WNT Signaling Pathway and Stem Cell Signaling Network

Masuko Katoh[1] and Masaru Katoh[2]

Abstract 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. Microtubule affinity–regulating kinase (PAR-1) family kinases, casein kinase Iε (CKIε), and FRAT are positive regulators of the canonical WNT pathway, whereas APC, AXIN1, AXIN2, CKIα, NKD1, NKD2, βTRCP1, βTRCP2, ANKRD6, Nemo-like kinase (NLK), and peroxisome proliferator–activated receptor γ (PPARγ) 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 NH_2-terminal kinase) or the Ca^{2+}-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 PPARγ 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 Iα (CKIα) and glycogen synthase kinase 3β (GSK3β) in the NH_2-terminal degradation box, which is polyubiquitinated by βTRCP1 or βTRCP2 complex for the following proteasome-mediated degradation (4). In the presence of canonical WNT signaling, Dishevelled (DVL) is phosphorylated by CKIα 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 CKIα and GSK3β 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 NH_2-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 Ca^{2+}-dependent effector molecules of noncanonical pathway (18, 19). Small G proteins are implicated in the cytoskeletal reorganization during invasion and metastasis. NLK

Authors' Affiliations: [1]M&M Medical BioInformatics, Hongo, Japan and [2]Genetics and Cell Biology Section, National Cancer Center Research Institute, Tokyo, Japan
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Requests for reprints: Masaru Katoh, Genetics and Cell Biology Section, National Cancer Center Research Institute, 5-1-1 Tsukiji, Tokyo 104-0045, Japan. Phone: 81-3-3542-2511; Fax: 81-3-3541-2685; E-mail: mkatoh-kkr@umin.ac.jp.

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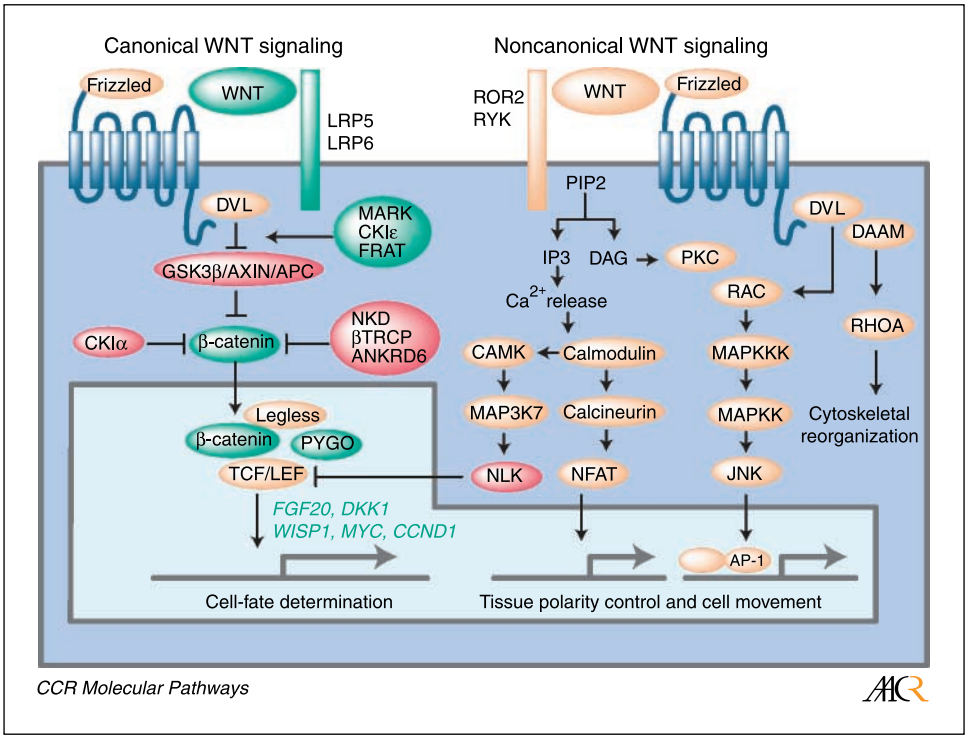

Fig. 1. Landscape of WNT signaling cascades. WNT signals are transduced to the canonical pathway for cell fate determination, and to the noncanonical pathway for control of cell movement and tissue polarity. Canonical WNT signals are transduced through Frizzled family receptors and LRP5/LRP6 coreceptor 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, CKIε, and FRAT are positive regulators of the canonical WNT pathway, whereas APC, AXIN1, AXIN2, CKIα, NKD1, NKD2, βTRCP1, βTRCP2, ANKRD6, NLK, and PPARγ 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, CKIα, GSK3β, 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 CKIα and GSK3β 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 GSK3β 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 GSK3β 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 (TGFβ/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 noncanonical 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 (PPARγ) 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. PPARγ counteracts the canonical WNT signaling during adipocyte differentiation and colorectal carcinogenesis. PPARγ agonist is one of the promising drugs to inhibit the canonical WNT signaling pathway; however, the mode of small molecules on PPARγ activity in adipose tissues is different from that in colorectal epithelium or colorectal cancer. Therefore, PPARγ 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 TGFβ/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 TGFβ/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 PPARγ 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.

References

1. Katoh M. *WNT* and *FGF* gene clusters. Int J Oncol 2002;21:1269–73.
2. Bhanot P, Brink M, Samos CH, et al. A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature 1996;382:225–30.
3. Pinson KI, Brennan J, Monkley S, et al. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 2000;407:535–8.
4. Price MA. CKI, there's more than one: casein kinase I family members in Wnt and Hedgehog signaling. Genes Dev 2006;20:399–410.
5. Wong HC, Bourdelas A, Krauss A, et al. Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. Mol Cell 2003;12:1251–60.
6. Tolwinski NS, Wehrli M, Rives A, et al. Wg/Wnt signal can be transmitted through arrow/LRP5, 6 and Axin independently of Zw3/Gsk3β activity. Dev Cell 2003;4:407–18.
7. Kramps T, Peter O, Brunner E, et al. Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear β-catenin-TCF complex. Cell 2002;109:47–60.
8. Katoh M, Katoh M. Identification and characterization

of human *BCL9L* gene and mouse *Bcl9l* gene *in silico*. Int J Mol Med 2003;12:643–9.
9. Chamorro MN, Schwartz DR, Vonica A, et al. *FGF20* and *DKK1* are transcriptional target of β-catenin and FGF20 is implicated in cancer and development. EMBO J 2005;24:73–84.
10. Pennica D, Swanson TA, Welsh JW, et al. *WISP* genes are members of the connective tissue growth factor family that are up-regulated in *Wnt1*-transformed cells and aberrantly expressed in human colon tumors. Proc Natl Acad Sci U S A 1998;95:14717–22.
11. He TC, Sparks AB, Rago C, et al. Identification of *c-MYC* as a target of the APC pathway. Science 1998;281:1509–12.
12. Tetsu O, McCormick F. β-Catenin regulates expression of *cyclin D1* in colon carcinoma cells. Nature 1999;398:422–6.
13. Oishi I, Suzuki H, Onishi N, et al. The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway. Genes Cells 2003;8:645–54.
14. Lu X, Borchers AG, Jolicoeur C, et al. PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. Nature 2004;430:93–8.
15. Lu W, Yamamoto V, Ortega B, et al. Mammalian Ryk is a Wnt coreceptor required for stimulation of neurite outgrowth. Cell 2004;119:97–108.
16. Boutros M, Paricio N, Strutt DI, et al. Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. Cell 1998;94:109–18.
17. Tao W, Pennica D, Xu L, et al. *Wrch-1*, a novel member of the *Rho* gene family that is regulated by Wnt-1. Genes Dev 2001;15:1796–807.
18. Ishitani T, Kishida S, Hyodo-Miura J, et al. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca^{2+} pathway to antagonize Wnt/β-catenin signaling. Mol Cell Biol 2003;23:131–9.
19. Dejmek J, Safholm A, Kamp Nielsen C, et al. Wnt-5a/Ca^{2+}-induced NFAT activity is counteracted by Wnt-5a/Yes-Cdc42-casein kinase Iα signaling in human mammary epithelial cells. Mol Cell Biol 2006;26:6024–36.
20. Katoh M. WNT/PCP signaling pathway and human cancer. Oncol Rep 2005;14:1583–8.
21. Kawano Y, Kypta R. Secreted antagonists of the Wnt signaling pathway. J Cell Sci 2003;116:2627–34.
22. Katoh M. Bioinformatics for cancer management in the post-genome era. Technol Cancer Res Treat 2006;5:169–76.
23. Lee FS, Lane TF, Kuo A, et al. Insertional mutagenesis identifies a member of the *Wnt* gene family as a candidate oncogene in the mammary epithelium of *int-2/Fgf-3* transgenic mice. Proc Natl Acad Sci U S A 1995;92:2268–72.
24. Katoh M, Katoh M. Cross-talk of WNT and FGF signaling pathways at GSK3β to regulate β-catenin and SNAIL signaling cascades. Cancer Biol Ther 2006;5:1059–64.
25. Suzuki H, Gabrielson E, Chen W, et al. A genomic screen for genes up-regulated by demethylation and histone deacetylase inhibition in human colorectal cancer. Nat Genet 2002;31:141–9.
26. Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991;253:665–9.
27. Satoh S, Daigo Y, Furukawa Y, et al. *AXIN1* mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat Genet 2000;24:245–50.
28. Katoh M, Katoh M. Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. Int J Mol Med 2006;17:681–5.
29. Robbins J, Blondel BJ, Gallahan D, et al. Mouse mammary tumor gene *int-3*: a member of the *Notch* gene family transforms mammary epithelial cells. J Virol 1992;66:2594–9.
30. Duncan AW, Rattis FM, DiMascio LN, et al. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immune 2005;6:314–22.
31. He X, Saint-Jeannet JP, Wang Y, et al. A member of the Frizzled protein family mediating axis induction by Wnt-5A. Science 1997;275:1652–4.
32. Swain RK, Katoh M, Medina A, et al. *Xenopus* frizzled-4S, a splicing variant of Xfz4, is a context-dependent activator and inhibitor of Wnt/β-catenin signaling. Cell Commun Signal 2005;3:12.
33. Bennett CN, Longo KA, Wright WS, et al. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci U S A 2005;102:3324–9.
34. Kanazawa A, Tsukada S, Kamiyama M, et al. Wnt5b partially inhibits canonical Wnt/β-catenin signaling pathway and promotes adipogenesis in 3T3–1 preadipocytes. Biochem Biophys Res Commun 2005;330:505–10.
35. Liu J, Wang H, Zou Y, et al. Functional interaction between peroxisome proliferators-activated receptor γ and β-catenin. Mol Cell Biol 2006;26:5827–37.
36. Shackleton S, Vaillant F, Simpson KL, et al. Generation of functional mammary gland from a single stem cell. Nature 2006;439:84–8.
37. McDonald SA, Preston SL, Lovell MJ, et al. Mechanisms of disease: from stem cells to colorectal cancer. Nat Clin Pract Gastroenterol Hepatol 2006;3:267–74.
38. van den Brink GR, Bleuming SA, Hardwick JC, et al. Indian Hedgehog is an antagonist of Wnt signaling in colonic epithelial cell differentiation. Nat Genet 2004;36:277–82.
39. Radtke F, Clevers H, Riccio O. From gut homoeostasis to cancer. Curr Mol Med 2006;6:275–89.
40. Barker N, Clevers H. Mining the Wnt pathway for cancer therapeutics. Nat Rev Drug Discov 2006;5:997–1014.

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