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REVIEW ARTICLE

The evolving roles of Wnt signaling in stem cell proliferation and differentiation, the development of human diseases, and therapeutic opportunities

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Abstract The evolutionarily conserved Wnt signaling pathway plays a central role in development and adult tissue homeostasis across species. Wnt proteins are secreted, lipid-modified signaling molecules that activate the canonical (β-catenin dependent) and non-canonical (β-catenin independent) Wnt signaling pathways. Cellular behaviors such as proliferation, differentiation, maturation, and proper body-axis specification are carried out by the canonical pathway, which is the best characterized of the known Wnt signaling paths. Wnt signaling has emerged as an important factor in stem cell biology and is known to affect the self-renewal of stem cells in various tissues. This includes but is not limited to embryonic, hematopoietic, mesenchymal, gut, neural, and epidermal stem cells. Wnt signaling has also been implicated in tumor cells that exhibit stem cell-like properties. Wnt signaling is crucial for bone formation and presents a potential target for the development of therapeutics for bone disorders. Not surprisingly, aberrant Wnt signaling is also associated with a wide variety of diseases, including cancer. Mutations of Wnt pathway members in cancer can lead to unchecked cell proliferation, epithelial—mesenchymal transition, and metastasis. Altogether, advances in the understanding of dysregulated Wnt signaling in disease have paved the way for the development of novel therapeutics that target components of the Wnt pathway. Beginning with a brief overview of the mechanisms of canonical and non-canonical Wnt, this review aims to summarize the current knowledge of Wnt signaling in stem cells, aberrations to the Wnt pathway associated with diseases, and novel therapeutics targeting the Wnt pathway in preclinical and clinical studies.

Introduction

The first *Wnt* gene was discovered in mouse mammary epithelial tumor cells and was termed *int-1*.[1] In time, sequencing would reveal the drosophila homolog of the mouse *int-1* proto-oncogene (*Dint-1*) and the Drosophila segment polarity gene wingless (*Wg*) were identical. Moreover, the discovery that *Dint-1* was homologous to *Wg* added to the notion that *int*/*Wg* played an important role in development.[2] The int/Wg family later became known as the Wnt family due to the fusion of the names wingless and integrated.[3] Multiple distinct Wnt signaling pathways have been identified to date, including the canonical (β-catenin-dependent) and the non-canonical (or β-catenin-independent) paths. Non-canonical Wnt signaling can be further divided into the planar cell polarity and Wnt/Ca^{2+} paths.[4] As a result of the wide range of diverse biological functions attributed to Wnt signaling, dysregulations can lead to the development of many pathologies. In the past decade, the Wnt pathway has also emerged as a potential target in cancer, disease therapy, and tissue regeneration. While Wnt signaling has been studied for decades and numerous elucidations have been made in that time, there exists a need to compile this information in a cohesive, up-to-date summary. Therein, the purpose of this review is to summarize the current knowledge of Wnt in stem cells, Wnt pathway-associated diseases, and pharmacologic targeting of the Wnt pathway.

Overview of canonical Wnt signaling

The canonical path is the most thoroughly characterized Wnt pathway and is crucial for inducing cell proliferation, differentiation, maturation, and proper body-axis specification (Fig. 1). Canonical Wnt signaling is activated via the Wnt1 class ligands, which include Wnt2, Wnt3, Wnt3a, and Wnt8a.[5,6] Canonical Wnt ligands bind to the N-terminal extracellular cysteine-rich domain of Fz or Fzd (Frizzled) receptors as well as LRP5/6 (low-density-lipoprotein-receptor-related proteins 5 and 6) coreceptors. When active, canonical Wnt signaling stimulates target gene transcription via the binding of β-catenin to the TCF/LEF (T-cell factor/lymphoid enhancer factor) family of transcription factors.[7] Moreover, the mammalian TCF/LEF family is composed of four nuclear factors, TCF1, LEF1, TCF3, and TCF4.[8] For a full explanation of the detailed canonical and two non-canonical Wnt signaling pathways, please refer to our other work: The Wnt signaling pathway: Multilayered mediators, molecular basis of signaling circuitry and crosstalk with other major signaling pathways (Fig. 1).

In the absence of Wnt ligands, a destruction complex is responsible for clearing β-catenin (Fig. 2). The destruction complex is composed of adenomatous polyposis coli (APC) protein, Axin, serine/threonine kinase glycogen synthase kinase 3 (GSK-3), casein kinase 1 (CK1), the E3-ubiquitin ligase β-TrCP, and protein phosphatase 2A (PP2A). CK1 first phosphorylates (or "primes") β-catenin at the Ser45 residue, enabling GSK-3 to phosphorylate the Ser33, Ser37, and Thr41 residues, creating a binding site for β-TrCP.[9] Phosphorylated Ser37 and Ser33 are recognized by the F-box protein β-TrCP for ubiquitination (Fig. 2).[10] β-TrCP complexes with Skp1/cullin machinery to ubiquitinate β-catenin, which leads to its proteasomal degradation.[9]

In the absence of β-catenin, the TCF/LEF complex is bound to the transducing-like enhancer protein (TLE/Groucho), which recruits histone deacetylases (HDACs) to repress gene transcription.[11] In the presence of Wnt

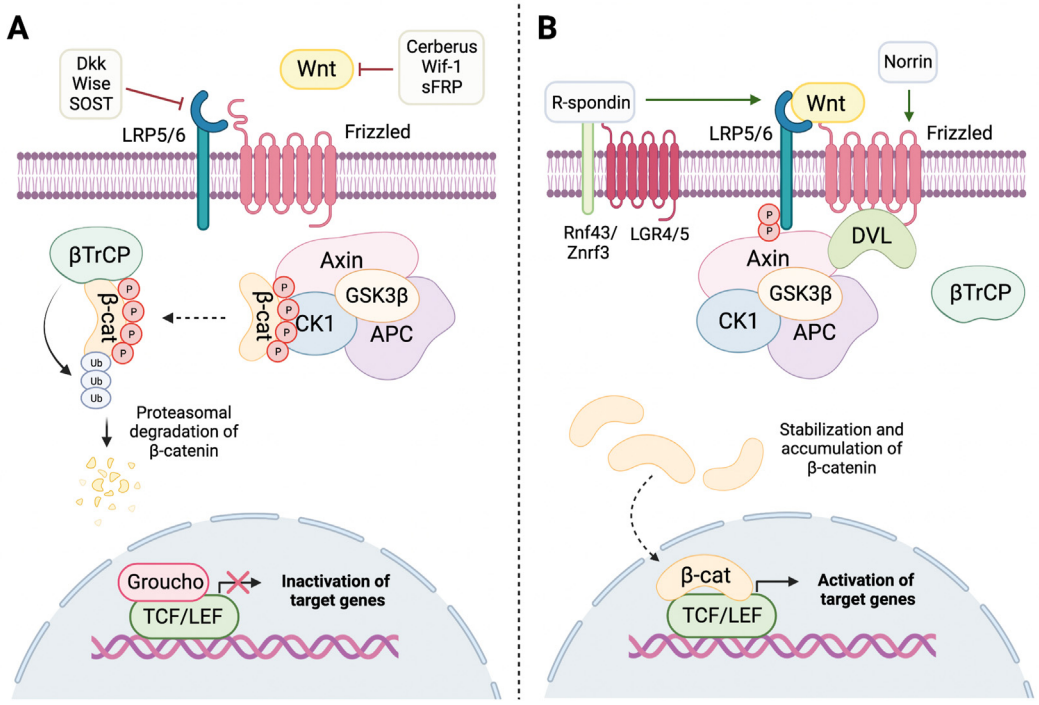

Figure 1 The simplified canonical Wnt/β-catenin signaling pathway. **(A)** In the absence of Wnt ligands, a destruction complex of adenomatous polyposis coli (APC) protein, Axin, serine/threonine kinase glycogen synthase kinase 3β (GSK-3β), and casein kinase 1 (CK1) phosphorylates β-catenin. Following phosphorylation, the E3-ubiquitin ligase β-TrCP ubiquitinates β-catenin, leading to its proteasomal degradation. The TCF/LEF transcription factor complex remains bound to the transducing-like enhancer protein (TLE/Groucho), and gene transcription does not proceed. **(B)** In the presence of Wnt ligands, Fz receptors and LRP5/6 coreceptors recruit the disheveled protein (Dsh/Dvl), phosphorylating the cytoplasmic tails of LRP5/6. LRP5/6 binds Axin, causing the destruction complex to disassemble and freeing β-catenin. β-Catenin is stabilized and accumulates in the cytoplasm before translocating to the nucleus to stimulate gene expression. Wherein, β-catenin binds to TCF/LEF, displacing TLE/Groucho, and target gene transcription proceeds. A number of extracellular regulators also play a role in modulating canonical Wnt signals. R-spondin ligands potentiate Wnt signaling through interaction with LGR4/5 and inhibition of Rnf43/Znrf3 (a transmembrane E3 ubiquitin ligase) mediated degradation of Fzd. Norrin is capable of binding to Fz receptor subtype-4 (Fz4) in order to activate the canonical pathway in an LRP5-dependent manner. Dkk family proteins and SOST bind to the LRP5/6 co-receptor to prevent the binding to Wnt. Wif-1, Cerberus, and members of the sFRP family sequester Wnts in the extracellular space to prevent the triggering of signaling.

ligands, Fz receptors and LRP5/6 coreceptors recruit the disheveled protein (Dsh/Dvl), phosphorylating the cytoplasmic tails of LRP5/6. LRP5/6 binds Axin, causing the destruction complex to disassemble and freeing the β-catenin. β-Catenin is stabilized as a result, accumulating in the cytoplasm and translocating to the nucleus to regulate target gene expression.[7,12–15] β-Catenin binding to TCF/LEF displaces TLE/Groucho and recruits histone-modifying activators such as CBP/p300, Pygo, BCL9, and BRG1.[11]

Overview of non-canonical Wnt signaling

Non-canonical Wnt signaling functions independent of the cytoplasmic stabilization of β-catenin and is involved in processes such as cell polarization and migration. Non-canonical Wnt is characterized by two unique paths: the Wnt/planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway. Moreover, non-canonical Wnt is known to utilize the Wnt5a type ligands which include Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, and Wnt11.[4,5] The Wnt/PCP pathway is an important regulator of cellular polarization for proper development and function.[16] On the other hand, the Wnt/Ca^{2+} path has been characterized as an important organizer during early embryogenesis.[17]

In the Wnt/PCP cascade (Fig. 3), Wnt signaling is transduced through Fzd receptors and co-receptors such as protein tyrosine kinase 7 (PTK7), tyrosine kinase-like orphan receptor (ROR1/ROR2), and tyrosine kinase related receptor (RYK).[18–20] Additionally, the Wnt/PCP pathway is also known to utilize the Celsr1 and Vangl2 receptors, although the exact ligand-receptor binding interactions have not been fully clarified.[21] As a result of Wnt binding to Fzd receptors, Dvl becomes phosphorylated, leading to Inversin (Invs) recruitment.[22] Smad ubiquitination regulatory factor (Smurf) is then recruited by the phosphorylated Dvl to Par6. Smurf is responsible for the ubiquitination of Wnt/PCP antagonizing Prickle, thereby targeting Prickle for proteasomal destruction.[23] Dvl also associates with DAAM (Disheveled-associated activator of morphogenesis), which mediates actin polymerization through the activation of profilin protein.[24] Dvl and DAAM, respectively, stimulate the activation of the GTPases RHOA and Rac1.[25] RHOA, in turn, activates ROCK (Rho kinase). ROCK mediates actin

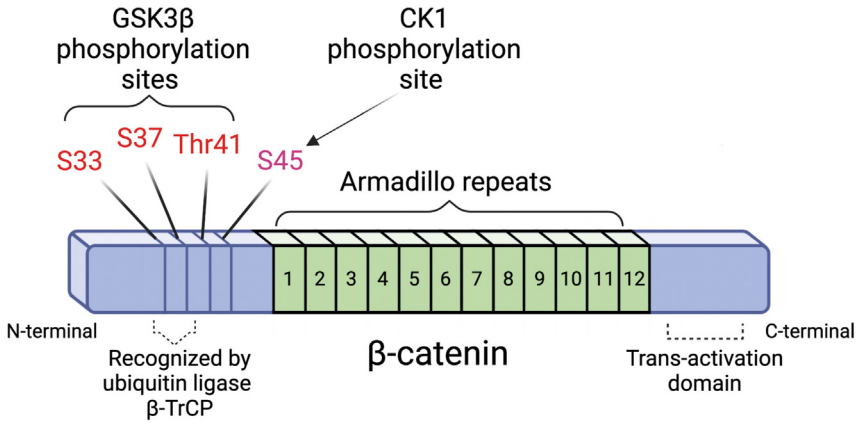

Figure 2 GSK3β and CK1 phosphorylation sites on β-catenin. Prior to ubiquitination by β-TrCP, β-catenin is phosphorylated at 4 key residues. Destruction complex member CK1 first phosphorylates β-catenin at the Ser45 residue, enabling GSK3β to phosphorylate the Ser33, Ser37, and Thr41 residues. The result is the creation of a binding site for β-TrCP. Thereafter, phosphorylated Ser37 and Ser33 on β-catenin are recognized by the F-box protein β-TrCP for ubiquitination, leading to the subsequent proteasomal destruction of β-catenin.

polymerization through its activation of mitogen-activated protein kinase (MRLC).[26] Moreover, Rac1 activates JNK (c-Jun N-terminal kinase) which phosphorylates c-Jun, resulting in c-Jun activation.[27] Furthermore, c-Jun, a well-characterized protein of the activator protein-1 (AP-1) complex,[28] moves to the nucleus in order to initiate target gene expression.[29] JNK also phosphorylates and activates CapZ-interacting protein (CapZIP), which serves to remodel actin filament assembly.[30] Altogether, Wnt/PCP effectors influence the cytoskeletal rearrangements needed for cell polarity and cell motility.

The Wnt/Ca^{2+} pathway functions to regulate endoplasmic reticulum (ER) calcium release in order to control intracellular calcium levels (Fig. 4). Wnt/Ca^{2+} signaling is primarily initiated by the binding of the Wnt5a ligand and Fzd2 receptor.[31] Wnt/Fzd interaction, along with the Ror1/Ror2 co-receptor,[32] leads to the co-stimulation of Dvl and heterotrimeric G-protein to activate phospholipase C (PLC).[33] PLC cleaves phosphatidylinositol-4,5-bisphosphate (PtdInsP2) into diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (InsP3/IP3). DAG activates protein kinase C (PKC) to stimulate CDC42.[21] CDC42 serves to mediate cellular polarity during development.[34,35] InsP3 binds to InsP3R receptors on the ER surface, initiating the release of Ca^{2+} ions, thereby increasing cytoplasmic levels of calcium.[21] The calcium sensor stromal interaction molecule 1/2 (STIM1/2) detects decreases in lumen ER Ca^{2+} concentration before activation of the Orai family proteins to mediate store-operated Ca^{2+} entry.[36] Sarcoplasmic/ER Ca^{2+} ATPases (SERCAs) also pump Ca^{2+} back from the cytosol into the ER.[21] Increased cytoplasmic Ca^{2+} concentration activates the phosphatase calcineurin as well as calcium calmodulin-mediated kinase II (CAMKII). Calcineurin activates the nuclear factor of activated T cells (NFAT), leading to target gene transcription.[37,38] CAMKII activates TGFβ-activated protein kinase 1 (TAK1),[39] which then activates Nemo-like kinase (NLK). NLK is responsible for the phosphorylation of TCF, thereby inhibiting the formation of the B-catenin/TCF complex and preventing gene transcription.[40]

Wnt secretion and extracellular regulators

The human genome encodes 19 glycoprotein Wnt ligands, 350—400 amino acids in length, that are capable of binding Fzd receptors to trigger complex signaling cascades.[14,41] The specificity of the Wnt ligands depends on the cell-specific context and the receptors and downstream molecules expressed.[42] In addition to glycosylation, Wnt proteins also undergo post-translational palmitoylation via the membrane-bound O-acyltransferase Porcupine (PORCN) in the endoplasmic reticulum.[43] For example, Wnt3a requires Porc-dependent palmitoylation at Ser209 prior to secretion.[44] In order to be secreted, Wnt proteins must also be bound to the carrier protein Wntless (Wls), also known as Evenness interrupted (Evi), and escorted from the Golgi apparatus to the plasma membrane for secretion.[45] After delivering Wnt proteins to the plasma membrane, the retromer complex recycles Wls from endocytic vesicles for return back to the Golgi.[46]

A number of extracellular regulators capable of modulating Wnt signaling have been identified. Wnt antagonists include secreted Frizzled-related proteins (sFRP), Wnt inhibitory factor (Wif-1), Cerberus, Dickkopf (DKK), and Wise/sclerostin (SOST) families.[47] Dickkopf family proteins and SOST bind to the LRP5/6 co-receptor to prevent the binding to Wnt.[48,49] Wif-1, Cerberus, and members of the sFRP family sequester Wnts in the extracellular space to prevent the triggering of signaling.[50—52] On the other hand, two families of proteins are known to activate canonical Wnt signaling: Norrin and R-spondin (Rspo).[47] Rspo ligands potentiate Wnt signaling through interaction with LGR4/5 and inhibition of Rnf43/Znrf3 (a transmembrane E3 ubiquitin ligase) mediated degradation of Fzd.[53—55] Interestingly, it was recently demonstrated that Rspo2 and Rspo3, but not Rspo1 or Rspo4, can potentiate WNT/β-catenin signaling in the absence of LGRs.[56] Furthermore, Norrin is a unique non-Wnt ligand that binds to Fz receptor subtype-4 (Fz4) in order to activate the canonical pathway in an LRP5-dependent manner.[57]

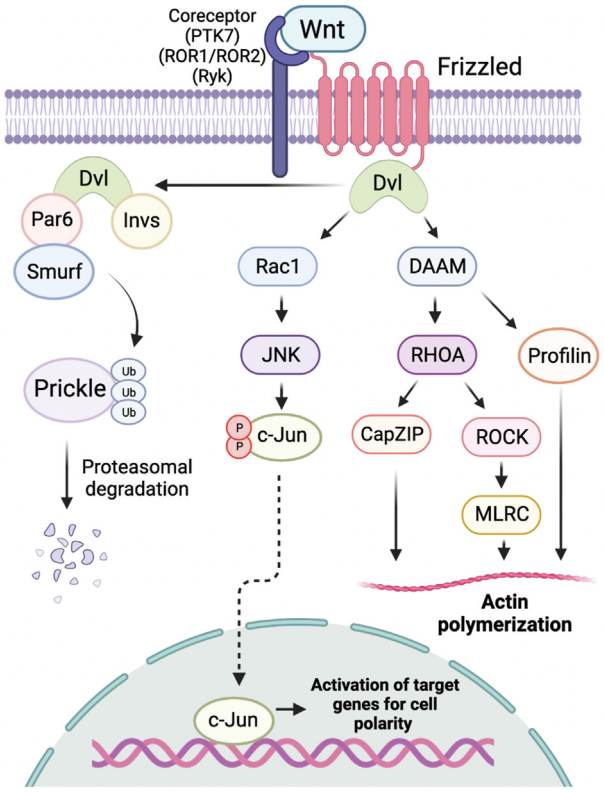

Figure 3 The non-canonical Wnt/PCP pathway. Wnt signaling is transduced through Fzd receptors and co-receptors such as protein tyrosine kinase 7 (PTK7), tyrosine kinase-like orphan receptor (ROR1/ROR2), and tyrosine kinase related receptor (RYK). As a result, Dvl is phosphorylated, leading to Inversin (Invs) recruitment. Smad ubiquitination regulatory factor (Smurf) is then recruited by the phosphorylated Dvl to Par6. Smurf is responsible for the ubiquitination of Wnt/PCP antagonizing Prickle, targeting Prickle for proteasomal destruction. Dvl also associates with DAAM (Disheveled-associated activator of morphogenesis), which mediates actin polymerization through the activation of profilin protein. Dvl and DAAM, respectively, stimulate the activation of the GTPases RHOA and Rac1. RHOA, in turn, activates ROCK (Rho kinase). ROCK mediates actin polymerization through its activation of mitogen-activated protein kinase (MRLC). Moreover, Rac1 activates JNK (c-Jun N-terminal kinase) which phosphorylates c-Jun, resulting in c-Jun activation. Furthermore, c-Jun, a well-characterized protein of the activator protein-1 (AP-1) complex, moves to the nucleus in order to initiate target gene expression. JNK also phosphorylates and activates CapZ-interacting protein (CapZIP), which serves to remodel actin filament assembly. Altogether, Wnt/PCP effectors influence the cytoskeletal rearrangements needed for cell polarity and cell motility.

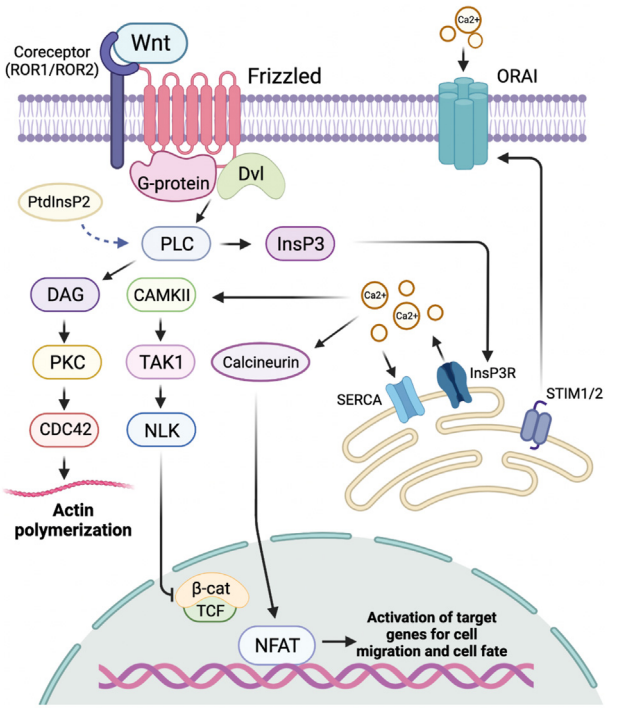

Figure 4 The non-canonical Wnt/Ca^{2+} signaling pathway. Wnt/Ca^{2+} signaling is primarily initiated by the binding of the Wnt5a ligand and Fzd2 receptor. Wnt/Fzd interaction, along with the Ror1/Ror2 co-receptor, leads to the co-stimulation of Dvl and heterotrimeric G-protein to activate phospholipase C (PLC). PLC cleaves phosphatidylinositol-4,5-bisphosphate (PtdInsP2) into diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (InsP3/IP3). DAG activates protein kinase C (PKC) to stimulate CDC42, which serves to mediate cellular polarity during development. InsP3 binds to InsP3R receptors on the ER surface, initiating the release of Ca^{2+} ions, thereby increasing cytoplasmic levels of calcium. The calcium sensor stromal interaction molecule 1/2 (STIM1/2) detects decreases in lumen ER Ca^{2+} concentration before activation of the Orai family proteins to induce store-operated Ca^{2+} entry. Sarcoplasmic/ER Ca^{2+} ATPases (SERCAs) also pump Ca^{2+} back from the cytosol into the ER. Increased cytoplasmic Ca^{2+} concentration activates the phosphatase calcineurin as well as calcium calmodulin-mediated kinase II (CAMKII). Calcineurin activates the nuclear factor of activated T cells (NFAT), leading to target gene transcription. CAMKII activates TGFβ-activated protein kinase 1 (TAK1), which then activates Nemo-like kinase (NLK). NLK is responsible for the phosphorylation of TCF, thereby inhibiting the formation of the β-catenin/TCF complex and preventing canonical Wnt target gene transcription.

Wnt signaling in stem cell self-renewal and lineage differentiation

Stem cells are a group of unspecialized cells that usually arise from a single cell and are responsible for tissue development and regeneration.[58] They are capable of self-renewal into at least one identical daughter cell, preserving the stem cell pool throughout life. Moreover, self-renewal involves cell division accompanied by maintenance of an undifferentiated state.[59] Stem cells are organized at the apex of a developmental hierarchy and are capable of differentiation towards more specialized cell types. Through the process of differentiation, developmental potency is reduced at each step of maturity.[60] As a result of their biological capabilities, stem cells have been extensively studied and characterized due to widespread interest in their use for disease research.[61–63] In this review, we

discuss the current evidence that canonical and non-canonical Wnt signaling cascades play important roles in stem cells of various tissues.

Wnt signaling in embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent cells, commonly derived from the inner cell mass of pre-implantation embryos, that are capable of generating all 3 germ layers (ectoderm, endoderm, and mesoderm).[64] Moreover, ESCs exist in two distinct states of pluripotency. Naïve pluripotent stem cells (PSC) correspond to an earlier developmental state and resemble cells from the preimplantation embryo. By contrast, primed PSCs resemble a later developmental stage found in the post-implantation epiblast.[65] Xu et al recently discovered naïve human ESCs (hESCs) secrete Wnts that activate autocrine or paracrine Wnt/β-catenin signaling to promote efficient self-renewal as well as prevent the transition to the primed state. The authors demonstrated that recombinant canonical Wnt3a, but not non-canonical Wnt5a, rescues self-renewal in naïve hESCs affected by inhibited Wnt secretion. While Wnt/β-catenin signaling is an important factor regulating the self-renewal of naïve hESCs, it was not required for the expression of pluripotency markers. Furthermore, it was discovered that the inhibition of Wnt/β-catenin signaling induced protein and metabolic changes associated with the naïve to primed state transition in hESCs.[66]

The role of Wnt signaling in ESC self-renewal and differentiation remains complex. Many studies have reported that Wnt/β-catenin signaling is a key player in mouse ESC (mESC) self-renewal and maintenance of pluripotency.[67–69] However, there are conflicting reports on the role of Wnt signaling in the self-renewal of human ESCs. Sato et al found that Wnt pathway activation by 6-bromoindirubin-3′-oxime (BIO), a pharmacological inhibitor of glycogen synthase kinase-3β (GSK3β), sufficiently maintained self-renewal in both hESCs and mESCs. Canonical Wnt activation also maintained an undifferentiated phenotype in both types of ESCs, as well as preserved the expression of the pluripotent state-specific transcription factors Oct-3/4, Rex-1, and Nanog.[70] By contrast, Dravid et al demonstrated that the addition of recombinant Wnt3a to undifferentiated human ESCs led not only to cell proliferation but also differentiation. Subsequent reporter assays revealed Wnt/β-catenin-mediated transcriptional activation was minimal in undifferentiated hESCs but greatly up-regulated during differentiation.[71] Similarly, further study has demonstrated that activation of Wnt/β-catenin signaling in hESCs does not promote self-renewal, but rather, drives the induction of mesoderm lineage genes.[72] Consistent with these findings, other studies have also shown Wnt signaling is required for ESC meso/endodermal differentiation.[73,74]

The embryonic stem cell cycle is unique in that it is composed mainly of the S phase with a short G1 phase. Evidence suggests that as ESCs differentiate, their cell cycle structure changes to incorporate a significantly longer G1 phase.[75,76] De Jaime-Soguero et al sought to determine whether canonical Wnt/TCF1 activity controls the cell cycle of mESCs. The authors activated the Wnt pathway by treating mESCs with the GSK3β inhibitor BIO, and in contrast to the known mitogenic effects of canonical Wnt signaling on somatic stem cells,[77] they found that canonical Wnt induced the expression of negative regulators of the cell cycle. This occurred via TCF1 recruitment at the promoter regions of cell cycle repressor genes such as the *Ink4/Arf locus*. Wnt/TCF1 signaling in mESCs, thereby increasing the expression of tumor suppressors such as Cdkn2a (p16Ink4a, p19Arf) and Cdkn2b (p15Ink4b), leading to a reduction of cell proliferation and an increase in the number of cells in G1.[78] Taken together, these findings suggest that Wnt/TCF1 activity triggers an antiproliferative effect in mESCs without perturbing pluripotency. Evenness interrupted (Evi), also known as wntless, is a Wnt-binding protein required for the secretion of Wnt ligands.[79] Through the generation of Evi-deficient and Evi-overexpressing mESCs (modeling Wnt depleted and overexpressed lines respectively), it was discovered that autocrine Wnt signaling is necessary for mESC survival and genomic stability. Thereby, the inhibition of autocrine Wnt signaling in mESCs resulted in mitotic defects, suggesting Wnt signaling plays an important role in chromosomal stability.[80]

Wnt signaling in hematopoietic stem cells

Hematopoietic stem cells (HSCs) reside primarily in the bone-marrow (BM) microenvironment and give rise to a variety of blood and immune cells.[81] Interactions between HSCs and the niche constituents regulate the careful balance between self-renewal and lineage differentiation. Hematopoietic stem cells and progenitor cells (HSPCs) are divided into three multipotent populations: long-term hematopoietic stem cells (LT-HSCs), short-term hematopoietic stem cells (ST-HSCs), and multipotent progenitor cells (MPPs). MPP populations give rise to two oligopotent progenitor cell lines, common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs).[82] Moreover, hematopoietic development occurs in two distinct phases: primitive and definitive. Primitive hematopoiesis occurs early in development and primarily generates nucleated embryonic erythrocytes that sustain an organism during early development. On the other hand, definitive hematopoiesis gives rise to all HSPCs, which are the source of mature blood and immune cells.[83] Sturgeon et al conducted a study of Wnt/β-catenin in human pluripotent stem cells (hPSCs) to discern the role of Wnt signaling in deriving primitive and definitive hematopoietic progenitors *in vitro*. Measured by T-cell differentiation potential, they found Wnt signaling inhibits primitive hematopoiesis but appears to enhance definitive hematopoiesis.[84] Interestingly, studies of mouse embryonic stem cells revealed Wnt signaling stimulates the specification of the nucleated primitive erythroid lineage within the blood islands of the yolk sac. Primitive erythrocytes are the predominant circulating blood cells in the fetus until enucleated erythrocytes can be produced by the fetal liver.[85,86]

A number of studies have implicated both the canonical and non-canonical Wnt signaling pathways in the maintenance of HSCs (Fig. 5). Canonical Wnt signaling via Wnt3a has been shown to play a role in HSC self-renewal capability, whereby, Wnt3a deficiency led to reductions in both the number of HSCs in fetal mouse liver and long-term

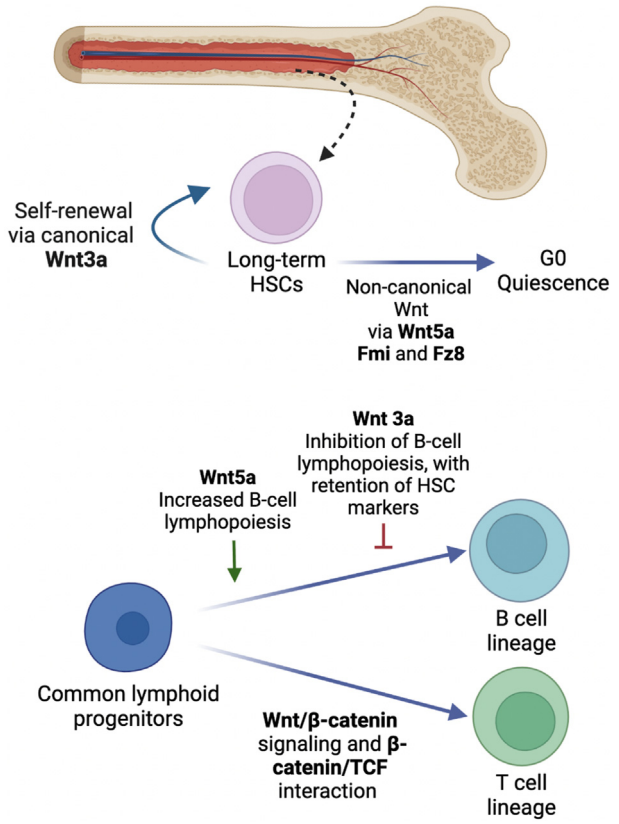

Figure 5 Wnt signaling in multipotent hematopoietic stem cells. Canonical Wnt signaling via Wnt3a has been shown to play a role in HSC self-renewal capability. Wnt3a deficiency led to a reduction in both the number of HSCs in fetal mouse liver and long-term repopulation capacity. Moreover, Wnt5a has been shown to induce HSC quiescence through the activation of the non-canonical pathway. Furthermore, in another study, *Fmi* and *Fz8* knockout resulted in the decline of quiescent LT-HSC populations, indicating non-canonical Wnt signaling plays an important role in the maintenance of LT-HSCs. Studies have shown that Wnt/β-catenin signaling and β-catenin/TCF interaction are required for normal T-cell development. Hossain et al demonstrated the disruption of β-catenin and TCF interaction hinders T-cell survival due to increased susceptibility of thymocytes and activated T-cells to apoptosis. It has been reported that canonical and non-canonical Wnt ligands have differing effects on B-cell development. Canonical Wnt signaling, through Wnt3a, has been shown to inhibit B-cell lymphopoiesis and promote the retention of HSC markers. On the other hand, Wnt5a, which may oppose the canonical path or act via the non-canonical cascade, increased B-cell lymphopoiesis.

repopulation capacity, as measured by secondary transplantation assays.[87] It was found that the overexpression of activated β-catenin expanded the pool of HSCs in culture. By contrast, the ectopic expression of Wnt pathway inhibitors, an Fz ligand-binding domain or Axin, reduced both HSC growth *in vitro* and reconstitution *in vivo*.[88] Sugimura et al investigated the role of non-canonical Wnt signaling in HSC self-renewal and maintenance via *Flamingo* (*Fmi*) and *Frizzled* (*Fz*) *8* knockout. Fmi is known to regulate Fz8 distribution at the interface between HSCs and N-cadherin^{+} osteoblasts that enrich osteoprogenitors. They discovered that *Fmi* and *Fz8* knockout resulted in the decline of quiescent LT-HSC populations, indicating non-canonical Wnt signaling plays an important role in the maintenance of LT-HSCs. Mechanistically, the authors determined that non-canonical Wnt signaling, mediated by Fmi and Fz8, maintains quiescent long-term HSCs by suppressing the Ca^{2+} NFAT interferon-gamma (IFNγ) pathway and by antagonizing canonical Wnt signaling.[89] Moreover, Wnt5a has been shown to induce HSC quiescence through the activation of the non-canonical pathway as well as inhibit Wnt3a-mediated canonical Wnt signaling.[90] An additional study revealed Wnt5a acts through the Ryk receptor to induce HSC quiescence and enhance short-term and long-term hematopoietic repopulation.[91] Further study of the non-canonical Ryk receptor found that Ryk-deficient HSCs from fetal liver exhibited diminished quiescence, leading to proliferation-induced apoptosis and decreased self-renewal.[92]

The role of Wnt signaling in lymphopoiesis is an area of interest. Studies have shown that Wnt/β-catenin signaling and β-catenin/TCF interaction are required for normal T-cell development.[93,94] Similarly, Hossain et al demonstrated the significance of the interaction between β-catenin and TCF in transgenic mice expressing ICAT (an inhibitor of β-catenin and TCF). ICAT expression resulted in increased susceptibility of thymocytes and activated T-cells to apoptosis. These results suggest that in adult mice, disruption of β-catenin and TCF interaction hinders T-cell survival.[95] Malhotra et al reported differing effects of canonical and non-canonical Wnt ligands on B-cell development. Canonical Wnt signaling, through Wnt3a, inhibited B-cell lymphopoiesis and promoted the retention of HSC markers. On the other hand, Wnt5a, which may oppose the canonical path or act via the non-canonical cascade, increased B-cell lymphopoiesis.[96] When coupled to β-catenin, lymphoid enhancer-binding factor 1 (LEF1) functions as an important nuclear mediator of Wnt signaling.[97] Moreover, LEF1 has been shown to be transiently expressed during the early stages of B lymphocyte differentiation.[98] It was also discovered that Wnt5a regulates B-cell proliferation and acts as a tumor suppressor through the non-canonical Wnt/Ca^{2+} pathway. Wnt5a hemizygous mice developed myeloid leukemias and B-cell lymphomas and displayed loss of Wnt5a function in tumor tissue. Moreover, the deletion or loss of expression of the *Wnt5a* gene was noted in human primary leukemias, highlighting the role of Wnt5a as a tumor suppressor in hematopoietic malignancies.[99] Wilson et al showed that conditional elimination of canonical Wnt effector c-Myc in mouse BM resulted in cytopenia, the accumulation of self-renewing HSCs *in situ*, and failure of normal HSC differentiation.[100] These results suggest c-Myc controls the balance between HSC self-renewal and differentiation in the BM niche.

Interestingly, while several studies have implicated canonical Wnt signaling in lymphopoiesis, others report no defects following the deletion of β-catenin. Using the Mx1-Cre system, Cobas et al inactivated β-catenin in murine BM progenitor cells. Surprisingly, they observed that BM chimeras showed no defects in hematopoiesis or lymphopoiesis despite the absence of β-catenin.[101] In a subsequent

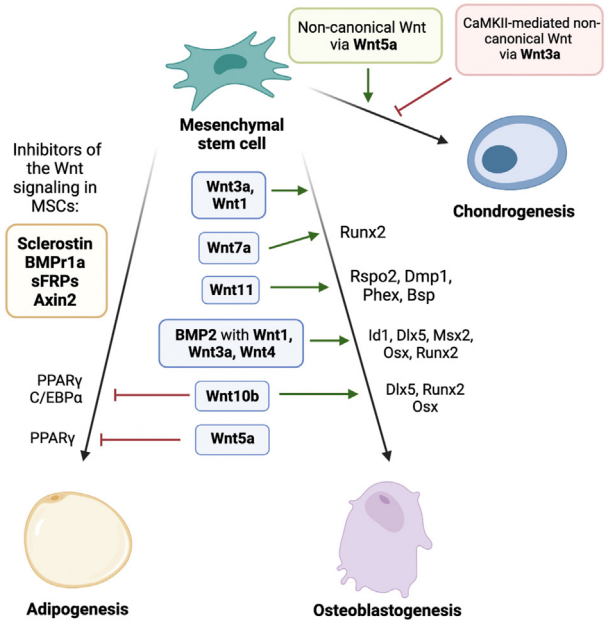

Figure 6 The influence of Wnt signaling on adipogenesis and osteogenesis in MSCs. Non-canonical Wnt signaling via Wnt5a was shown to be important to the chondrogenic differentiation of MSCs. Wnt5a enhanced cartilage formation, collagen fiber rearrangement, and glycosaminoglycan and collagen deposition *in vivo*. Wnt3a plays a dual role in modulating chondrogenesis. When acting via the canonical Wnt pathway, Wnt3a promotes MSC proliferation. By contrast, Wnt3a also inhibits MSC chondrogenesis via the CaMKII-mediated non-canonical Wnt pathway. In no specific order, the following Wnt pathway ligands have been shown to affect the osteoblastogenic differentiation of MSCs. Wnt3a and Wnt1 are capable of stimulating osteoblastogenesis through β-catenin activation. Wnt7a enhances the differentiation of MSCs into osteoblasts via enhancing TCF-1 binding to the promoter region of Runx2. Wnt11 increases the expression of Rspo2 as well as osteoblast-associated genes Dmp1 (dentin matrix protein 1), Phex (phosphate-regulating endopeptidase homolog), and Bsp (bone sialoprotein). BMP2 stimulates LRP5 expression and inhibits β-TrCP expression, leading to an increase in β-catenin levels in osteoblasts and the promotion of osteogenic differentiation. BMP2 also increases expression of the canonical Wnt ligands Wnt1, Wnt3a, and Wnt4 which function to increase transcription of osteogenic genes *Id1*, *Dlx5*, *Msx2*, *Osx*, and *Runx2*. Wnt10b was found to shift cell fate toward the osteoblast lineage by induction of the osteoblastogenic transcription factors Runx2, Dlx5, and osterix. Moreover, Wnt10b suppresses the adipogenic transcription factors peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer-binding protein a (C/EBPa). The non-canonical Wnt5a ligand inhibits PPARγ activation, thereby suppressing adipogenesis and promoting the osteogenic differentiation of MSCs. By contrast, Wnt inhibition has been shown to play a role in adipogenesis. The Wnt/β-catenin inhibitor sFRP1 is endogenously expressed by mature adipocytes in human adipose tissue and several sFRPs are associated with adipocyte dysfunction in obesity. Expressed by mature osteocytes but not by early osteocytes or osteoblasts, sclerostin (SOST) binds to the Wnt co-receptors LRP5/6, thereby antagonizing Wnt signaling with the effect of

experiment, Koch et al examined whether the persistence of normal hematopoiesis and lymphopoiesis could be explained by the compensatory function of γ-catenin, a close homolog of β-catenin. However, double-deficient β- and γ-catenin HSCs did not exhibit an impaired ability to differentiate into all myeloid, erythroid, and lymphoid lineages.[102] In contrast to a previous study, these results suggested canonical Wnt signaling (through β- or γ-catenin) is not essential for hematopoiesis or lymphopoiesis. In accordance with this notion, Jeannet et al also showed that hematopoiesis and lymphopoiesis were found to be normal in the absence of β- and γ-catenin. However, unexpectedly, *ex vivo* reporter assays showed Wnt signal transmission was maintained in double-deficient HSCs,[103] suggesting redundancy of Wnt signaling molecules or perhaps that HSCs may still transduce canonical Wnt signals despite the loss of β- and γ-catenin via alternative or compensatory pathways.

Wnt signaling mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells found in several tissues such as bone, cartilage, and fat. MSCs have the ability to self-renew and also exhibit multilineage differentiation into osteocytes, chondrocytes, and adipocytes.[104] Human MSCs are commonly isolated from adult tissues, namely from bone marrow and adipose.[105,106] MSC multi-lineage specification is controlled by several pathways, one of which is Wnt signaling (Fig. 6).[107] Shen et al showed the overexpression of microRNA 1—2 in mouse-derived bone-marrow MSCs led to their differentiation into cardiomyocytes via the activation of Wnt/β-catenin signaling.[108] Wnt signaling also plays an important role in the regulation of chondrogenesis. Noncanonical Wnt signaling via Wnt5a was shown to be important to the chondrogenic differentiation of MSCs. In a rabbit model, Wnt5a enhanced cartilage formation, collagen fiber rearrangement, and glycosaminoglycan and collagen deposition *in vivo*.[109] Misexpression of Wnt5a in developing chicken limb resulted in a delay of chondrocyte maturation as well as growth plate shortening.[110] Wnt3a plays a dual role in modulating chondrogenesis. When acting via the canonical Wnt pathway, Wnt3a promotes MSC proliferation. By contrast, Wnt3a inhibits MSC chondrogenesis via the CaMKII-mediated non-canonical Wnt pathway.[111]

Wnt signaling is an important regulator of osteoblast differentiation and skeletal development (Fig. 6). Sclerostin is a small protein expressed by the *SOST* gene located on the chromosomal region 17q12-21 which codes for sclerostin. Expressed by mature osteocytes but not by early osteocytes or osteoblasts, sclerostin binds to the Wnt co-receptors LRP5/6,

inhibiting bone formation and indirectly promoting bone resorption. BMP signaling may also restrict Wnt signaling through induction of SOST, as He et al found that BMP receptor type 1a (BMPr1a) induces the expression of SOST to limit cancellous bone accrual. Furthermore, a novel role for Axin2 in adipogenesis has also been described, as it was discovered that the adipogenic transcription factor PPARγ transcriptionally activates the destruction complex member Axin2, thereby impairing Wnt signaling via β-catenin degradation.

thereby antagonizing Wnt signaling with the effect of inhibiting the bone formation and indirectly promoting bone resorption.[49,112] The deletion of β-catenin in early osteoblast progenitors and precursors led to a failure of osteoblast differentiation as well as a conversion to a chondrocyte fate.[113] Studies have found that Wnt/β-catenin signaling via the Wnt10b ligand facilitates osteogenesis and increases bone mass, as supported by findings that the deletion of Wnt10b leads to the reduction of trabecular bone mass, bone mineral density, and serum osteocalcin.[114,115] It was also revealed that Wnt10b-null mice exhibit age-dependent loss of bone mass as well as a reduction in the number of bone marrow-derived mesenchymal progenitors.[116]

Wnt3a and Wnt1 are also capable of stimulating osteoblastogenesis through β-catenin activation.[117] The canonical Wnt pathway is known to up-regulate the expression of the Cbfa1/Runx2 transcription factor required for osteoblastogenesis.[118] Wnt7a enhances the differentiation of MSCs into osteoblasts via enhancing TCF-1 binding to the promoter region of Runx2.[119] Wnt11 increases the expression of Rspo2 as well as osteoblast-associated genes Dmp1 (dentin matrix protein 1), Phex (phosphate-regulating endopeptidase homolog), and Bsp (bone sialoprotein).[120] Moreover, β-catenin deletion from differentiated osteoblasts has been shown to lead to osteopenia. By contrast, Wnt/β-catenin signaling regulates the osteoblast expression of osteoprotegerin, an inhibitor of osteoclast differentiation and function.[121] *Axin2*-knockout mice experienced significantly increased trabecular bone mass, osteoblast proliferation, along with decreased osteoclast formation. These results demonstrate the negative regulation of bone remodeling by Axin2.[122] Interestingly, non-canonical Wnt5a cooperates with Wnt/β-catenin signaling to promote osteoblastogenesis via the up-regulation of Lrp5/6 in osteoblast lineage cells.[123]

Crosstalk between the bone morphogenetic protein (BMP) and Wnt/β-catenin signaling pathways stimulates osteoblast differentiation and bone formation.[124] Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta (TGF-β) family of cytokines and are vital in embryogenesis and homeostasis, and are known to induce bone formation.[125] BMP2 stimulates LRP5 expression and inhibits β-TrCP expression, leading to an increase in β-catenin levels in osteoblasts and the promotion of osteogenic differentiation. BMP2 also increases expression of the canonical Wnt ligands Wnt1, Wnt3a, and Wnt4 and exhibits a cooperative effect with Wnt3a to increase transcription of osteogenic genes (*Id1*, *Dlx5*, *Msx2*, *Osx*, and *Runx2*).[126,127] Furthermore, Wnt11 is known to promote the BMP-induced expression of alkaline phosphatase and mineralization.[120] Tang et al determined Wnt3a and BMP9 act synergistically to induce alkaline phosphatase (ALP) activity in MSCs. The knockdown of β-catenin also showed that the BMP9-induced osteogenic differentiation of MSCs depended on functional canonical Wnt signaling.[128] BMP signaling may also restrict Wnt signaling through induction of SOST, as He et al found that BMP receptor type 1a (BMPr1a) induces the expression of SOST to limit cancellous bone accrual.[129]

The formation of osteoblasts (osteogenesis) occurs at the expense of adipocyte differentiation (adipogenesis) and is regulated by canonical Wnt/β-catenin signaling. Wnt10b was found to shift cell fate toward the osteoblast lineage by induction of the osteoblastogenic transcription factors Runx2, Dlx5, and osterix. Moreover, Wnt10b also suppresses the adipogenic transcription factors peroxisome proliferator-activated receptor (PPARγ) and CCAAT/enhancer-binding protein a (C/EBPa).[115,130] The non-canonical Wnt5a ligand inhibits PPARγ activation, thereby suppressing adipogenesis and promoting the osteogenic differentiation of MSCs. Wnt5a deficiency in osteoblast-lineage cells in mice has been shown to cause low bone mass.[123] Prior to differentiation into mature adipocytes, MSCs must first differentiate into an intermediate cell type called preadipocytes.[131] Evidence suggests Wnt signaling promotes stem cell maintenance, rather than differentiation, of preadipocytes in adipose tissue, with Wnt10a and Wnt6 ligands shown to have similar effects. Cawthorne et al demonstrated that overexpression of Wnt10a resulted in stabilized β-catenin, suppression of preadipocyte differentiation as well as the stimulation of osteoblast formation. The authors found that the knockdown of endogenous Wnt6 is associated with greater preadipocyte differentiation and impaired osteoblastogenesis.[132] Furthermore, human preadipocytes secrete the Wnt antagonist Dkk1 to promote differentiation into adipocytes during early adipogenesis.[133] Pygo2 is a nuclear component of Wnt signaling believed to promote β-catenin-LEF1/TCF transcriptional activation.[134] Xie et al identified that Pygo2 expression and Wnt/β-catenin signaling declined during preadipocyte differentiation into adipocytes. Furthermore, the authors demonstrated that ectopic expression of Pygo2 reduced adipocyte differentiation.[135] Song et al sought to determine whether interrupting Wnt signaling via knockout of *β-catenin* from osteoblasts would promote bone marrow adiposity. The authors conducted knockout of *β-catenin* in mouse preosteoblasts and conducted lineage tracing, both *in vivo* and *in vitro*, to find that the loss of β-catenin from preosteoblasts caused a cell-fate shift of these cells from osteoblasts to adipocytes. Thereby, the observed cell-fate switch contributed to an increase in bone marrow adiposity and low bone mass in knockout mice.[136]

By contrast, Wnt inhibition has been shown to play a role in adipogenesis. The Wnt/β-catenin inhibitor sFRP1 is endogenously expressed by mature adipocytes in human adipose tissue and several sFRPs are associated with adipocyte dysfunction in obesity.[137] Mori et al demonstrated that sFRP5 inhibition of Wnt signaling in a mouse model suppressed adipocyte mitochondrial metabolism to stimulate maximal adipocyte growth. By contrast, the authors found that mice lacking sFRP5 were resistant to adipocyte growth despite a diet intended to induce obesity.[138] Increased levels of circulating sFRP2 have been linked to increased adipose tissue mass as well as vascular endothelial growth factor (VEGF) expression, suggesting sFRP2 may play a role in regulating adipose tissue blood supply and expansion.[139] Lagathu et al discovered that sFRP1 expression in human adipose tissue peaked in patients with mild obesity, but gradually fell in those with morbidly obese.[140] Furthermore, a novel role for Axin2 in adipogenesis has also been described, as it was discovered that the adipogenic transcription factor PPARγ transcriptionally activates the destruction complex member Axin2, thereby impairing Wnt signaling via β-catenin degradation.[141]

Wnt signaling intestinal stem cells

The intestine is the body's primary site of nutrient uptake. Furthermore, intestinal epithelial cells greatly contribute to the maintenance of the symbiotic relationship between gut microbiota and the host by constructing mucosal barriers.[142,143] The single-layered intestinal epithelium is exposed to continuous mechanical, chemical, and biological insults and necessary tissue replenishment is fueled by continuously dividing intestinal stem cells (ICSs) that reside at the bottom of crypts.[144] The Wnt target gene and transmembrane receptor, Lgr5, has been identified as a stem cell marker for cycling crypt base columnar cells (CBCs).[145] CBCs may either self-renew or give rise to specialized cell types (including the absorptive enterocytes and secretory goblet cells, Paneth cells, and enteroendocrine cells).[146] Moreover, CBCs generate rapidly proliferating transit-amplifying (TA) cells that move upwards from the crypt base. During their upward migration, TA cells differentiate into specialized cell lineages, eventually moving from the crypt area to the villus.[147] A notable exception to this upward migration is the antimicrobial Paneth cell, which travels downward to the base during its differentiation.[148] Usually located at the +4 position from the crypt base, label-retaining cells (LRCs) are functionally distinct from crypt base stem cells. LRCs are slow-cycling, quiescent stem cells that have been shown to be precursors of the secretory Paneth and enteroendocrine cell lineages. Moreover, LRCs are capable of rapid proliferation following damage.[149]

The Wnt pathway is an important regulator of crypt homeostasis, as active Wnt signaling is believed to stimulate crypt stem cell proliferation (Fig. 7).[150] The loss of Wnt effector TCF4 leads to the depletion of the intestinal stem cell compartment, as mice lacking TCF4 were born with intestinal epithelium composed entirely of differentiated, non-dividing villus cells.[151] Consistent with these findings and in support of the notion that canonical Wnt signals are crucial for ISC homeostasis, ectopic expression of the Wnt inhibitor Dkk1 in transgenic mice led to the loss of Lgr5-expressing crypt stem cells as well as cells of the secretory lineages.[152] On the other hand, constitutive activation of the B-catenin/TCF4 complex via the loss of the APC tumor suppressor transforms the colonic epithelium, leading to colorectal carcinogenesis.[153] Crypt stem cells receive Wnt3 signals from neighboring daughter Paneth cells. Wnt3 from Paneth cells takes part in a signaling loop, as its expression is necessary for Paneth cell induction and maturation.[154] Moreover, culturing of Lgr5 stem cells with Paneth cells or exogenous Wnt3a has been shown to improve growth efficiency.[155] Interestingly, Farin et al demonstrated Wnt3 was dispersible for the maintenance of ISCs *in vivo*, suggesting the redundancy of Wnt signals from other sources in ISC homeostasis. By contrast, Wnt3 was required for the growth of cultured crypt organoids *in vitro* and exogenous Wnt2b supplementation, or co-culture with mesenchymal cells restored organoid growth.[156] Foxl1-expressing mesenchymal cells have been identified as an essential source of Wnt2b, Wnt4, Wnt5a, and Wnt-activating Rspo3 within the intestinal stem cell niche. Aoki and colleagues demonstrated that diphtheria toxin-mediated loss of Foxl1 cells resulted in the shortening of the intestine as well as decreased epithelial cell proliferation, villi length, and crypt depth.[157]

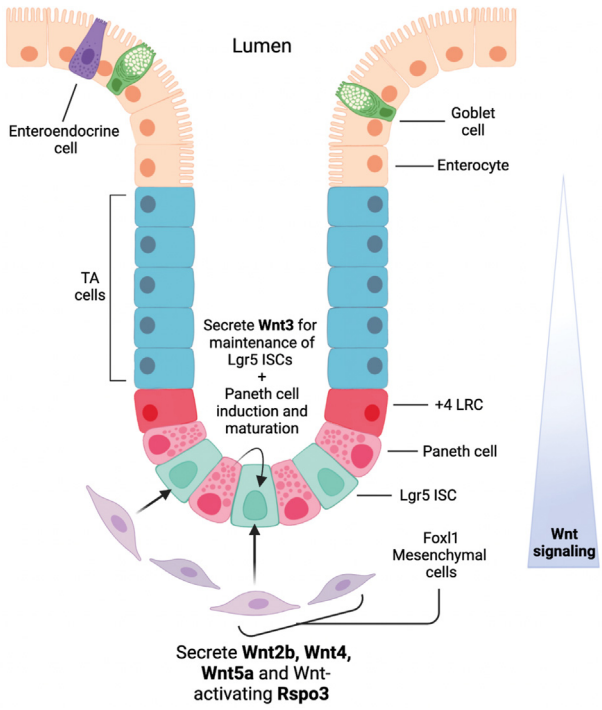

Figure 7 Wnt signaling in crypt intestinal stem cells. The Wnt target gene and transmembrane receptor Lgr5, is a stem cell marker for cycling crypt base columnar cells (CBCs). CBCs may either self-renew or give rise to specialized absorptive and secretory cell types. CBCs generate rapidly proliferating transit amplifying (TA) cells that move upwards from the crypt base before differentiating into specialized cell lineages. Usually located at the +4 position from the crypt base, label-retaining cells (LRCs) are slow cycling, quiescent stem cells. Active Wnt signaling stimulates crypt stem cell proliferation. Crypt stem cells receive Wnt3 signals from neighboring daughter Paneth cells. These signals play a role in Paneth cell induction and maturation, as well as Lgr5 stem cell growth efficiency. Interestingly, Farin et al demonstrated Wnt3 was dispersible for the maintenance of ISCs *in vivo*, suggesting the redundancy of Wnt signals from other sources in ISC homeostasis. As such, Foxl1-expressing mesenchymal cells have been identified as an essential source of Wnt2b, Wnt4, Wnt5a, and Wnt-activating Rspo3 within the intestinal stem cell niche. Aoki and colleagues demonstrated that diphtheria toxin-mediated loss of Foxl1 cells resulted in the shortening of the intestine as well as decreased epithelial cell proliferation, villi length, and crypt depth.

Wnt signaling has also been implicated in intestinal epithelium regeneration following damage. CD44 is both a Wnt target gene and a positive regulator, modulating LRP6 membrane localization and activation.[158] *CD44* knockout in intestinal epithelium had the effect of reducing Wnt/β-catenin signaling in the crypts, thereby reducing the expression of Wnt target genes as well as the number of Paneth cells. Moreover, mice lacking CD44 were given dextran sulfate sodium (DSS)-induced colitis and showed more severe

intestinal inflammation and delayed regeneration.[159] With these results, Walter et al demonstrated the importance of CD44 and Wnt signal to intestinal epithelial regeneration. Inflammation frequently involves the production of tumor necrosis factor (TNF) by macrophages.[160] Bradford et al investigated the role of TNF signaling on Wnt/β-catenin-mediated intestinal stem and progenitor cell expansion in mouse models. They found that TNF-deficient mice exhibited impaired Wnt/β-catenin signaling, less intestinal epithelial cell proliferation, and increased cell apoptosis. TNF-deficient mice given chronic DSS-induced colitis also exhibited delayed ulcer healing and more mucosal inflammation, suggesting TNF and Wnt/β-catenin signaling contribute to the intestinal epithelial healing process following injury.[161] Besides, the ablation of epithelial WNT secretion diminished crypt expansion and ISC activation following rotavirus-induced epithelial cell injury, supporting the importance of Wnt signals in injury repair.[162] Additionally, stem cells ensure homeostasis and chromosomal stability by preserving telomere length through the use of telomerase reverse transcriptase (TERT).[163] Distinguished from the previously described $Lgr5^+$ ISCs and +4 label-retaining stem cells, $TERT^+$ intestinal stem cells are slowly cycling cells capable of regeneration upon tissue injury and differentiation into all intestinal cell types.[164] Wnt signaling is crucial for $TERT^+$ stem cell activation in response to tissue injury, as *β-catenin* knockout has been shown to impair the irradiation-induced quiescence exit of $TERT^+$ stem cells, subsequently hindering intestinal epithelial regeneration.[165]

Wnt signaling neural stem cells

Neural stem cells (NSCs) are a group of progenitor cells that give rise to the neural and glial cell components of the central nervous system (CNS). Adult NSCs may either undergo self-renewal to maintain the population of multipotent progenitors or they may differentiate into specialized cell types. Notably, NSCs are present in the CNS during embryonic development as well as in the adult brains of mammals. There are two neurogenic niches of the mammalian brain where NSCs are known to reside: the subventricular zone (SVZ) lining the lateral ventricles of the cerebral cortex and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus.[166–168] Similarly, adult neurogenesis is restricted in the mammalian brain to the SGZ and SVZ regions.[169] A lineage-tracing experiment with Axin2 conducted on developing mouse embryos found that early ectodermal, neuroepithelial, and radial glial cells of the central nervous system are Wnt/β-catenin responsive. The authors also discovered that progeny from $Axin2^+$ cells gave rise to populations of adult neural stem cells in the SVZ and dentate gyrus of the hippocampus.[170] These results are consistent with the notion Wnt signaling is involved in neural stem and progenitor cell development. It has also been reported that Wnt signaling is implicated in the self-renewal of neural progenitors. Kalani et al demonstrated the addition of soluble Wnt protein increased stem cell clonal outgrowth in cultures of multipotent Wnt-responsive cells isolated from the SVX of developing E14.5 mouse brain. Cell treatment with exogenous Wnt3a protein led to a twofold increase in colony initiation efficiency. In contrast, the addition of Wnt inhibitor Dkk resulted in a fivefold decrease in colony initiation,[171] indicating Wnt signaling is required for the efficient cloning and expansion of developing neural progenitors. TLX (also known as NR2E1) is a known regulator of NSC self-renewal and neurogenesis.[172] Qu et al demonstrated that TLX activates the canonical Wnt pathway via Wnt7a in adult mouse NSCs. Moreover, lentiviral transduction of active beta-catenin in neurogenic areas of TLX-null murine brains led to increased cell proliferation. By contrast, the deletion of Wnt7a or lentiviral transduction of Wnt inhibitor axin resulted in decreased proliferation of progenitor cells in adult neurogenic regions,[173] demonstrating the importance of Wnt/β-catenin to NSC proliferation and self-renewal.

Wnt signaling regulates multiple aspects of adult hippocampal neurogenesis. Rodent and human adult hippocampal progenitors (AHPs) both produce endogenous Wnt proteins as well as possess the intracellular components necessary to respond to Wnt signals. Moreover, the cessation of endogenous Wnt signaling via the application of Wnt antagonists, axin, or truncated cadherin (β-catenin sequestration), decreased the population of hippocampal progenitor cells while an increased fraction committed to a cell fate.[174] These findings suggest autocrine Wnt signaling serves to maintain multipotent rodent and human neural hippocampal progenitor cells. Moreover, Wnt5a has been shown to play a role in adult hippocampal neurogenesis by activating the non-canonical Wnt pathway. The Wnt5a ligand is expressed in the axons of developing rat hippocampal neurons, where it is involved in neural differentiation and synaptic modulation.[175] Moreover, Wnt5a knockdown was found to impair neuronal differentiation of hippocampal progenitors both *in vivo* and *in vitro*. Activated by the Wnt5a ligand, the non-canonical Wnt/JNK and Wnt/CaMKII pathways were found to be key players in the neuronal differentiation of progenitor cells in the adult hippocampus.[176] In addition, it was found that paracrine Wnt3 secreted from hippocampal astrocytes promoted neuronal differentiation via the canonical Wnt/β-catenin pathway. The overexpression of Wnt3 caused an increase in AHP neurogenesis *in vitro* and *in vivo*. Conversely, blocking Wnt3 signaling in AHPs reduced neurogenesis *in vitro* and abolished it *in vivo*.[177] A further study discovered the deletion of Wnt3a in developing mouse brains resulted in the under-proliferation of progenitor cells resulting in a tiny or altogether missing hippocampus.[178] Moreover, Okamoto et al showed that Wnt3 production by hippocampal astrocytes decreases with age and correlates with impaired neurogenic differentiation.[179] Together, these findings suggest Wnt signaling is crucial for the normal growth of the hippocampus.

Wnt signaling in epidermal stem cells

Skin is the largest organ in the body and is constituted of three primary layers, the epidermis, the dermis, and the hypodermis. Multipotent epidermal stem cells (SCs) reside in the stratum basalis (basal cell layer) of the epidermis as well as within hair follicles (HFs).[180,181] Wnt/β-catenin signaling has been shown to play a crucial role in epidermal SC development and maintenance. Neural induction is the

developmental process by which cells from the ectoderm acquire a neural fate as opposed to an epidermal cell fate.[182] In a study of neural and epidermal fates in chick embryos, Edlund et al discovered that Wnt signaling leads to a block of neural specification and the induction of epidermal lineage. Specifically, the authors found Wnt signals block the response of ectoderm to fibroblast growth factor (FGF) signals, permitting the expression of bone morphogenetic proteins (BMPs) to direct cells toward an epidermal fate. On the other hand, a lack of exposure of ectoderm to Wnt signals permits cell response to FGFs, thereby inducing a neural fate.[183] Wnt signaling is also implicated in epidermal stratification. In a mouse model ablating GPR177, a trans-membrane cargo protein required for Wnt secretion,[184] Zhu et al demonstrated epidermal production of Wnts is essential for the formation of the spinous layer of the epidermis. Moreover, the authors found that signaling of epidermal Wnt to the underlying dermis activated a BMP-FGF signaling cascade required for the epidermal stratification process.[185]

Hair is unique to mammals and serves several functions including thermoregulation, protection, and sensory activity. Hair follicles give rise to terminally differentiated keratinocytes, comprising the hair shaft.[186] HFs themselves develop from thickenings in ectodermal tissue called placodes (also known as hair germs or buds depending on the developmental stage).[187] HF morphogenesis can be broadly classified into three phases, induction, organogenesis, and cytodifferentiation.[188] Wnt/β-catenin plays a role during the induction phase early in HF morphogenesis as Wnt signals have been found to be critical in placode formation. To determine whether WNT signals are required for the initiation of follicular development, Andl et al ectopically expressed the Wnt inhibitor Dkk1 in the skin of transgenic mice. The authors found this resulted in a complete failure of placode formation.[189] Moreover, investigating the importance of Wnt/β-catenin in mouse fetuses, Atit et al found that dorsal hair placodes were absent in β-catenin loss-of-function mutants.[190] Together, these results support the notion that β-catenin is required for the induction of hair placodes.

HF regeneration occurs via repetitive cycles of growth (anagen), apoptosis-driven regression (catagen), and rest (telogen).[191] The bulge region of HFs, a convex protrusion of the outer root sheath in the most distal permanent portion of the follicle, houses multipotent bulge stem cells. Upon activation, bulge stem cells proliferate, forming the hair germ, and extend downwards to the dermal papilla at the base of the follicle. Activated matrix keratinocytes that have migrated out of the bulge to colonize the matrix area will terminally differentiate into cell lineages of the hair shaft and the inner root sheath.[186] The HF bulge is normally a Wnt-restricted environment during the telogen phase of the hair follicle cycle, with microarrays showing the expression of Wnt-inhibitory factors. Despite this, bulge stem cells express several Frizzled receptors (Fzd3, F2d7, and Fzd2), elucidating their potential to receive Wnt signals.[192] These findings could explain how bulge cells, in relative quiescence and in the absence of Wnt/β-catenin signals, may maintain an undifferentiated state.

Moreover, it was discovered that Wnt/β-catenin is a crucial player in bulge stem cell lineage. The absence of β-catenin in bulge stem cells led to their failure to differentiate into follicular keratinocytes. Instead, they adopted an epidermal phenotype.[193] Furthermore, LEF1 has been shown to be crucial for the development of hair follicles.[194,195] In a study of gain of function mutations and inducible loss of function mutations, Lowry et al demonstrated that β-catenin-mediated activation of LEF1/TCF complexes and subsequent up-regulation of target genes led to the conversion of transiently amplifying cells to activated cells of the newly developing hair follicle.[196] Wnt signaling is also involved in the promotion of the anagen growth phase. A study regulating β-catenin expression in the skin of a murine model showed that chronic β-catenin activation in telogen HFs resulted in an exaggerated anagen phase. On the other hand, transient activation of β-catenin in telogen follicles produced a normal anagen, indicating Wnt/β-catenin signaling has a critical role in the promotion of the telogen-anagen transition.[197]

Wnt signaling in cancer stem cells

Cancer stem cells (CSCs) represent a subset of tumor cells that are capable of tumor initiation, stem cell-like self-renewal, proliferation, and aberrant differentiation to heterogeneous cancer cell types.[198,199] The CSC model remains somewhat controversial, as not all cancers express CSC markers.[200] CSCs are also unique among tumor cells as they are known to be resistant to many anti-cancer treatments.[201,202] CSCs exist at the top of the tumor cell hierarchy and reside in the CSC niche within the tumor microenvironment. The CSC niche, composed of bone marrow-derived stromal cells as well as normal tissues,[203] is responsible for maintaining CSC properties, providing protection from the immune system, and facilitating metastasis.[204] CSCs have been implicated in driving tumor metastasis by induction of the epithelial—mesenchymal transition (EMT). The transition to migratory mesenchymal cells allows for enhanced tumor invasion and secondary site colonization.[205] Another characteristic of CSCs is their utilization of similar signaling pathways and gene expression patterns as normal stem cells to promote malignant growth.[206] Aberrant regulation of canonical and non-canonical WNT signaling occurs in many human malignancies.[207] As a result, mutations to the Wnt pathway play important roles in the induction of various cancers.[11] Dysregulated Wnt/β-catenin signaling also plays a role in cancer cell resistance to cytotoxic chemotherapy.[208,209] In this portion of the review, we will discuss the evidence that Wnt signaling is an important regulator of CSC biology.

Leucine rich repeat containing G protein-coupled receptor 5 (LGR5) is a cell surface GPCR that binds R-spondin ligands and activates the Frizzled/LRP6 complex together with Wnt ligands. Thereby, LGR5 and R-spondin ligands function to modulate Wnt signaling.[210] LGR5 was recently identified as a CSC marker[211] and is known to be up-regulated in many human cancers such as breast, gastric, cervical, and colorectal cancers.[212—215] Notably, LGR5 was found to promote CSC self-renewal and tumorigenicity in breast cancer through activation of Wnt/β-catenin signaling.[212] Furthermore, elevated LGR5 expression in cervical cancer stem cells resulted in increased tumor

sphere-forming efficiency in addition to conferring chemoresistance to cisplatin treatment.[214]

A key characteristic of normal stem cell and CSC maintenance is the ability to ensure chromosomal stability by preserving telomere length through the use of TERT, a catalytic subunit of telomerase.[163] A link between TERT and Wnt/β-catenin signaling has been described whereby β-catenin regulates TERT expression in a variety of cell lines.[216–218] Activation of the Wnt/β-catenin pathway via Wnt3a has been shown to enhance leukemic stem/progenitor cell self-renewal potential in acute myeloid leukemia (AML) and acute T-lymphoblastic leukemia (T-ALL) cell lines.[219] Furthermore, Cai et al demonstrated that blocking Wnt signaling with DKK-1 reduced the self-renewing capacity of tumorsphere stem cells from MKN-45 human gastric cancer cells. By contrast, the addition of lithium chloride improved gastric cancer cell renewal capacity, indicating a role for Wnt signaling in the maintenance of gastric cancer stem cells.[220] Wnt/β-catenin signaling has been implicated in the self-renewal and expansion of hepatocellular carcinoma (HCC)-initiating cells with stem/progenitor features.[221] Interestingly, it was found that the expression of non-canonical Wnt5a inhibited the effects of Wnt/β-catenin signaling in HCC cells.[222]

Wnt signaling up-regulates transcriptional factors which facilitate EMT induction and tumor metastasis. Among these, slug (also known as snail2) and snail (snail1) belong to the snail family of transcriptional factors and are known as major EMT inducers expressed in breast, ovarian, and colon cancers.[223] Wu et al demonstrated the link between canonical Wnt signaling and slug activity. In the absence of Wnt/β-catenin signaling, the cytosolic slug is phosphorylated by GSK3β and subsequently undergoes β-Trcp1 dependent ubiquitination and proteasomal degradation. When present, Wnt signaling inhibited GSK3β-mediated phosphorylation, thereby stabilizing the slug and permitting the induction of EMT programs.[224] Twist is a key transcriptional factor for the initiation of EMT in breast cancer.[225] Howe et al demonstrated that Twist expression is up-regulated in murine mammary epithelial cells in response to Wnt1. The authors observed elevated Twist in nearly 70% of mammary tumors in Wnt1 transgenic mice.[226] Altogether, these findings indicate a link between Wnt1 and twist expression contributing to mammary tumorigenesis. Moreover, the non-canonical ligands Wnt5a/Wnt5b and Fzd2 receptors are highly expressed in metastatic breast liver, lung, and colon cancer cell lines. Wnt5a/5b binds to Fzd2 receptors, leading to the phosphorylation of transcription factor and driver of EMT, STAT3.[227] Wnt5a has also been linked to EMT in ovarian and nasopharyngeal cancers.[228,229] On the other hand, the role of Wnt5a is complex as Cheng et al demonstrated Wnt5a inhibited cell proliferation and EMT in colon cancer cells.[230]

Studies have described a relationship between non-coding microRNAs (miRs) and Wnt signaling in CSCs. Whereby, miRs provide intrinsic modulation of Wnt signaling, and are implicated in CSC maintenance of stem cell traits. For example, assays revealed miR-217 expression was increased in HCC tissues and cells. MiR-217 induced stem cell-like traits in HCC cells through the activation of Wnt signaling via the targeting of Wnt inhibitor DKK1.[231] Moreover, miR-1207 is up-regulated in ovarian cancer cells and promotes stem cell traits by activating Wnt signaling via the targeting of negative Wnt regulators SFRP1, AXIN2, and ICAT.[232] Lastly, miR-21 expression is increased in colon cancer cells and induces stemness via the suppression of transforming growth factor β receptor 2 (TGFβR2) as well as increases in levels of β-catenin, TCF/LEF activity, c-Myc expression, and cyclin-D1 expression.[233]

Wnt signaling in human diseases

Wnt signaling has a wide range of functions in development. As mentioned previously, the canonical pathway is vital for inducing cell proliferation, differentiation, maturation, as well as proper body-axis specification. On the other hand, the non-canonical pathway is involved in cell polarization and migration. Both the canonical and non-canonical pathways perform a wide array of functions across species, and their dysregulation can lead to numerous diseases. Several genetic changes in Wnt signaling components can cause human diseases (Table 1). Although not exhaustive, we will cover many important diseases in this section, with a particular focus on the more thoroughly understood canonical Wnt signaling pathway.

Table 1 List of genetic diseases caused by mutations in the Wnt pathway.

Implicated member of the Wnt pathway	Associated disease
APC	Familial adenomatous polyposis
Axin2	Colorectal cancer
Axin1	Hepatocellular carcinoma
	Prostate cancer
DVL1	Robinow syndrome
Fzd4	Familial exudative vitreoretinopathy
Lef1	Human sebaceous tumors
LGR4	Obesity
LRP5	Osteoporosis pseudoglioma syndrome
	Early-onset osteoporosis
LRP6	Early-onset coronary artery disease
	Late-onset alzheimer's disease
Norrin	Familial exudative vitreoretinopathy
	Norrie disease
PORCN	Focal dermal hypoplasia
RNF43	Hereditary sessile serrated adenomas
	Colorectal adenocarcinomas
Sclerostin (SOST)	Sclerosteosis
	Van Buchem disease
TCF7L2	Type II diabetes
Wnt1	Osteogenesis imperfecta
	Early-onset osteoporosis
Wnt3	Tetra-Amelia
Wnt4	SERKAL syndrome
Wnt5a	Robinow syndrome
Wnt7a	Fuhrmann syndrome
Wnt10B	Oligodontia
	Early-onset obesity
Wnt11	Hepatocellular carcinoma

Wnt signaling in cancer

Control of Wnt signaling is crucial in the prevention of a diverse range of cancers (Fig. 8).[234,235] Moreover, mutations of Wnt pathway members can lead to EMT, unchecked cell proliferation, and metastasis. Overexpression of Wnt pathway members FZD7, LRP6, and TCF7 has been observed in triple-negative breast cancer (TNBC).[236] TNBC is an aggressive variant of breast cancer that lacks the expression of estrogen receptors, progesterone receptors, and human epidermal growth factor receptors (HER-2) seen in other breast cancer subtypes.[237] Moreover, transcriptional knockdown of LRP6 or FZD7 has been shown to reduce TNBC tumor growth *in vivo*.[238] APC is an important component of the β-catenin destruction complex in canonical Wnt signaling. Mutations in APC have been characterized by familial adenomatous polyposis, a hereditary condition involving thousands of colonic polyps.[239] However, somatic mutations in a single *APC* gene are responsible for over 80% of colorectal tumors, and over 60% in those with two *APC* gene mutations.[240] APC is expressed in the basolateral membrane of colorectal epithelial cells and is suggested to cause apoptosis to control cell death and regulate cell number. APC functions as a tumor-suppressing "gatekeeper" gene of colonic epithelial cell proliferation, maintaining cell number, and preventing uninhibited cell division. This may also explain how APC mutations do not result in cancer in other organs despite being ubiquitously expressed.[241] Overall, the loss of APC causes constitutive activation of the β-catenin/TCF4 complex, which can lead to the transformation of colonic epithelium.[242]

Axin proteins are negative regulators of Wnt/β-catenin signaling and help comprise the β-catenin destruction complex. Mutations in Axin2, leading to the stabilization of β-catenin and activation of Wnt/β-catenin signaling, have been linked to CRC susceptibility.[243,244] Yet, findings by Wu et al suggest the role of Axin2 may be more complex. Axin2 was actually found to promote CRC oncogenesis in mice by up-regulating the transcriptional repressor Snail1, leading to increased EMT and metastasis. Interestingly, these findings suggest that rather than acting as a tumor suppressor, Axin2 may be involved in promoting oncogenic activity.[245] Mutations in Axin1, on the other hand, have been associated with HCC and prostate cancer.[246,247]

Perhaps unsurprisingly, mutations in the *β-catenin* gene are also associated with cancer. Mutations that removed the Ser/Thr regulatory residues on the N-terminus of β-catenin were found in CRC tumors with intact *APC* genes.[248] Melanoma was one of the first cancers to be associated with β-catenin dysregulation.[249] However, the exact role of β-catenin in melanoma is unclear, as subsequent studies have linked activated Wnt/β-catenin signaling to decreased proliferation and suppression of invasion.[250,251]

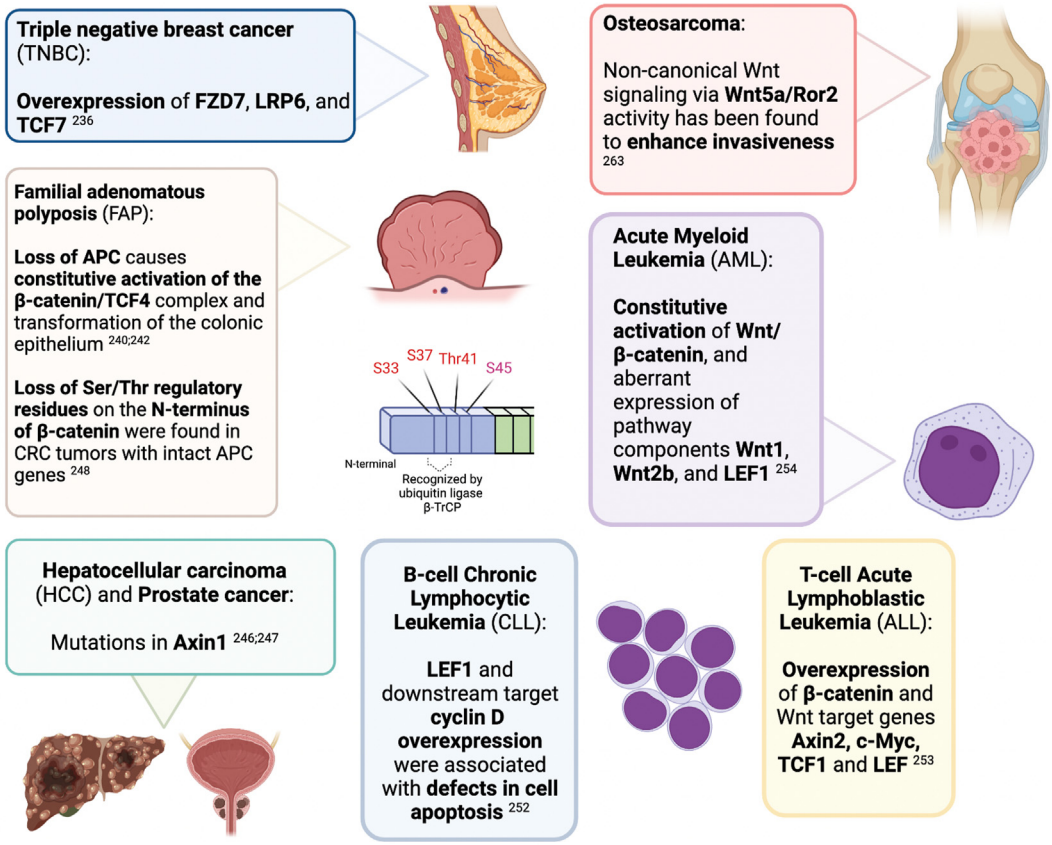

Figure 8 Aberrant activation of Wnt signaling in cancer. Wnt signaling plays numerous roles in normal development. The canonical pathway is vital for inducing cell proliferation, differentiation, and maturation, as well as proper body-axis specification. The non-canonical pathway is involved in cell polarization and migration. As such, the dysregulations of Wnt can lead to the development of diseases. Above are examples of aberrations to the WNT pathway that have been associated with cancer.

Furthermore, canonical Wnt signaling has been shown to drive oncogenesis in a number of leukemia subtypes. Wnt signaling components, LEF1 and downstream target cyclin D1, were overexpressed in cell lines of B cell chronic lymphocytic leukemia (CLL), and their expression was associated with defects in cell apoptosis.[252] An examination of Wnt/β-catenin signaling in T-cell acute lymphoblastic leukemia (T-ALL) patients showed that β-catenin was upregulated in nearly 85% of the childhood T-ALL patients. Moreover, Wnt target genes Axin2, c-Myc, TCF1, and LEF were also highly expressed. When β-catenin was silenced, T-ALL cancer cells showed higher rates of apoptosis. Together, these results suggest Wnt/β-catenin signaling plays a role in a notable subset of T-ALL cancers.[253] Furthermore, Wnt signaling is required for the pathogenesis of AML. A significant portion of AML cases was found to exhibit constitutive activation of Wnt/β-catenin signaling, as well as the aberrant expression of pathway components Wnt1, Wnt2b, and LEF1.[254] A study of the role of LEF1 dysregulation in murine HSCs showed that mice transduced to express LEF1 or a constitutive active LEF1 mutant developed abnormal hematopoietic differentiation leading to AML.[255] Leukemia stem cells (LSC) are a subset of AML cells with stem-like properties of self-renewal and proliferation, as well as the ability to initiate and maintain leukemia *in vivo*.[256] Wang et al have shown that canonical Wnt signals are required for the self-renewal of LSCs in mouse models of AML.[257] Epigenetic regulation of the Wnt pathway in AML can occur via methylation of Wnt antagonists. Valencia et al used a methylation-specific PCR approach to study the methylation status of a number of Wnt antagonists (including sFRP1, sFRP2, sFRP4, sFRP5, Dkk1, and Dkk3). The authors discovered aberrant methylation of Wnt antagonists in up to 64% of AML marrow samples.[258]

One-third of human sebaceous tumors were found to contain inactivating mutations of LEF1, one of the nuclear factors of the TCF/LEF family. The mutations stimulated the expression of sebocyte markers and prevented β-catenin from binding to LEF1 and activating target gene transcription. Interestingly, the LEF1 mutations characterized in human sebaceous tumors are among the first tumor-associated mutations that inactivate Wnt signaling.[259] As previously mentioned, dysregulation of the Wnt pathway may also occur via epigenetic means, as the hypermethylation of the promoter regions of Wnt inhibitors SFRP, DKK, and WIF-1 were associated with a worse prognosis of both ALL and AML.[260] Ring finger protein 43 (RNF43) is a negative regulator of the canonical Wnt pathway, and in the absence of R-spondin ligands, degrades Fzd receptors.[55] RNF43 mutations have been associated with hereditary sessile serrated adenomas (SSAs) and colorectal adenocarcinomas.[261,262] Moreover, Non-canonical signaling via Wnt5a/Ror2 activity has been found to enhance the invasiveness of osteosarcoma cells.[263] Non-canonical Wnt11 acts as a tumor suppressor during hepatocarcinogenesis, as the loss of Wnt11 expression led to malignancy via canonical and non-canonical paths.[264]

Wnt signaling in bone and skeletal disorders

The Wnt pathway plays a significant role in bone formation and, as will be discussed later in this review, presents a potential target for the development of therapeutics for bone disorders.[41,265,266] The importance of Wnt signaling in bone formation is highlighted by the *SOST* gene located on chromosome chromosomal region 17q12-21 which codes for sclerostin. Expressed by mature osteocytes, sclerostin is a small protein that functions as an antagonist of Wnt signaling by binding to the Wnt co-receptors LRP5/6 with the effect of inhibiting bone formation and indirectly promoting bone resorption. Moreover, mutations in *SOST* can cause sclerosteosis as well as van Buchem disease (VBD).[267,268] Sclerosteosis and VBD are rare autosomal recessive sclerosing bone dysplasias that result from bone overgrowth due to osteoblast hyperactivity. Sclerosteosis is known to be caused by loss-of-function mutations in the *SOST* gene which encodes sclerostin. VBD results from a deletion of regulatory elements for SOST transcription, leading to reduced sclerostin expression.[269]

Osteoporosis is a condition characterized by low bone mineral density (BMD) resulting from altered bone microstructure, that ultimately predisposes patients to low-impact, fragility fractures.[270] Osteogenesis imperfecta, also known as brittle bone disease, is marked by the early onset of osteoporosis and increased bone fragility.[271] While most osteogenesis imperfecta cases are caused by dominant mutations to the *COL1A1* or *COL1A2* genes involved in the production of type-1 collagen, a number of studies have also linked mutations in Wnt1 to autosomal recessive osteogenesis imperfecta.[272–274] Laine et al discovered a heterozygous missense mutation of Wnt1 in adults with dominantly inherited early-onset osteoporosis.[275] Furthermore, a heterozygous missense mutation in LRP5 that reduces the activity of canonical Wnt signaling was linked to juvenile-onset primary osteoporosis, without the features of osteogenesis imperfecta.[276] Yu et al identified autosomal-dominant missense mutations in Wnt10B that were associated with oligodontia, a severe form of tooth agenesis.[277] Furthermore, partial loss of Wnt7A function has been linked to Fuhrmann syndrome, a skeletal dysplasia characterized by limb aplasia or hypoplasia as well as joint abnormalities.[278] Dysregulation of Wnt signaling has not only been implicated in disorders of the axial skeleton. It is well established that Saethre Chotzen Syndrome,[279] characterized by a mutation in the *Twist1* gene (a downstream target of Wnt), results in multi-suture craniosynostosis. Behr and colleagues[280] have elegantly demonstrated that canonical Wnt signaling determines cranial suture fate in the mice and perturbations in such signaling, whether via continuous activation or inhibition, yield differential patterns of closure in posterior frontal and sagittal sutures.

Wnt signaling in ocular disorders

In humans, autosomal dominant and recessive loss of function mutations in the Frizzled 4 (Fzd4) receptor cause a disease known as familial exudative vitreoretinopathy (FEVR). The expressivity of the disease can be highly variable but, generally, FEVR results in lack of retinal angiogenesis. This can lead to neovascularization, fibrosis, retinal detachment, and even retinal dysplasia among other symptoms, clarifying the importance of Fzd4 receptors in retinal vascular development.[281] X-linked mutations in the

Norrin disease protein (*NDP*) gene, which codes for norrin (a ligand of FZD4),[282] cause X-linked FEVR and Norrie disease.[281] Norrie disease is characterized by retinal hypovascularization,[283] as the lack of Norrin-induced Wnt signaling is integral to proper retinal vascular development.[282] The overactivation of Wnt/β-catenin signaling also plays a role in the development of age-related macular degeneration through increasing angiogenic factors.[284,285] Furthermore, LRP5 loss of function mutations has also been linked to osteoporosis pseudoglioma syndrome, a rare disease characterized by reduced bone formation and congenital vision loss. Patients born with osteoporosis pseudoglioma syndrome may experience blindness as well as severe disruptions of ocular structure blood vessel development.[286]

Diabetic retinopathy (DR) is a microvascular disease and a major complication of diabetes mellitus (DM).[287] DR is also associated with increased Wnt/B-catenin signaling, as retinal sections taken from DR patients revealed elevated β-catenin and LRP5/6 levels. Moreover, the administration of Dkk1 reduced retinal inflammation caused by pro-inflammatory factors such as ICAM-1 and cyclooxygenase-2. Dkk1 also reduced retinal vascular leakage as well as ischemia-induced retinal neovascularization in DR models.[288] Analysis of the vitreous humor of patients with proliferative diabetic retinopathy revealed a correlation between LRP6 levels and VEGF levels.[289] Moreover, plasma levels of Wnt inhibitor Dkk1 were found to be lower in DR patients than in non-diabetic controls, further implicating Wnt signaling in the pathogenesis of DR.[290] The cornea is the outermost part of the eye and provides both refractive and protective functions. It is a unique tissue without vascularization and maintaining its transparency and avascular nature is important to visual acuity.[291] Corneal neovascularization (CNV) is a sight-threatening condition that involves the growth of blood vessels within the cornea. Insults associated with CNV include hypoxia, infection, inflammation, and chronic contact lens wear.[292] Aberrant Wnt signaling has also been associated with CNV, as components of the Wnt pathway have been shown to be increased in animal models of CNV. Following the induction of suture-induced CNV in rats, phosphorylated LRP6 and nuclear accumulation of β-catenin were found to be increased in endothelial cells and neo-vascularized corneal tissue.[293] Moreover, similar results have been demonstrated in the corneas of mice and rats following alkali burns.[294]

Wnt signaling in cardiovascular diseases

Atherosclerosis is an inflammatory disease characterized by the progressive accumulation of lipids and fibrous elements in large arteries,[295] and the Wnt pathway has been implicated in mechanisms contributing to the progression of atherosclerosis. Genetic investigations of families or populations that exhibited early onset coronary artery disease revealed a number of LRP6 mutations associated with the increased risk of elevated low-density-lipoprotein-cholesterol.[296–298] Furthermore, murine and human macrophages and atherosclerotic lesions were found to have elevated levels of Wnt5a expression.[299] Wnt5a contributes to inflammation in atherosclerosis by inducing the gene expression of cyclooxygenase-2 and inflammatory cytokines in human aortic endothelial cells.[300] Dkk-1 is also elevated in atherosclerotic lesions and Ueland et al identified Dkk-1 as a novel mediator in platelet-mediated endothelial cell activation and release of pro-inflammatory cytokines.[301] By contrast, Dkk-3 has been linked to a potential atheroprotective role against atherosclerosis in human subjects. Wherein, Yu et al found that plasma Dkk-3 levels were inversely associated with carotid artery intima-media thickness and carotid atherosclerosis.[302] β-Catenin is capable of up-regulating genes that promote vascular smooth muscle cell proliferation, such as cyclin D1, while also decreasing the level of cell cycle inhibitor p21.[303] Moreover, Wnt4 expression was also found to be elevated in proliferating vascular smooth muscle cells.[304]

The Wnt pathway has been shown to be activated following myocardial infarction (MI). Reporter analysis of Wnt signaling in the myocardium following MI, *in vivo*, showed that active Wnt signaling increased in the cardiomyocytes at the infarct border, from 7 days post-MI onwards.[305] Meyer discerned that the cardiac microenvironment employs non-canonical Wnt signals to activate monocytes after MI. The authors also discovered an up-regulation of Wnt pathway inhibitor Wif1 in cardiomyocytes. Knockout studies revealed the deletion of *Wif1* led to more recruited monocytes, increased scar size, and reduced ejection fraction 4 weeks post MI. By contrast, cardiomyocyte-specific Wif1 overexpression attenuated the monocyte response and improved cardiac function after MI.[306] Furthermore, the expression of Wnt pathway inhibitors such as sFRP1 and sFRP5 has been shown to protect against further injury by mitigating the inflammatory response post-MI. When expressed in bone marrow cells (but not cardiomyocytes or endothelial cells), sFRP-1 reduced neutrophil infiltration after ischemia, lessened scar formation, and improved cardiac hemodynamic parameters.[307] Similarly, knockout of *sFRP5* in an ischemic heart resulted in greater infiltration of Wnt5a-positive macrophages and increased inflammatory cytokine and chemokine expression compared to wild-type mice.[308]

LRP5/Wnt3 signaling has been linked to valvular calcification and stenosis due to osteoblast differentiation in calcified aortic valves.[309] Arrhythmogenic cardiomyopathy (AC) is a disease of life-threatening ventricular arrhythmias and progressive dystrophy of the ventricular myocardium.[310] A role for GSK-3β in the pathogenesis of AC has been described, as it has been shown that GSK-3β localization occurs in intercalated disks of myocardium in mouse models of AC.[311] Wnt signaling has been linked to cardiac remodeling that can result in the progression of heart failure. The inactivation of Wnt inhibiting sFRP1 in the hearts of aged mice led to increased expression of ligands Wnt1, Wnt3, Wnt7b, Wnt16, and Wnt target genes Wisp1 and LEF1. Moreover, *sFRP1* knockout mice exhibited ventricular dilation and hypertrophy as well as depressed cardiac function and fibrosis. Further study revealed the down-regulation of sFRP-1 gene expression in human hearts with dilated cardiomyopathy and ischemic cardiomyopathy, suggesting similarity with the phenotype observed in *sFRP1* knockout mice.[312] Hou et al discovered that β-catenin is stabilized and translocated to the nucleus of cardiomyocytes in human ischemic and dilated

cardiomyopathy, as well as in mouse desmin cardiomyopathy. β-Catenin/transcription factor 7-like 2 (TCF7L2), of the TCF/LEF family of transcription factors, interacts with β-catenin to up-regulate c-Myc, resulting in cardiac hypertrophy. TCF7L2 knockdown was found to suppress these activities, suggesting TCF7L2 mediates the c-Myc up-regulation and abnormal cardiac remodeling seen in heart failure.[313]

Wnt signaling in metabolic disorders

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder primarily characterized by two factors, defective insulin secretion by β-cells in the pancreas and the inability of insulin-sensitive tissues to respond appropriately to insulin.[314] TCF7L2, a downstream effector of the Wnt/β-catenin signaling pathway, is a potent risk locus for T2DM.[315] A large human genome review and meta-analysis of different populations found that under a multiplicative genetic model, four variants of *TCF7L2* gene are associated with T2DM. The results of this study suggested the *TCF7L2* gene may be involved in nearly 1/5 of all T2DM cases.[316] While the exact mechanisms by which TCF7L2 polymorphisms affect T2DM are still not well understood, a number of association studies have helped to elucidate this role. Zhou et al utilized RNA sequencing to identify a TCF7L2-regulated transcriptional network responsible for insulin production and processing in rodent and human pancreatic islets. Wherein, ISL LIM homeobox 1 (Isl1) was found to be the primary target of TCF7L2. Isl1, in turn, mediates proinsulin production and processing via the regulation of PCSK1, PCSK2, SLC30A8, MAFA, PDX1, and NKX6.1.[317] Inactivation of TCF7L2 in the pancreatic pericytes of transgenic mice led to poor glucose tolerance as a result of β-cell dysfunction and impaired glucose-stimulated insulin secretion. These findings were accompanied by the discovery that TCF7L2-dependent pericytic expression of secreted factors such as BMP4 promoted β-cell function. Moreover, exogenous BMP4 rescued the impaired glucose-stimulated insulin secretion of transgenic mice, highlighting the importance of TCF7L2 activity in pancreatic β-cells.[318] Further studies in mice have shown that TCF7L2 loss-of-function in pancreatic β cells results in defective glucose-stimulated insulin secretion and a decrease in β cell mass.[319,320]

Obesity is characterized by the accumulation of excess energy into white adipose tissue. By contrast, brown adipose tissue (BAT) is primarily energy-dissipating and protects the body from cold through thermogenesis.[321,322] The loss of functioning Wnt10B leads to an inability to activate canonical Wnt signaling and block adipogenesis and, as Christodoulides et al discovered, mutations in Wnt10B are associated with early-onset obesity.[323] LGR4, a receptor for R-spondin ligands, has been implicated in energy expenditure by driving the conversion of white fat to brown fat. Wang et al demonstrated that LGR4 homozygous mutant mice exhibit reduced adiposity, greater energy expenditure, and higher expression of BAT markers. Mutant mice also resisted dietary and leptin mutant-induced obesity. Moreover, the ablation of LGR4 potentiated BAT differentiation. The authors also identified a functional low-frequency missense LGR4 variant associated with an increased risk of obesity.[324] Recently, *LGR4* gene polymorphisms found in Chinese nuclear families with female children were related to obesity phenotypes, including body mass index and trunk fat mass.[325]

Members of the sFRP family act as adipokines and have been associated with adipocyte dysfunction in obesity.[137] sFRP5 is an anti-inflammatory adipokine secreted by adipocytes and its potential role in obesity is an area of interest. In mouse models of obesity and diabetes, sFRP5 was shown to reduce adipose tissue inflammation with the overall effect of improving metabolic dysfunction by sequestering non-canonical Wnt5a and preventing the activation of the JNK pathway in macrophages and adipocytes.[326] Schulte et al discerned that obesity is associated with elevated serum levels of pro-inflammatory Wnt5a in humans. The authors also found that nutritional therapy and weight loss increaseses serum concentrations of sFRP5 in subjects with obesity.[327] A large population-based study discovered that serum sFRP5 was negatively associated with multiple risk factors for T2DM and cardiovascular diseases. At the same time, higher levels of sFRP5 were associated with lower odds of developing prediabetes/T2DM.[328] Another study found that subjects with T2DM exhibited elevated Wnt5a levels that positively correlated with IL-6 and triglyceride concentrations.[329] However, in contrast to results in mice implicating sFRP5 in metabolic dysfunction, Ehrlund et al found that sFRP5 was not actively secreted from human white adipose tissue and its expression was not influenced by obesity.[137] Taken together, these conflicting results suggest that the exact role of sFRP5 in obesity requires further elucidation.

Wnt signaling in neurological disorders

Wnt/β-catenin signals are important for the function of the adult nervous system and are critical for adult neurogenesis and synaptic maintenance.[330] Alzheimer's disease (AD) is a neurodegenerative disease characterized by the presence of amyloid-β plaques tau neurofibrillary tangles. AD patients experience progressive impairment of behavioral and cognitive functions.[331] Genetic analysis revealed variations in LRP6 were associated with late-onset AD, supporting the notion aberrant Wnt signaling is involved in AD.[332] Canonical Wnt signaling inhibition by Dkk1 results in increased GSK3-β activity and decreased cytoplasmic β-catenin protein levels. Similarly, a study of prefrontal lobe structures in AD patients found that brains with AD exhibited decreased β-catenin levels and increased phosphorylation of GSK3β compared to age-matched controls.[333] Neurofibrillary tangles are a characteristic lesion found in AD and are composed of hyper-phosphorylated forms of the microtubule-associated protein tau.[334] Several studies have implicated GSK3-β and GSK3-α as important tau-kinases in AD.[335–337] Moreover, autosomal dominant AD involves mutations of presenilin (PSEN) genes. Takashima et al discovered mutant PSEN1 binds both GSK3β and tau protein and that the association of GSK3-β with mutant PSEN1 prompts the phosphorylation of tau.[338] Furthermore, Purro et al showed that amyloid-β increases the levels of Dkk1 in the hippocampus, which in turn modulates synaptic loss

through the dispersal of synaptic components.[339] Further study revealed the induced expression of Dkk1 in the hippocampus of transgenic mouse mode triggers cognitive impairments such as synapse loss as well as learning and memory deficits. However, following the loss of synaptic connectivity, reactivation of the Wnt pathway by cessation of Dkk1 expression notably restored synapse number, synaptic plasticity, and long-term memory.[340] Moreover, the administration of lithium (which rescues the Wnt pathway by inhibiting GSK3-β) and rosiglitazone in mouse models of AD also resulted in spatial memory improvement, possibly due to the reactivation of Wnt signaling.[341]

Parkinson's disease (PD) is a common neurodegenerative disorder marked by motor symptoms such as tremors, rigidity, bradykinesia/akinesia, and postural instability. The substantia nigra is a midbrain dopaminergic nucleus crucial for modulating motor movement. Pathologically, PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta as well as the deposition of intra-cytoplasmic inclusions (Lewy bodies) containing ubiquitin and α-synuclein (α-syn).[342] Leucine-rich repeat kinase 2 (LRRK2) mutations have been linked with pathologies of α-syn and identified as a genetic risk factor for developing PD.[343] Berwick et al found that over-expressed LRRK2 binds to and represses β-catenin activity, suggesting LRRK2 may contribute to the β-catenin destruction complex. By contrast, the authors showed that the loss of LRRK2 led to increased canonical Wnt signaling both *in vitro* and *in vivo*.[344] Together, these results indicate decreased Wnt/β-catenin signaling may play a role in PD pathogenesis. Wnt/β-catenin signaling plays an important role in midbrain dopaminergic (mDA) neuron induction, as Wnt1 regulates the establishment of the dopaminergic progenitors in mammalian ventral midbrain.[345] A previous study has shown that the inactivation of Wnt1 in murine embryonic stem cell lines leads to the loss of the midbrain region.[346] Conversely, expression of ectopic Wnt1 has been shown to induce the overproliferation of precursor cells in the caudal midbrain.[347] Furthermore, Schulte et al have reported Wnt-3a enhances the proliferation of midbrain dopaminergic precursors and that Wnt-5a increases differentiation of mDA cells as well as disheveled phosphorylation.[348] Administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) induces a PD-like phenotype and MPTP mouse models have been used to replicate the pathophysiology of PD.[349] Interestingly, L'Episcopo et al discovered MPTP-reactive astrocytes express Wnt1 and engage in the rescue of mesencephalic dopaminergic neurons from MPP (the active metabolite of MPTP) induced neurotoxicity.[350] Of note, aging impairs Wnt-mediated self-protection, and neurogenesis/neurorepair capacity in the ventral midbrain, as astrocyte-derived Wnt signaling is down-regulated with age while the expression of endogenous Wnt antagonists such as Dkk1 is up-regulated.[351]

Wnt signaling in other human diseases

Wnt signaling is known to play a critical role in embryonic development and has been implicated in a number of developmental disorders. For example, Wnt/β-catenin signaling is necessary for proper lung development. β-Catenin inactivation in mouse foregut endoderm leads to the absence of both the lungs and trachea. By contrast, activated β-catenin has been linked to Nkx2.1 expression, an early marker of trachea and lung development.[352] Bronchopulmonary dysplasia is a chronic lung disease in infants caused by prenatal and/or postnatal injury to developing lungs. It was discovered that Wnt signaling is up-regulated in hyperoxia-induced arrest in alveolar development and subsequent bronchopulmonary dysplasia in neonatal rats.[353] Wnt signaling also plays a major role in nephrogenesis. Failures of Wnt signaling may result in congenital anomalies of the kidney and urinary tract.[354] Furthermore, *β-catenin* knockout mice exhibited hypoplastic kidneys with reduced renal function, absence of the superficial layer of renal tubules, a thin cortex, and underdeveloped capillary tufts in the glomeruli.[355] Additionally, *LRP6* knockout in mouse embryos resulted in hypoplastic kidneys and cyst development.[356] Highlighting the important role of Wnt signaling in human organogenesis, loss of function missense mutations in Wnt4 were described in SERKAL syndrome, an autosomal recessive syndrome characterized by female-to-male sex reversal as well as kidney, adrenal, and lung dysgenesis.[357] Mutations in Fzd1 and Fzd2 are known to cause cleft palate and ventricular septal defects in mice. These mutations also affected neural tube closure and inner ear development. Fzd7 mutations were shown to cause ventricular septal defects but were more commonly associated with a kinked tail phenotype.[358] Loss-of-function mutations in Wnt3 have been linked to Tetra-Amelia, which is characterized by the congenital absence of all four limbs.[359] DVL1 frameshift mutations have been identified and associated with autosomal dominant Robinow syndrome, a rare disorder of mesomelic limb shortening, genital hypoplasia, and distinct facial features.[360] Additionally, missense mutations in Wnt5a have also been associated with Robinow syndrome.[361]

Dupuytren's disease is a fibroproliferative disorder that causes the palmar fascia to thicken and shorten. Patients with Dupuytren's present with nodules on the palm that can progress to contracture deformities of the hand.[362] Studies have noted increased β-catenin expression in Dupuytren's disease.[363,364] A genome-wide association study identified multiple loci involved in the Wnt signaling pathway that were associated with susceptibility to Dupuytren's disease. Wherein, polymorphisms in *Wnt2*, *Wnt4*, *Wnt7B*, *R-spo2*, and *sFRP4* genes were linked to the process of fibromatosis seen in Dupuytren's.[365] Further study revealed a significantly higher expression of Wnt7B and β-catenin in nodules from Dupuytren's patients compared to controls, adding to the notion of canonical Wnt activation in Dupuytren's.[366] Furthermore, X-linked mutations in PORCN (an o-acyltransferase critical for cellular export of Wnt signaling proteins) are known to cause focal dermal hypoplasia, a multisystem disorder characterized by developmental abnormalities of the skin, skeletal system, eyes, and face.[367,368] With respect to other fibrotic diseases, psoriasis is a chronic immune-mediated skin condition characterized by inflammation, uncontrolled keratinocyte proliferation, and erythematous scaly plaques.[369] Studies of lesional and uninvolved psoriatic skin have revealed the increased expression of Wnt5a in psoriatic lesions.[370,371] By contrast,

the expression of soluble Wnt inhibitors such as WIF-1, sFRP1, sFRP2, sFRP4, and sFRP5 as well as Dkk1, Dkk2, and Dkk3 were down-regulated in psoriatic skin.[371] Together, these results suggest non-canonical signaling via Wnt5a may play a role in the pathogenesis of psoriasis.

Dysregulation of Wnt signaling is also associated with idiopathic pulmonary fibrosis (IPF), a disease characterized by progressive and chronic lung scarring that is detrimental to lung function. Although the exact role of Wnt signaling is not well understood, current research suggests that the crosstalk between Wnt and TGF-β underlies the development of IPF. β-catenin and LRP5 promote fibrosis by increasing TGF-β, while TGF-β can activate canonical Wnt signaling in fibroblasts.[372] However, it has been reported that β-catenin in the alveolar epithelium may be implicated in a protective role against lung fibrosis, so the exact role of Wnt signaling in IPF remains complicated and requires further delineation.[373] Aberrant Wnt signaling is also involved in the development of renal cysts, as the deletion of APC in mice led to increased β-catenin levels and cyst formation.[374]

Targeting Wnt signaling as novel therapeutics

Wnt signaling is involved in a multitude of physiological processes and, unsurprisingly, aberrant Wnt signaling is associated with a wide variety of diseases, including cancer. Advances in the understanding of Wnt signaling have enabled the creation of novel therapeutics that target components of the Wnt pathway. There is a wide array of Wnt-targeting therapeutics in preclinical studies or clinical trials that are under development for numerous disease applications. In this review, we will outline the drugs for Wnt-related bone disease that are approved or in clinical trials as well as the anti-cancer therapeutics in preclinical and clinical studies.

Wnt-targeting therapeutics for bone and skeletal diseases

Osteoporosis is a common bone disease characterized by low BMD that predisposes patients to low-impact, fragile fractures. Osteogenesis imperfecta, also known as brittle bone disease, is marked by early-onset osteoporosis, increased bone fragility, bone deformity, and short stature. The risk of developing osteoporosis increases with age and it was estimated osteoporosis causes greater than 8.9 million fractures worldwide each year, with fractures commonly occurring in the hip, vertebrae, and distal forearm.[270] Moreover, the incidence of osteoporotic fracture in women is higher than that in men.[375] Postmenopausal women are susceptible to developing osteoporosis, in particular, due to a decline in estrogen levels and progressive loss of its anti-osteoclastic and osteoanabolic effects.[376,377] Osteoporotic fractures can lead to a significant decrease in quality of life, while increasing morbidity and disability.[378] A recent retrospective cohort study of 3992 patients admitted to the hospital due to osteoporotic hip fracture in 17 years reported an increase in mortality for patients with hip fracture and a higher mortality rate in men than in women. Moreover, the risk of death was highest immediately after fracture occurrence.[379] The pain, disability, and costs associated with osteoporosis reflect the essential need for effective measures to prevent fractures. Important contributing factors to the ability of bone to withstand trauma include density, size, micro-, and macro-architecture as well as turnover.[380] Current therapies for osteoporosis include antiresorptive treatments and anabolic drugs. Antiresorptive drugs are more widely utilized and include bisphosphonates (first-line drugs for osteoporosis that work by inducing osteoclasts apoptosis), selective estrogen receptor modulators, and RANKL inhibitors (drugs that inhibit osteoclast activity by direct binding to RANKL). By contrast, current anabolic therapies include parathyroid hormone analogues and the recently approved romosozumab (a monoclonal antibody targeting sclerostin).[381]

Due to its role in osteogenesis and bone metabolism, the Wnt pathway presents a promising therapeutic target for the treatment of osteoporosis and osteogenesis imperfecta (Table 2). Encoded by the *SOST* gene, sclerostin is a small protein that antagonizes Wnt signaling by binding to the Wnt co-receptors LRP5/6 with the effect of inhibiting bone formation and indirectly promoting bone resorption. In this review, we will discuss the monoclonal antibodies targeting sclerostin currently in clinical trials as well as the efficacy and safety of romosozumab (Evenity), the first FDA-approved sclerostin inhibitor for the treatment of osteoporosis in postmenopausal women with a high risk of fracture. Approved by the FDA in 2019, romosozumab is a fully humanized and specific monoclonal IgG2 antibody against sclerostin that works by binding to sclerostin and preventing its inhibition of the Wnt signaling pathway.[382] It is also the first anabolic medication for osteoporosis that increases bone formation while decreasing bone resorption.[383] The Fracture Study in Postmenopausal Women with Osteoporosis (FRAME) phase III study demonstrated that romosozumab treatment was associated with a lower risk of vertebral fracture than placebo at 12 months and, following

Table 2 List of therapeutic antibodies targeting sclerostin for osteoporosis.

Drug (Monoclonal Antibody)	Treatment or Indication	Phase of Clinical Trial	Trial Identifier(s)
Romosozumab	Postmenopausal Osteoporosis	FDA approved (2019)	NCT01575834, NCT01631214, NCT00896532
	Osteogenesis Imperfecta	Phase I	NCT04545554
Blosozumab	Postmenopausal Osteoporosis	Phase II	NCT01742078, NCT01144377
SHR-1222	Postmenopausal Osteoporosis	Phase I	NCT04435158
Setrusumab (BPS-804)	Osteogenesis Imperfecta	Phase II	NCT01417091, NCT03118570

the transition to Denosumab (a RANKL inhibitor), at 24 months.[384] The romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis (ARCH) phase III study compared a regimen of romosozumab transitioning to alendronate (bisphosphonate drug) versus an alendronate treatment alone. Results of the trial showed the risk of new vertebral fracture was reduced by 48% in the romosozumab-alendronate group when compared with the alendronate—alendronate group.[385] A phase II study found that romosozumab does not exhibit long-lasting effects after its discontinuation. In the absence of another treatment for osteoporosis, the BMD of study subjects returned to close to baseline 1 year after discontinuation of romosozumab.[386] The primary safety concern of romosozumab use is potential cardiovascular events. Due in part to the results of the ARCH study that showed an imbalance between groups in cardiovascular-related adverse events, a boxed warning for romosozumab was included that outlined the potential risk of MI, stroke, and cardiovascular death associated with romosozumab use.[383]

Blosozumab is another monoclonal antibody against sclerostin that has undergone clinical trials. Structurally diverse from romosozumab, it is also designed to treat postmenopausal osteoporosis.[381] As of this writing, blosozumab has undergone phase I and II clinical trials. Blosozumab was well tolerated in the phase I study that demonstrated dose-dependent increases in bone formation biomarkers and BMD after single and multiple doses of the drug.[387] The phase II study showed a dose-dependent increase in bone formation markers as well as a reduction in bone resorption, as assessed by CTX (a biochemical marker of bone resorption). Moreover, compared with placebo, blosozumab treatment resulted in significant dose-related increases in the spine, femoral neck, and total hip BMD. Furthermore, mild injection site reactions were reported more frequently with blosozumab compared to placebo.[388] In a follow-up study 1 year after discontinuing treatment, women initially treated with blosozumab 270 mg Q2W and blosozumab 180 mg Q2W showed lumbar spine BMD that remained significantly greater than placebo.[389] SHR-1222 is the newest monoclonal antibody targeting sclerostin in postmenopausal osteoporosis to enter clinical trials. It is currently in its phase I trial and no clinical data has been published up to this point. Setrusumab (BPS-804) is a monoclonal antibody against sclerostin used to treat osteogenesis imperfecta that has been tested in a phase IIa clinical trial. A study of 14 adult patients with osteogenesis imperfecta demonstrated that BPS-804 was generally well tolerated and treatment stimulated bone formation, reduced bone resorption (as measured by CTX levels), and increased lumbar spine BMD (by 4% on day 141 compared with baseline).[390]

Anti-cancer therapeutics targeting Wnt signaling

As previously mentioned, aberrations of the Wnt pathway are implicated in numerous cancers. Research in this area has paved the way for the development of anti-cancer therapeutics that target the Wnt pathway. Many have entered clinical trials, either as monotherapies or in combination with chemotherapy (Table 3).

Porcupine (PORCN) inhibitors prevent the post-translational palmitoylation of Wnt ligands. Thereby, PORCN inhibitors work to block the secretion of Wnt ligands and the triggering of Wnt signaling, which can have implications for the control of aberrant cell growth in cancer.[391] Current applications of PORCN inhibitors in clinical trials include solid tumors, gastrointestinal cancers, pancreatic cancer, BRAF-mutant CRC, melanoma, and TNBC among others. The interaction between cAMP response element binding (CREB) binding protein (CBP) and β-catenin is believed to activate genes linked to cell proliferation,[392] and inhibitors are under development. Moreover, small molecule inhibitors of TCF4/β-catenin interaction that prevent the transcription of Wnt target genes have demonstrated anti-tumor activity in preclinical experiments.[393,394] Signal transduction through the non-canonical Wnt co-receptor, tyrosine kinase-like orphan receptor (ROR), is overexpressed in a number of hematologic malignancies and solid tumors. β-Catenin independent Wnt/ROR signaling has been linked to processes such as tumor cell proliferation, survival, invasion, or treatment resistance. As a result, targeting ROR1/ROR2 is a point of interest for anti-cancer therapies and several drugs are in clinical trials.[395]

Tankyrase enzymes are negative regulators of the β-catenin destruction complex that mediate the ubiquitination and degradation of the scaffolding protein Axin.[396] A number of tankyrase inhibitors have been in development and several have shown promising anti-tumor activity in preclinical experiments.[397,398] However, none have entered clinical trials as of this writing. Foxy-5 (a formylated Wnt5A-derived six amino acid peptide) functions as a Wnt5a mimicking peptide that has exhibited anti-metastatic effects in preclinical experiments.[399,400] Foxy-5 has also undergone phase I clinical trials for the treatment of metastatic breast, colon, and prostate cancer. LY2090314 is a GSK-3β inhibitor that activates the Wnt/β-catenin pathway. Preclinical studies of LY2090314 have demonstrated the inhibition of tumor growth in melanoma and neuroblastoma.[401,402] LY2090314 has also undergone clinical trials both as a single therapy for leukemia and in combination with other chemotherapies for advanced cancers.

Besides small molecule inhibitors, antibody-based therapeutics have also been developed to target Wnt signaling. DKN-01 is an IgG4 monoclonal antibody that targets Dkk1. While the specific mechanism is unknown, Dkk1 is thought to play a role in tumor immune resistance. Elevated levels of Dkk1 have also been associated with poor prognosis in gynecologic and gastroesophageal cancers.[403] Vantictumab (OMP-18R5) is a monoclonal antibody that binds to FZD1, FZD2, FZD5, FZD7, and FZD8 receptors through a conserved epitope within the extracellular domain. Preclinical testing in a xenograft model demonstrated reduced tumor growth and tumor-initiating cell frequency and synergistic activity with other chemotherapeutic agents.[404] OMP-18R5 has undergone phase I clinical trials as both a single therapy and in combination with other agents. Furthermore, OMP-54F28 is a fused protein of truncated FZD8 and IgG1 Fc region that antagonizes Wnt signaling by competing with the native Fzd8 receptor for ligands. Preclinical experiments have demonstrated inhibition of tumor growth in an MMTV-Wnt1-induced tumor model as well as synergy with gemcitabine in a pancreatic cancer xenograft model.[405]

Table 3 List of anti-cancer Wnt-targeting therapeutics.

Mode of Action	Drug(s)	Cancer (Indication)	Phase	Identifier
PORCN Inhibitor	LGK974	Pancreatic cancer, BRAF mutant CRC, Melanoma, TNBC, HNSCC, Cervical squamous cell cancer, Esophageal squamous cell cancer, Lung squamous cell cancer	Phase I	NCT01351103
	WNT974 (with LGX818 and Cetuximab)	Metastatic CRC	Phase I/II	NCT02278133
	ETC-1922159 (single agent and with Pembrolizumab)	Advanced solid tumors	Phase I	NCT02521844
	CGX1321	GI Cancer	Phase I	NCT03507998
	CGX1321 (with Pembrolizumab or Encorafenib + Cetuximab)	Solid tumors, GI cancer	Phase I	NCT02675946
	RXC004	Advanced solid tumors	Phase II	NCT04907851
	RXC004 (with Nivolumab)	Advanced solid tumors	Phase I	NCT03447470
CBP/β-catenin antagonist	PRI-724	Advanced or metastatic pancreatic adenocarcinoma	Phase I	NCT01764477
	PRI-724	Acute myeloid leukemia, Chronic myeloid leukemia	Phase I/II	NCT01606579
ROR1- and ROR2-antagonist	Cirmtuzumab	Chronic Lymphocytic Leukemia	Phase I	NCT02222688
	Cirmtuzumab (with ibrutinib)	B-cell chronic lymphocytic leukemia, Small lymphocytic lymphoma, Mantle cell lymphoma, Marginal zone lymphoma	Phase I/II	NCT03088878
	Zilovertamab vedotin	Breast cancer, Lung cancer, Pancreatic cancer, Gastric cancer	Phase II	NCT04504916
	CCT301-59	Solid tumors, Soft tissue sarcoma, Gastric cancer, Pancreatic cancer, Bladder cancer	Phase I	NCT03960060
Wnt5a peptide mimic	Foxy-5	Metastatic Breast cancer, Metastatic Colon cancer, Metastatic Prostate cancer	Phase I	NCT02655952
	Foxy-5	Colon cancer	Phase II	NCT03883802
GSK-3β inhibitor	LY2090314 (with pemetrexed and carboplatin)	Advanced cancer	Phase I	NCT01287520
	LY2090314	Leukemia	Phase II	NCT01214603
mAb targeting Dkk1	DKN-01	Multiple myeloma, Solid tumors, Non-small cell lung cancer	Phase I	NCT01457417
	DKN-01 (with lenalidomide/ dexamethasone)	Multiple myeloma	Phase I	NCT01711671
	DKN-01 (with Nivolumab)	Advanced Biliary Tract Cancer	Phase II	NCT04057365
	DKN-01 (with Gemcitabine/Cisplatin)	Carcinoma of intrahepatic and extra-hepatic biliary system, Carcinoma of gallbladder, Cholangiocarcinoma	Phase I	NCT02375880
LRP5/6 antagonist	BI 905677	Solid tumors	Phase I	NCT03604445
mAb targeting FZD receptors	OMP-18R5	Solid tumors	Phase I	NCT01345201
	OMP-18R5 (with Docetaxel)	Solid tumors	Phase I	NCT01957007
	OMP-18R5 (with Paclitaxel)	Metastatic breast cancer	Phase I	NCT01973309
	OMP-18R5 (with Nab-Paclitaxel and Gemcitabine)	Stage IV pancreatic cancer	Phase I	NCT02005315

(*continued on next page*)

Table 3 (*continued*)

Mode of Action	Drug(s)	Cancer (Indication)	Phase	Identifier
Truncated FZD8 fused to IgG1 Fc	OMP-54F28	Solid tumors	Phase I	NCT01608867
	OMP-54F28 (with Sorafenib)	Hepatocellular cancer	Phase I	NCT02069145
	OMP-54F28 (with Paclitaxel and Carboplatin)	Ovarian cancer	Phase I	NCT02092363
	OMP-54F28 (with Nab-Paclitaxel and Gemcitabine)	Stage IV pancreatic cancer	Phase I	NCT02050178

Lastly, there are numerous repurposed drugs (*e.g.*, niclodamide and pyrvinium) and chemical/natural products (*e.g.*, ginsenoside Rg3, resveratrol, and tetrandrine) that have been shown to target various components of Wnt signaling in cancer cells as a part of their modes of action, although they may not exclusively impact Wnt signaling.[235,406–413] It is thus anticipated more Wnt signaling-targeting chemicals and compounds will be identified and subsequently developed as novel anticancer agents.

Conclusions and future directions

Since its discovery nearly 40 years ago, research into the evolutionarily conserved Wnt pathway has broadened our understanding of stem cell self-renewal, tissue homeostasis, and diseases. Research has also revealed the underlying mechanisms of the canonical and the non-canonical paths. The study of Wnt signaling has not only increased our knowledge of physiological development and homeostasis but also of disease pathology when components of Wnt signaling experience dysregulation. These developments in our understanding of Wnt signaling have paved the way for the creation of novel therapeutics. The development of romosozumab (a monoclonal antibody targeting sclerostin) illustrates the therapeutic potential in targeting the Wnt pathway in human diseases. While many anti-cancer treatments targeting the Wnt pathway have entered the early phases of clinical trials, they face a significant challenge in side effects on normal tissue. As a result, further study is warranted in the area of cancer-specific regulators of Wnt signaling that may prove to be druggable targets of Wnt-associated cancers.

Conflict of interests

All authors declare no conflict of interests.

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