

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).

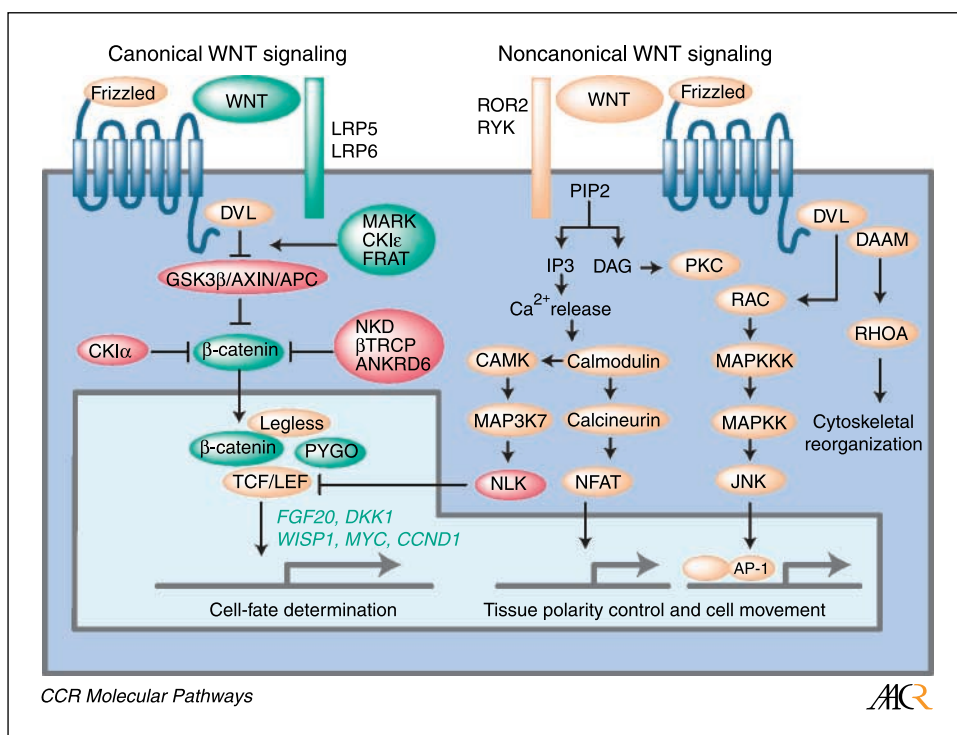
Noncanonical WNT signaling pathway. Noncanonical WNT signals are transduced through FZD family receptors and coreceptors, such as ROR2 and RYK (13–15). Small G proteins (RHOA, RHOU, RAC, and CDC42) and c-jun NH₂-terminal kinase are the DVL-dependent effector molecules of the noncanonical pathway (16, 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.

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/Gsk3 β 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, Jolicœur 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 DJ, 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/Ca²⁺* pathway to antagonize *Wnt/ β -catenin* signaling. *Mol Cell Biol* 2003;23:131–9.
 19. Dejmek J, Saffholm A, Kamp Nielsen C, et al. *Wnt-5a/Ca²⁺*-induced NFAT activity is counteracted by *Wnt-5a/Yes-Cdc42-casein kinase I α* 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 *GSK3 β* 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 homeostasis 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.