

WNT Signaling Pathway and Stem Cell Signaling Network

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Abstract WNT signals are transduced to the canonical pathway for cell fate determination, and to the non-canonical pathway for control of cell movement and tissue polarity. Canonical WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 coreceptor to the β -catenin signaling cascade. Microtubule affinity – regulating kinase (PAR-1) family kinases, casein kinase I ϵ (CKI ϵ), and FRAT are positive regulators of the canonical WNT pathway, whereas APC, AXIN1, AXIN2, CKI α , NKD1, NKD2, β TRCP1, β TRCP2, ANKRD6, Nemo-like kinase (NLK), and peroxisome proliferator – activated receptor γ (PPAR γ) are negative regulators. Nuclear complex, consisting of T-cell factor/lymphoid enhancer factor, β -catenin, BCL9/BCL9L, and PYGO, activates transcription of canonical WNT target genes such as *FGF20*, *DKK1*, *WISP1*, *MYC*, *CCND1*, and *Glucagon* (*GCG*). Noncanonical WNT signals are transduced through Frizzled family receptors and ROR2/RYK coreceptors to the Dishevelled-dependent (Rho family GTPases and c-jun NH₂-terminal kinase) or the Ca²⁺-dependent (NLK and nuclear factor of activated T cells) signaling cascades. WNT signals are context-dependently transduced to both pathways based on the expression profile of WNT, SFRP, WIF, DKK, Frizzled receptors, coreceptors, and the activity of intracellular WNT signaling regulators. Epigenetic silencing and loss-of-function mutation of negative regulators of the canonical WNT pathway occur in a variety of human cancer. WNT, fibroblast growth factor (FGF), Notch, Hedgehog, and transforming growth factor β /bone morphogenetic protein signaling network are implicated in the maintenance of tissue homeostasis by regulating self-renewal of normal stem cells as well as proliferation or differentiation of progenitor (transit-amplifying) cells. Breakage of the stem cell signaling network leads to carcinogenesis. Nonsteroidal anti-inflammatory drugs and PPAR γ agonists with the potential to inhibit the canonical WNT signaling pathway are candidate agents for chemoprevention. ZTM000990 and PKF118-310 are lead compounds targeted to the canonical WNT signaling cascade. Anti-WNT1 and anti-WNT2 monoclonal antibodies show *in vitro* effects in cancer treatment. After the optimization, derivatives of small-molecule compound and human monoclonal antibody targeted to the WNT signaling pathway could be used in cancer medicine.

WNT Signaling Pathway

Overview. The human *WNT* gene family consists of 19 members, encoding evolutionarily conserved glycoproteins with 22 or 24 Cys residues (1). WNT signals are transduced to the canonical pathway for cell fate determination, and to the noncanonical pathway for control of cell movement and tissue polarity (Fig. 1).

Canonical WNT signaling pathway. Canonical WNT signals are transduced through Frizzled (FZD) family receptors and LRP5/LRP6 coreceptor to the β -catenin signaling cascade (2, 3). In the absence of canonical WNT signaling, β -catenin complexed with APC and AXIN is phosphorylated by casein kinase I α (CKI α) and glycogen synthase kinase 3 β (GSK3 β) in the NH₂-terminal degradation box, which is polyubiquitinated

by β TRCP1 or β TRCP2 complex for the following proteasome-mediated degradation (4). In the presence of canonical WNT signaling, Dishevelled (DVL) is phosphorylated by CKI α for high-affinity binding to FRAT. Because canonical WNT signal induces the assembly of FZD-DVL complex and LRP5/6-AXIN-FRAT complex (5, 6), β -catenin is released from phosphorylation by CKI α and GSK3 β for stabilization and nuclear accumulation. Nuclear β -catenin is complexed with T-cell factor/lymphoid enhancer factor (TCF/LEF) family transcription factors and also with Legless family docking proteins (BCL9 and BCL9L) associated with PYGO family coactivators (PYGO1 and PYGO2; refs. 7, 8). The TCF/LEF- β -catenin-Legless-PYGO nuclear complex is the effector of the canonical WNT signaling pathway to activate the transcription of target genes such as *FGF20*, *DKK1*, *WISP1*, *MYC*, and *CCND1* (9–12).

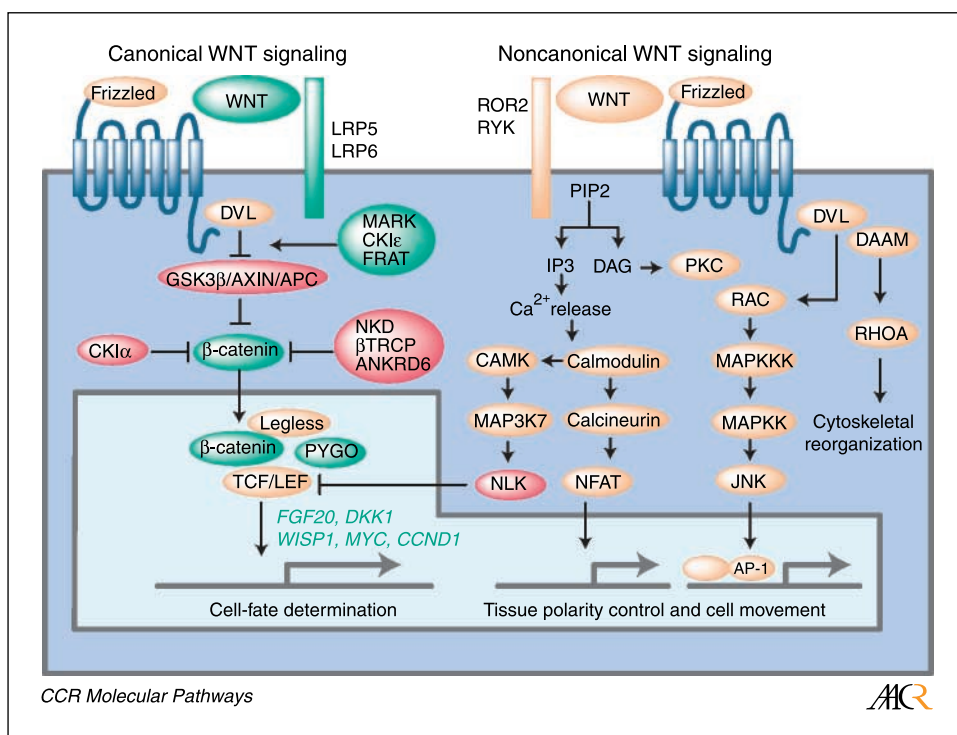
Noncanonical WNT signaling pathway. Noncanonical WNT signals are transduced through FZD family receptors and coreceptors, such as ROR2 and RYK (13–15). Small G proteins (RHOA, RHOU, RAC, and CDC42) and c-jun NH₂-terminal kinase are the DVL-dependent effector molecules of the noncanonical pathway (16, 17), whereas Nemo-like kinase (NLK) and nuclear factor of activated T cells (NFAT) are the Ca²⁺-dependent effector molecules of noncanonical pathway (18, 19). Small G proteins are implicated in the cytoskeletal reorganization during invasion and metastasis. NLK

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Fig. 1. Landscape of WNT signaling cascades. WNT signals are transduced to the canonical pathway for cell fate determination, and to the noncanonical pathway for control of cell movement and tissue polarity. Canonical WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 coreceptor to the β -catenin signaling cascade. Noncanonical WNT signals are transduced through Frizzled family receptors and ROR2/RYK coreceptors to the DVL-dependent (Rho family GTPases and JNK) or the Ca^{2+} -dependent (NLK and NFAT) signaling cascades. Microtubule affinity – regulating kinase (*MARK*; *PAR-1*) family kinases, *CKI ϵ* , and *FRAT* are positive regulators of the canonical WNT pathway, whereas *APC*, *AXIN1*, *AXIN2*, *CKI α* , *NKD1*, *NKD2*, β *TRCP1*, β *TRCP2*, *ANKRD6*, *NLK*, and *PPAR γ* are negative regulators. *FGF20*, *DKK1*, *WISP1*, *MYC*, *CCND1*, and *Glucagon (GCG)* are target genes of the canonical WNT signaling pathway. WNT signals are context-dependently transduced to both pathways based on the expression profile of WNT, *SFRP*, *WIF*, *DKK*, Frizzled receptors, coreceptors, and the activity of intracellular WNT signaling regulators.



phosphorylates TCF/LEF family transcription factors to inhibit the canonical WNT signaling pathway. NFAT transcription factor is implicated in the convergent extension during early embryogenesis as well as in the metastasis during carcinogenesis. Noncanonical WNT signaling pathway, transduced to a variety of DVL- or Ca^{2+} -dependent cascades, is overlapping with the planar cell polarity signaling pathway (20).

WNT Signaling Pathway and Carcinogenesis

Secreted-type WNT signaling inhibitors. *SFRP1*, *SFRP2*, *SFRP3*, *SFRP4*, *SFRP5*, *WIF1*, *DKK1*, *DKK2*, *DKK3*, and *DKK4* are secreted-type WNT signaling inhibitors (21). *SFRP* family members and *WIF1* are WNT antagonists that inhibit WNT binding to FZD family receptors. *DKK* family members interact with LRP5/LRP6 coreceptor and trigger its endocytosis to prevent formation of the WNT-FZD-LRP5/LRP6 complex for the canonical WNT signaling.

Intracellular-type canonical WNT signaling inhibitors. *APC*, *AXIN1*, *AXIN2*, *CKI α* , *GSK3 β* , *NKD1*, *NKD2*, *ANKRD6*, and *NLK* are negative regulators of the canonical WNT signaling pathway. *APC*, *AXIN1*, and *AXIN2* are scaffold proteins of the β -catenin destruction complex, whereas *CKI α* and *GSK3 β* are serine/threonine kinases that phosphorylate β -catenin to trigger degradation.

Canonical WNT signaling activation during carcinogenesis. Genetic predisposition, environmental factor, and aging are risk factors of human cancer (22). Transcriptional activation of canonical WNTs occurs during tissue regeneration associated with chronic persistent inflammation, and up-regulation of *Wnt1*, *Wnt3*, or *Wnt10b* due to mouse mammary tumor virus (MMTV) integration leads to mammary carcinogenesis (23, 24).

Epigenetic silencing of *SFRP1*, *SFRP2*, *DKK1*, *WIF1*, and *AXIN2* genes occurs in premalignant tissues associated with

chronic inflammation or in human cancer, whereas mutation of *APC*, *AXIN1*, and *AXIN2* genes occurs in human cancer (24–27). Genes encoding canonical WNT signaling inhibitors are down-regulated during carcinogenesis due to epigenetic silencing and genetic alteration.

Network between WNT and Other Oncodevelopmental Pathways

Fibroblast growth factor signaling pathway. Fibroblast growth factor (FGF) signals are transduced through FGF receptors to the phosphatidylinositol 3-kinase-AKT, Ca^{2+} , and mitogen-activated protein kinase signaling cascades. Mammary carcinogenesis in MMTV-*Wnt1* transgenic mice is accelerated by MMTV integration around *Fgf3-Fgf4* or *Fgf8* loci, and that in MMTV-*Fgf3* transgenic mice by MMTV integration around *Wnt1-Wnt10b* locus (23, 24). *FGF20* is up-regulated in human colorectal cancer as the target gene of the canonical WNT signaling pathway (9). Although the mechanisms are not the same, FGF and canonical WNT signaling pathways inhibit *GSK3 β* activity and activate Ca^{2+} signaling. FGF and canonical WNT signaling pathways converge to the β -catenin signaling cascade and the epithelial-mesenchymal transition signaling cascade through *GSK3 β* down-regulation (24), and also to the Ca^{2+} -dependent NFAT signaling cascade. Cross-talk of FGF and WNT signaling pathways in tumors leads to more malignant phenotype through the potentiation of β -catenin, epithelial-mesenchymal transition, and NFAT signaling cascades.

Notch signaling pathway. Notch ligand binding to Notch family receptor induces transcriptional activation of Notch target genes through the nuclear translocation of Notch intracellular domain. Among Notch ligand genes, *JAG1* gene is predicted as an evolutionarily conserved target of the canonical WNT signaling pathway based on the conservation of double

TCF/LEF-binding sites within the 5' promoter region of mammalian *JAG1* orthologues (28). Notch4 is activated due to MMTV integration during mammary carcinogenesis (29), wherein WNT signaling pathway is also activated. Notch and WNT signaling pathways are both necessary for the self-renewal of hematopoietic stem cells (30). Notch and WNT signaling pathways synergize to inhibit terminal differentiation of intestinal epithelial cells partially through down-regulation of ATOH1/HATH1 bHLH transcription factor. Together, these facts indicate that Notch and WNT signaling pathways keep the homeostasis of stem and progenitor (transit-amplifying) cells through the inhibition of terminal differentiation.

Other signaling pathways. WNT signaling pathway also networks with Eph, vascular endothelial growth factor, Hedgehog, and transforming growth factor β /bone morphogenetic protein (TGF β /BMP) signaling pathways, which are reviewed elsewhere.

Recent Advances in WNT Signaling Pathway

Context-dependent WNT signaling. During the early period of WNT research when β -catenin and TCF/LEF were not the "star players" of the canonical WNT signaling pathway, *Xenopus* axis duplication assay was mainly used to measure the canonical WNT signaling activity. Because injection of synthetic *Wnt1* or *Wnt3a* mRNA into the ventral marginal zone of *Xenopus* embryos at the four-cell stage induces axis duplication, *Wnt1* and *Wnt3a* were previously characterized as the canonical WNTs (1). Recently, *Wnt1* and *Wnt3a* were characterized to activate not only the canonical WNT signaling pathway but also the noncanonical WNT signaling pathway based on the cell or tissue type (15, 17). On the other hand, *Wnt4*, *Wnt5a*, and *Wnt11* were previously characterized as noncanonical WNTs. Although injection of *Wnt4*, *Wnt5a*, or *Wnt11* mRNA did not induce *Xenopus* axis duplication, co-injection of one of these noncanonical *Wnt* mRNA with *FZD5* mRNA did induce *Xenopus* axis duplication (31, 32). Therefore, WNT signals are context-dependently transduced to the canonical and non-canonical pathways based on the expression profile of WNT, SFRP, WIF, DKK, FZD family receptors, coreceptors, and the activity of cytoplasmic WNT signaling regulators.

Peroxisome proliferator-activated receptor γ . WNT10B activates the canonical WNT signaling pathway to inhibit adipogenesis (33), whereas *Wnt5b* partially inhibits the canonical WNT signaling pathway to promote adipogenesis (34). Peroxisome proliferator-activated receptor γ (PPAR γ) is a nuclear receptor predominantly expressed in adipose tissues to induce adipocyte differentiation and β -catenin degradation (35), and is also expressed in intestinal epithelium. PPAR γ counteracts the canonical WNT signaling during adipocyte differentiation and colorectal carcinogenesis. PPAR γ agonist is

one of the promising drugs to inhibit the canonical WNT signaling pathway; however, the mode of small molecules on PPAR γ activity in adipose tissues is different from that in colorectal epithelium or colorectal cancer. Therefore, PPAR γ agonist inhibiting the canonical WNT signaling pathway in human colorectal epithelium should be developed for clinical application to cancer patients.

Cancer stem cells. Hematologic stem cells and mammary gland stem cells have been isolated for their characterization (30, 36), whereas intestinal stem cells remain to be isolated (37). However, it is generally believed that the WNT, FGF, Notch, Hedgehog, and TGF β /BMP signaling network is implicated in the maintenance of tissue homeostasis by regulating self-renewal of normal stem cells as well as proliferation or differentiation of progenitor cells (9, 28, 30, 36–39). Breakage of the regulation for "stem cell signaling" network in normal stem cells leads to the transformation to cancer stem cells. Alternatively, acquisition of self-renewal potential in progenitor cells due to epigenetic change or genetic alteration of stem cell signaling-related genes gives rise to cancer stem cells. Detailed analyses on the dysregulation of WNT, FGF, Notch, Hedgehog, and TGF β /BMP signaling pathways in cancer stem cells derived from a various type of human tissues or organ should be systematically investigated for better understanding of cancer stem cells themselves as well as the scenario of carcinogenesis.

Clinical Implications

Prevention. *Helicobacter pylori*, hepatitis viruses, and papilloma viruses are causative agents for gastric cancer, liver cancer, and cervical cancer, respectively. Because chronic persistent inflammation is a causative factor for a variety of human cancer, eradication and vaccination are realistic methods for cancer prevention. However, eradication and/or vaccination could fail to prevent cancer in cases with persistent inflammation with tissue reorganization and epigenetic changes. Because up-regulation of WNT family ligands and down-regulation of WNT inhibitors occur during the early stage of carcinogenesis, nonsteroidal anti-inflammatory drugs and PPAR γ agonists with the potential to inhibit the canonical WNT signaling pathway are candidate agents for chemoprevention.

Therapeutics. Small-molecule compounds and human (or humanized) monoclonal antibodies are promising drugs in the post-genome era (22). ZTM000990 and PKF118-310 are lead compounds targeted to the canonical WNT signaling cascade, whereas anti-WNT1 and anti-WNT2 monoclonal antibodies show *in vitro* effects in cancer treatment (40). After the optimization, derivatives of small-molecule compound and human monoclonal antibody targeted to the WNT signaling pathway could be used in cancer medicine.

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