, PDAC

AML: acute myeloid leukemia; BCa: breast cancer; CML: chronic myelogenous leukemia; CRC: colorectal cancer; HCC: hepatocellular carcinoma; HNSCC: head and neck squamous cell carcinoma; NSCLC: non-small-cell lung carcinoma; PCa: prostate cancer; PDAC: pancreatic ductal adenocarcinoma.

treatment. Wnt5a mimetic Foxy5 is undergoing clinical trials as it can activate non-canonical Wnt5a pathways to either inhibit tumors that highly rely on canonical Wnt/β-catenin signaling for their growth or to reduce cell motility to inhibit metastasis.[175–179] The small molecule OMP54F28 is a Fzd8 containing fusion protein that competes with native membrane Fzd8 receptor for Wnt binding and dampens signal transduction. It decreases tumor size and the number of tumor initiating cells in mice xenografts of multiple cancer types and is currently under clinical trials.[180–184] OMP-131R10, a monoclonal antibody, specifically binds to R-spondin3 to release RNF43 allowing it to mediate Fzd ubiquitination and degradation, thereby preventing Wnt pathway activation.[44,185] Other compounds like OTSA101, OMP-18R5, and PF-06647020 are monoclonal antibodies that target specific membrane Fzd receptors or co-receptor PTK7 to block signal initiation of the canonical Wnt signaling or non-canonical signaling, respectively.[186–188] The small molecule PRI-724 was discovered to inhibit self-renewal transcriptome by blocking the interaction between β-catenin and Tcf, and it was shown to decrease tumor burden in ALL and metastatic CRC.[189,190]

Conclusions

Decades ago, the mammalian *Wnt* gene and its *Drosophila* homolog *Wingless* gene were found to promote oncogenesis and embryogenic development, respectively.[19] Since then, studies of Wnt signaling have elucidated their complex roles in different developmental stages and their intricate role in promoting or inhibiting tumor growth in different malignancies. However, a lot still needs to be determined in order to allow us to continue to generate targeted therapies with high efficacy and low off-target effects. Further studies in this area will strengthen our understanding of both developmental and cancer biology. Also, research in Wnt pathways in the context of cancer helps us understand how tumor cells hijack developmental pathways to sustain their growth when faced with severe survival stresses.

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