SPANDIDOS PUBLICATIONS

Hedgehog signaling pathway and gastrointestinal stem cell signaling network (Review)

YURIKO KATOH[1] and MASARU KATOH[2]

[1]M&M Medical BioInformatics, Hongo 113-0033; [2]Genetics and Cell Biology Section, National Cancer Center Research Institute, Tokyo 104-0045, Japan

Received September 19, 2006; Accepted October 10, 2006

Abstract. Hedgehog, BMP/TGFß, FGF, WNT and Notch signaling pathways constitute the stem cell signaling network, which plays a key role in a variety of processes, such as embryogenesis, maintenance of adult tissue homeostasis, tissue repair during chronic persistent inflammation, and carcinogenesis. Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH) bind to PTCH1/PTCH or PTCH2 receptor to release Smoothened (SMO) signal transducer from Patched-dependent suppression. SMO then activates STK36 serine/threonine kinase to stabilize GLI family members and to phosphorylate SUFU for nuclear accumulation of GLI. Hedgehog signaling activation leads to GLI-dependent transcriptional activation of target genes, such as *GLI1, PTCH1, CCND2*, *FOXL1, JAG2* and *SFRP1*. GLI1-dependent positive feedback loop combined with PTCH1-dependent negative feedback loop gives rise to transient proliferation of Hedgehog target cells. Iguana homologs (DZIP1 and DZIP1L) and Costal-2 homologs (KIF7 and KIF27) are identified by comparative integromics. SHH-dependent parietal cell proliferation is implicated in gastric mucosal repair during chronic *Helicobacter pylori* infection. BMP-RUNX3 signaling induces *IHH* expression in surface differentiated epithelial cells of stomach and intestine. Hedgehog signals from epithelial cells then induces FOXL1-mediated *BMP4* upregulation in mesenchymal cells. Hedgehog signaling is frequently activated in esophageal cancer, gastric cancer and pancreatic cancer due to transcriptional upregulation of Hedgehog ligands and epigenetic silencing of *HHIP1/HHIP* gene, encoding the Hedgehog inhibitor. However, Hedgehog signaling is rarely activated in colorectal cancer due to negative regulation by the canonical WNT signaling pathway. Hedgehog signaling molecules or targets, such as SHH, IHH, HHIP1, PTCH1 and GLI1, are applied as biomarkers for cancer diagnostics, prognostics and therapeutics. Small-molecule inhibitors for SMO or STK36 are suitable to be used for treatment of Hedgehog-dependent cancer.

Correspondence to: Dr Masaru Katoh, Genetics and Cell Biology Section, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
E-mail: mkatoh-kkr@umin.ac.jp

Key words: Hedgehog, BMP, RUNX, WNT, morphogenesis, carcinogenesis, bioinformatics, integrome network

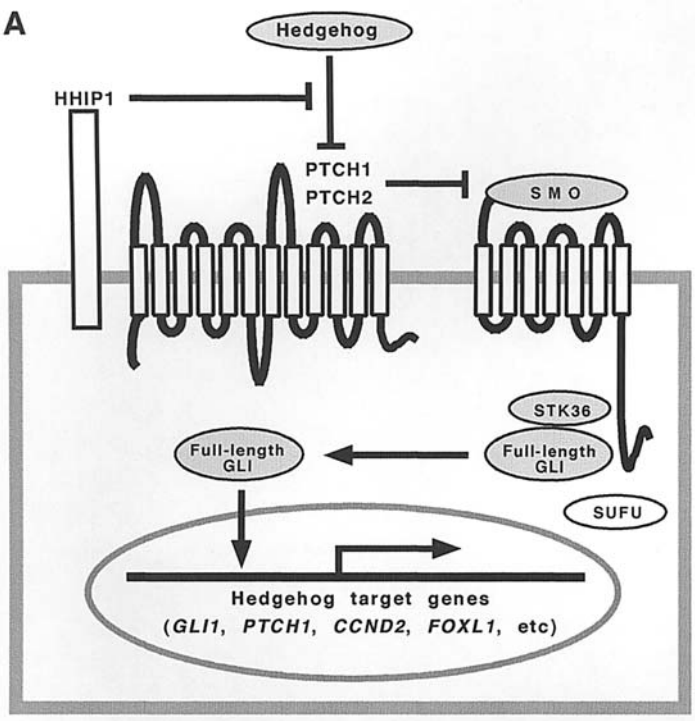
Contents

1. Overview of Hedgehog signaling pathway

Hedgehog signaling plays a key role in a variety of processes, such as embryogenesis, maintenance of adult tissue homeostasis, tissue repair during chronic persistent inflammation, and carcinogenesis (1-6). Hedgehog family ligands, Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH), undergo autoprocessing and lipid modification to generate mature peptides (7-9). Hedgehog acyltransferase (HHAT) is one of Hedgehog modifiers (10). Hedgehog family proteins with lipid modification are released from producing cells by Dispatched homologs (11,12).

In the absence of Hedgehog signaling, Patched family receptors (PTCH1/PTCH and PTCH2) inhibit the Smoothened (SMO) signal transducer (1-6,13-15). SMO inactivation leads to formation of the cytoplasmic GLI degradation complex, in which GLI family members (GLI1, GLI2 and GLI3) are phosphorylated by casein kinase Iα (CKIα), glycogen synthase kinase-3ß (GSK3ß) and protein kinase A (PKA) (16,17). Phosphorylated GLI is recognized by FBXW1/ßTRCP1 and FBXW11/ßTRCP2 (18-20) for ubiquitination, and ubiquitinated GLI is partially degraded to release its intact N-terminal half functioning as transcriptional repressor.

Hedgehog-binding to Patched family receptors releases the SMO signal transducer from Patched-dependent suppression. SMO then activates STK36 serine/threonine kinase to inhibit the assembly of GLI degradation complex for the stabilization of full-length GLI (21). Activated STK36 also phosphorylates SUFU to promote the nuclear accumulation of full-length GLI (22). Hedgehog signaling activation leads to GLI-dependent transcriptional activation of target genes, such as *GLI1, PTCH1, CCND2, FOXL1* and *JAG2* (14,23-25) (Fig. 1A).



B

Gene	Features	Genetic / Epigenetic / Transcriptional change in human tumors
SHH *IHH* *DHH*	Ligand	Transcriptional up-regulation
HHAT	Ligand modifier	
HHIP1	Inhibitor	Epigenetic silencing
PTCH1 *PTCH2*	Receptor	Loss-of-function mutation
SMO	Signal transducer	Gain-of-function mutation
STK36	Serine/Threonine kinase	
SUFU	GLI Regulator	Loss-of-function mutation
DZIP1 *DZIP1L*	GLI Regulator	
KIF7 *KIF27*	Kinesin	
FBXW1 *FBXW11*	Ubiquitin ligase component for GLI	
GLI1 *GLI2* *GLI3*	Transcription factor	Gene amplification Gene amplification Gene amplification

Figure 1. Hedgehog signaling pathway. (A), Overview of Hedgehog signaling. Hedgehog (SHH, IHH or DHH) binds to Patched family receptors (PTCH1 and PTCH2) to releases Smoothened (SMO) signal transducer from Patched-dependent suppression. SMO activates STK36 serine/threonine kinase to stabilize GLI family members (GLI1, GLI2 and GLI3) for nuclear translocation. Hedgehog signaling activates GLI-dependent transcription of target genes, such as *GLI1, PTCH1, CCND2,* and *FOXM1*. Positive regulators of Hedgehog signaling are shown in gray. (B), Human Hedgehog signaling components. Standard gene symbol, function, and epigenetic change or genetic alteration in human cancer for Hedgehog signaling-related genes are listed.

Hedgehog-dependent upregulation of the GLI1 transcription factor is a positive feedback mechanism of Hedgehog signaling. Hedgehog-dependent upregulation of PTCH1 is a negative feedback mechanism of Hedgehog signaling. In addition, Hedgehog-dependent upregulation of CCND2 and FOXM1 leads to proliferation of target cells through cell cycle progression. Therefore, GLI1-dependent positive feedback loop combined with PTCH1-dependent negative feedback loop gives rise to the transient proliferation of Hedgehog target cells.

Novel components of the Hedgehog signaling pathway have been identified based on comparative integromics. Zinc-finger and coiled-coil domain protein DZIP1 is the human ortholog of zebrafish Iguana regulating the intracellular distribution of GLI (26,27), and DZIP1L is the paralog of DZIP1 (Katoh Y, unpublished data). *Drosophila* Costal-2 (Cos2) interacts with *Drosophila* homologs of human SMO, GLI, STK36 and microtubule. Kinesin family members KIF27 and KIF7 are human homologs of *Drosophila* Cos2 (28,29).

2. Hedgehog signaling network in gastrointestinal tract

SHH and PTCH1 are expressed in the parietal cell-lineage of the stomach (30). Chronic persistent infection with *Helicobacter pylori* leads to chronic atrophic gastritis and gastric cancer (31,32). SHH-dependent parietal cell proliferation is implicated in the gastric mucosal repair during chronic *Helicobacter pylori* infection (6).

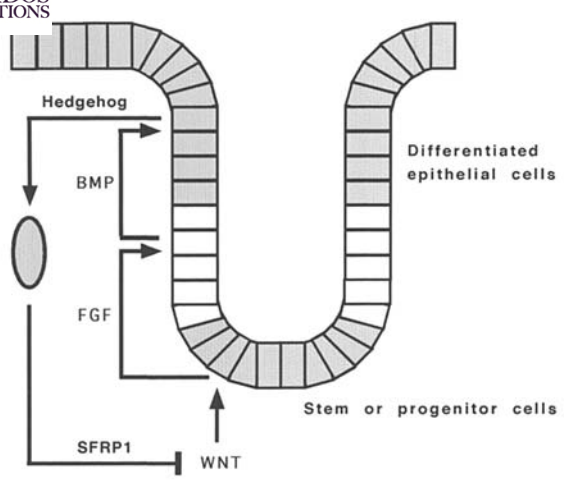
IHH and RUNX3 are expressed in the differentiated cells around the surface of the stomach and intestine (33,34). Hedgehog signals from epithelial cells then induces FOXL1-mediated *BMP4* upregulation in mesenchymal cells. BMP2 and BMP4, secreted from infiltrating inflammatory cells, activate BMP signaling in the surface epithelial cells in the gastric mucosa with *Helicobacter pylori* infection (35). RUNX family members interact with the BMP signaling SMAD protein complex to upregulate target genes (36). BMP-RUNX3 signaling induces *IHH* expression in the surface differentiated cells of the stomach and intestine (Fig. 2A).

WNT signaling plays a key role for the maintenance of intestinal epithelium (37-42). WNT signaling is activated in the progenitor (transit-amplifying) region around the bottom of intestinal crypt, while Hedgehog signaling in the differentiated region around the surface of intestinal villi (33). Hedgehog signaling inhibits the canonical WNT signaling and proliferation in intestinal epithelial cells (43). Hedgehog-dependent induction of WNT antagonist SFRP1 in mesenchymal cells partially explains the separation of the Hedgehog signaling activation domain from the WNT signaling activation domain (44).

Hedgehog signaling promotes epithelial proliferation in the esophagus, stomach and pancreas, but inhibits proliferation in the intestine due to its negative effects on the canonical WNT signaling pathway.

3. Oncogenic Hedgehog signaling activation

Carcinogenesis is a multi-stage disorder influenced by genetic predisposition, environmental factors, and aging (45). Transcriptional upregulation of genes encoding ligands for the onco-developmental pathways occurs during chronic inflammation



B

Type of cancer	Hedgehog ligand	Ligand inhibitor	Hedgehog signaling
Esophageal cancer	Expressed	Not examined	Activated
Gastric cancer	Expressed	Downregulated	Activated
Pancreatic cancer	Expressed	Downregulated	Activated
Colorectal cancer	Expressed	Downregulated	Inhibited

Figure 2. Hedgehog signaling network in the gastrointestinal tract. (A), Stem cell signaling network in the intestine. Canonical WNT signaling is activated in the progenitor (transit-amplifying) cells around the crypt base to activate FGF and Notch signaling pathways. BMP-RUNX3 signaling induces IHH expression in the differentiated cells around the villous surface. IHH induces SFRP1 expression in mesenchymal cells to inhibit the canonical WNT signaling pathway. Hedgehog-dependent SFRP1 induction keeps differentiated epithelial cells away from the effects of canonical WNT signaling. (B), Hedgehog signaling in gastrointestinal cancer. Hedgehog ligand upregulation and HHIP1 down-regulation occur relatively frequently in gastrointestinal cancer. Hedgehog signaling is activated in esophageal, gastric and pancreatic cancer, but rarely in colorectal cancer.

to repair the damaged tissues. Epigenetic silencing of genes encoding negative regulators of the onco-developmental pathways occurs during chronic persistent inflammation and/or aging to promote the repair process. Mutation, amplification or loss of genes encoding the onco-developmental signaling molecules then occurs to advance the multi-stage carcinogenesis.

HHIP1/*HHIP* gene encodes the Hedgehog-interacting protein functioning as Hedgehog inhibitor (46,47). *HHIP1* is up-regulated in basal cell carcinoma due to the negative feedback mechanism (48). On the other hand, *HHIP1* is down-regulated in a variety of tumors, such as gastric cancer, pancreatic cancer, colorectal cancer, and lung cancer (49). *HHIP1* down-regulation in pancreatic cancer is due to epigenetic CpG hypermethylation (50).

PTCH1 gene is mutated in the basal cell nevus syndrome, characterized by developmental abnormalities and basal cell carcinomas (13). *PTCH1* gene is also mutated in sporadic basal cell carcinoma. Loss-of-function mutation of *PTCH1* gene leads to carcinogenesis through constitutive Hedgehog signaling activation.

SMO gene is mutated in 10-20% of sporadic basal cell carcinomas (15). Gain-of-function mutation of *SMO* gene results in ligand-independent activation of the Hedgehog signaling pathway.

SUFU gene encodes a PEST domain protein implicated in the nuclear export of GLI family transcription factors (22). A subset of children with medulloblastoma carry germline and somatic mutations in *SUFU* gene with loss of heterozygosity of the wild-type *SUFU* allele.

GLI1, GLI2, and *GLI3* genes encode GLI family transcription factors functioning as Hedgehog effectors. *GLI1* gene is amplified in malignant glioma (16), *GLI2* gene in oral squamous cell carcinoma (51), and *GLI3* gene in alveolar rhabdomyosarcoma (52). Overexpression of GLI family members due to gene amplification leads to constitutive activation of the Hedgehog signaling pathway.

SMO and *GLI* family genes encoding a positive regulator of Hedgehog signaling pathway are proto-oncogenes, while *HHIP1, PTCH1* and *SUFU* genes encoding a negative regulator of Hedgehog signaling pathway are tumor suppressor genes (Fig. 1B).

4. Hedgehog signaling in gastrointestinal cancer

SHH and *IHH* were expressed in all of the six esophageal cancer cell lines, and *PTCH1* and *GLI1* in four of six esophageal cancer cell lines (53). *SHH* was expressed in primary esophageal cancer, and *SHH* gene was amplified in some of the cases. Elevated expression of Hedgehog target genes occured in 14 out of 22 primary esophageal cancer (54).

SHH, IHH, PTCH1 and *GLI1* were expressed in all of the six human gastric cancer cell lines (53). *SHH, PTCH1* and *GLI1* were expressed in 63 of 99 primary gastric cancer (55), while *HHIP1* was down-regulated in nine of ten primary gastric cancer (49).

SHH, IHH, PTCH1 and *GLI1* were expressed in five of six human pancreatic cancer cell lines (53). *IHH* is significantly upregulated in primary pancreatic cancer (56). CpG hyper-methylation of *HHIP1* promoter was detected in 13 out of 17 pancreatic cancer cell lines, and in 35 out of 75 primary pancreatic cancer (50).

SHH and *IHH* were expressed in all of the 11 human colorectal cancer cell lines (53), and *HHIP1* was down-regulated in 16 of 20 primary colorectal cancer (49). Despite *SHH/IHH* expression and *HHIP1* down-regulation, *PTCH1* is not expressed in colorectal cancer (53).

Hedgehog signaling is frequently activated in esophageal, gastric and pancreatic cancer due to transcriptional upregulation of Hedgehog ligands and epigenetic silencing of the *HHIP1* gene. However, Hedgehog signaling is rarely activated in colorectal cancer due to negative regulation by the canonical WNT signaling pathway (Fig. 2B).

5. Perspectives

Hedgehog and WNT counteract in the intestinal epithelium. Hedgehog inhibits WNT signaling in intestinal stem or progenitor cells partly due to SFRP1 induction in mesenchymal cells (Fig. 2A); however the mechanism of WNT-dependent Hedgehog signaling inhibition remains unclear. Expression profile, epigenetic change, and genetic alterations of Hedgehog signaling-related genes and phosphorylation status of Hedgehog signaling molecules should be investigated to explain why Hedgehog signaling is frequently inactivated in colorectal cancer.

High-throughput technologies and bioinformatics supervised by human intelligence are the driving forces for the phrmacogenomics and pharmacogenetics in the post-genome era (45). Because Hedgehog, BMP/TGFß, FGF, WNT and Notch signaling pathways constitute the stem cell signaling network (Fig. 2A), stem cell signaling molecules are applied as biomarkers or biomarker sets for clinical oncology. Hedgehog signaling molecules or targets, such as SHH, IHH, HHIP1, PTCH1 and GLI1, are applied as biomarkers for cancer diagnostics, prognostics and therapeutics.

Small-molecule inhibitors for seven-transmembrane proteins and protein kinases are promising drugs in the post-genome era (60). KADD-cyclopamine, SANT1-4 and Cur61414 have been developed as small-molecule SMO inhibitors (57-59). Because STK36 serine/threonine kinase functions as a positive regulator of the Hedgehog signaling pathway, small-molecule STK36 inhibitors could also be developed in the future.

References

1. Pasca di Magliano M and Hebrok M: Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer 3: 903-911, 2003.
2. Bijlsma MF, Spek CA and Peppelenbosch MP: Hedgehog: an unusual signal transducer. Bioessays 26: 387-394, 2004.
3. Lum L and Beachy PA: The Hedgehog response network: sensors, switches, and routers. Science 304: 1755-1759, 2004.
4. Hooper JF and Scott MP: Communicating with Hedgehogs. Nat Rev Mol Cell Biol 6: 306-317, 2005.
5. Briscoe J and Therond P: Hedgehog signaling: from the *Drosophila* cuticle to anti-cancer drugs. Dev Cell 8: 143-151, 2005.
6. Katoh Y and Katoh M: Hedgehog signaling in gastric cancer. Cancer Biol Ther 4: 1050-1054, 2005.
7. Marigo V, Roberts DJ, Lee SM, *et al*: Cloning, expression, and chromosomal location of *SHH* and *IHH*: two human homologues of the *Drosophila* segment polarity gene *hedgehog*. Genomics 28: 44-51, 1995.
8. Katoh Y and Katoh M: Identification and characterization of rat *Desert hedgehog* and *Indian hedgehog* genes *in silico*. Int J Oncol 26: 545-549, 2005.
9. Katoh Y and Katoh M: Comparative genomics on Sonic hedgehog orthologs. Oncol Rep 14: 1087-1090, 2005.
10. Chamoun Z, Mann RK, Nellen D, *et al*: Skinny hedgehog, an acyltransferase required for palmitoylation and activity of the hedgehog signal. Science 293: 2080-2084, 2001.
11. Burke R, Nellen D, Bellotto M, *et al*: Dispatched, a novel sterol-sensing domain protein dedicated to the release of cholesterol-modified hedgehog from signaling cells. Cell 99: 803-815, 1999.
12. Katoh Y and Katoh M: Identification and characterization of *DISP3* gene *in silico*. Int J Oncol 26: 551-556, 2005.
13. Johnson RL, Rothman AL, Xie J, *et al*: Human homolog of *patched*, a candidate gene for the basal cell nevus syndrome. Science 272: 1668-1671, 1996.
14. Carpenter D, Stone DM, Brush J, *et al*: Characterization of two patched receptors for the vertebrate hedgehog protein family. Proc Natl Acad Sci USA 95: 13630-13634, 1998.
15. Xie J, Murone M, Luoh SM, *et al*: Activating Smoothened mutations in sporadic basal-cell carcinoma. Nature 391: 90-92, 1998.
16. Kinzler KW, Bigner SH, Bigner DD, *et al*: Identification of an amplified, highly expressed gene in a human glioma. Science 236: 70-73, 1987.
17. Price MA: CKI, there's more than one: casein kinase I family members in Wnt and Hedgehog signaling. Genes Dev 20: 399-410, 2006.
18. Jiang J and Struhl G: Regulation of the Hedgehog and Wingless signaling pathways by the F-box/WD40-repeat protein Slimb. Nature 391: 493-496, 1998.
19. Koike J, Sagara N, Kirikoshi H, *et al*: Molecular cloning and characterization of the *ßTRCP2* gene on chromosome 5q35.1. Biochem Biophys Res Commun 269: 103-109, 2000.
20. Saitoh T and Katoh M: Expression profiles of *ßTRCP1* and *ßTRCP2*, and mutation analysis of *ßTRCP2* in gastric cancer. Int J Oncol 18: 959-964, 2001.
21. Osterlund T, Everman DB, Betz RC, *et al*: The *FU* gene and its possible protein isoforms. BMC Genomics 5: 49, 2004.
22. Taylor MD, Liu L, Raffel C, *et al*: Mutations in *SUFU* predispose to medulloblastoma. Nat Genet 31: 306-310, 2002.
23. Yoon JW, Kita Y, Frank DJ, *et al*: Gene expression profiling leads to identification of GLI1-binding elements in target genes and a role for multiple downstream pathways in GLI1-induced cell transformation. J Biol Chem 277: 5548-5555, 2002.
24. Hallikas O, Palin K, Sinjushina N, *et al*: Genome-wide prediction of mammalian enhancers based on analysis of transcription-factor binding affinity. Cell 124: 47-59, 2006.
25. Kasper M, Schnidar H, Neill GW, *et al*: Selective modulation of Hedgehog/GLI target gene expression in human keratinocytes. Mol Cell Biol 26: 6283-6299, 2006.
26. Moore FL, Jaruzelska J, Dorfman DM and Reijo-Pera RA: Identification of a novel gene, DZIP (DAZ-interacting protein), that encodes a protein that interacts with DAZ (deleted in azoospermia) and is expressed in embryonic stem cells and germ cells. Genomics 83: 834-843, 2004.
27. Sekimizu K, Nishioka N, Sasaki H, *et al*: The zebrafish *iguana* locus encodes Dzip1, a novel zinc-finger protein required for proper regulation of Hedgehog signaling. Development 131: 2521-2532, 2004.
28. Katoh Y and Katoh M: KIF27 is one of orthologs for *Drosophila* Costal-2. Int J Oncol 25: 1875-1880, 2004.
29. Katoh Y and Katoh M: Characterization of *KIF7* gene *in silico*. Int J Oncol 25: 1881-1886, 2004.
30. Van den Brink GR, Hardwick JC, Tytgat GN, *et al*: Sonic hedgehog regulates gastric gland morphogenesis in man and mouse. Gastroenterology 121: 317-328, 2001.
31. Katoh M and Terada M: Oncogenes and tumor suppressor genes. In: Gastric Cancer. Nishi M (ed). Springer-Verlag, Tokyo, pp196-208, 1993.
32. Katoh M and Katoh M: Pharmacogenomics on gastric cancer. Cancer Biol Ther 3: 566-567, 2004.
33. 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 36: 277-282, 2004.
34. Fukamachi H: Runx3 controls growth and differentiation of gastric epithelial cells in mammals. Develop Growth Differ 48: 1-13, 2006.
35. Bleuming SA, Kodach LL, Garcia Leon MJ, *et al*: Altered bone morphogenetic protein signalling in the *Helicobacter pylori*-infected stomach. J Pathol 209: 190-197, 2006.
36. Ito Y: Oncogenic potential of the *RUNX* gene family: overeiew. Oncogene 23: 4198-4208, 2004.
37. Katoh M: *WNT* and *FGF* gene clusters. Int J Oncol 21: 1269-1273, 2002.
38. Gregorieff A, Pinto D, Begthel H, *et al*: Expression pattern of WNT signaling components in the adult intestine. Gastroenterology 129: 626-638, 2005.
39. Katoh Y and Katoh M: FGF signaling inhibitor, SPRY4, is evolutionarily conserved target of WNT signaling pathway in progenitor cells. Int J Mol Med 17: 529-532, 2006.
40. Katoh M and Katoh M: Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. Int J Mol Med 17: 681-685, 2006.
41. Radtke F, Clevers H and Riccio O: From gut homoestasis to cancer. Curr Mol Med 6: 275-289, 2006.

M and Katoh M: Cross-talk of WNT and FGF signaling cascades. Cancer Biol Ther 5: 1059-1064, 2006.
43. Van den Brink GR and Hardwick JC: Hedgehog Wnteraction in colorectal cancer. Gut 55: 912-914, 2006
44. Katoh Y and Katoh M: WNT antagonist, SFRP1, is Hedgehog signaling target. Int J Mol Med 17: 171-175, 2006.
45. Katoh M: Bioinformatics for cancer management in the post-genome era. Technol Cancer Res Treat 5: 169-176, 2006.
46. Chuang PT and McMahon AP: Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. Nature 397: 617-621, 1999.
47. Katoh Y and Katoh M: Comparative genomics on HHIP family orthologs. Int J Mol Med 17: 391-395, 2006.
48. Bonifas JM, Pennypacker S, Chuang PT, *et al*: Activation of expression of hedgehog target genes in basal cell carcinomas. J Invest Dermatol 116: 739-742, 2001.
49. Olsen CL, Hsu PP, Glienke J, *et al*: Hedgehog-interacting protein is highly expressed in endothelial cells, but down-regulated during angiogenesis and in several human tumors. BMC Cancer 4: 43, 2004.
50. Martin ST, Sato N, Dhara S, *et al*: Aberrant methylation of the human *Hedgehog interacting protein (HHIP)* gene in pancreatic neoplasms. Cancer Biol Ther 4: 728-733, 2005.
51. Snijders AM, Schmidt BL, Fridlyand J, *et al*: Rare amplicons implicate frequent deregulation of cell fate specification pathways in oral squamous cell carcinoma. Oncogene 24: 4232-4342, 2005.
52. Gordon AT, Brinkschmidt C, Anderson J, *et al*: A novel and consistent amplicon at 13q31 associated with alveolar rhabdomyosarcoma. Genes Chromosomes Cancer 28: 220-226, 2000.
53. Berman DM, Karhadkar SS, Maitra A, *et al*: Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumors. Nature 425: 846-851, 2003.
54. Ma X, Sheng T, Zhang Y, *et al*: Hedgehog signaling is activated in subsets of esophageal cancers. Int J Cancer 118: 139-148, 2006.
55. Ma X, Chen K, Huang S, *et al*: Frequent activation of the hedgehog pathway in advanced gastric adenocarcinomas. Carcinogenesis 26: 1698-1705, 2005.
56. Kayed H, Kleeff J, Keleg S, *et al*: Indian hedgehog signaling pathway: expression and regulation in pancreatic cancer. Int J Cancer 110: 668-676, 2004.
57. Taipale J, Chen JK, Cooper MK, *et al*: Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. Nature 406: 1005-1009, 2000.
58. Chen JK, Taipale J, Young KE, *et al*: Small molecule modulation of Smoothened activity. Proc Natl Acad Sci USA 99: 14071-14076, 2002.
59. Williams JA, Guicherit OM, Zaharian BI, *et al*: Identification of a small molecule inhibitor of the hedgehog signaling pathway: effects on basal cell carcinoma-like lesions. Proc Natl Acad Sci USA 100: 4616-4621, 2003.
60. Noble ME, Endicott JA and Johnson LN: Protein kinase inhibitors: insights into drug design from structure. Science 303: 1800-1805, 2004.