

# Hedgehog signaling, epithelial-to-mesenchymal transition and miRNA (Review)

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**Abstract.** SHH, IHH, and DHH are lipid-modified secreted proteins binding to Patched receptors, and CDON, BOC or GAS1 co-receptors. In the absence of Hedgehog signaling, GLI1 is transcriptionally repressed, GLI2 is phosphorylated by GSK3 and CK1 for the FBXW11 ( $\beta$ TRCP2)-mediated degradation, and GLI3 is processed to a cleaved repressor. In the presence of Hedgehog signaling, Smoothed is relieved from Patched-mediated suppression due to the Hedgehog-dependent internalization of Patched, which leads to MAP3K10 (MST) activation and SUFU inactivation for the stabilization and nuclear accumulation of GLI family members. GLI activators then upregulate *CCND1*, *CCND2* for cell cycle acceleration, *FOXA2*, *FOXC2*, *FOXE1*, *FOXF1*, *FOXL1*, *FOXP3*, *POU3F1*, *RUNX2*, *SOX13*, *TBX2* for cell fate determination, *JAG2*, *INHBC*, and *INHBE* for stem cell signaling regulation. Hedgehog signals also upregulate *SFRP1* in mesenchymal cells for WNT signaling regulation. Epithelial-to-mesenchymal transition (EMT) during embryogenesis, adult tissue homeostasis and carcinogenesis is characterized by class switch from E-cadherin to N-cadherin. SNAI1 (Snail), SNAI2 (Slug), SNAI3, ZEB1, ZEB2 (SIP1), KLF8, TWIST1, and TWIST2 are EMT regulators repressing *CDH1* gene encoding E-cadherin. Hedgehog signals induce *JAG2* upregulation for Notch-CSL-mediated *SNAI1* upregulation, and also induce TGF $\beta$ 1 secretion for *ZEB1* and *ZEB2* upregulation via TGF $\beta$  receptor and NF- $\kappa$ B. TGF $\beta$ -mediated downregulation of miR-141, miR-200a, miR-200b, miR-200c, miR-205, and miR-429 results in upregulation of ZEB1 and ZEB2 proteins. Hedgehog signaling activation indirectly leads to EMT through FGF, Notch, TGF $\beta$  signaling cascades, and miRNA regulatory networks. miRNAs targeted to stem cell signaling components or EMT regulators are potent drug targets; however, off-target effects should be

strictly controlled before clinical application of synthetic miRNA. Peptide mimetic and RNA aptamer could also be utilized as Hedgehog signaling inhibitors or EMT suppressors.

## Contents

1. Introduction
2. Hedgehog signaling pathway
3. Epithelial-to-mesenchymal transition (EMT)
4. Hedgehog and EMT
5. MicroRNA (miRNA)
6. Perspectives

## 1. Introduction

Hedgehog family members are key regulators of embryogenesis, adult tissue homeostasis, and carcinogenesis (1-5). Genetic alterations and aberrant expression of Hedgehog signaling molecules in medulloblastoma, glioma, basal cell carcinoma, lung cancer, esophageal cancer, gastric cancer, pancreatic cancer, prostate cancer, and ovarian cancer have been reported, and reviewed elsewhere.

Hedgehog signaling cascade cross talks with WNT, Notch, EGF/FGF, and TGF $\beta$ /Activin/Nodal/BMP signaling cascades to constitute the stem cell signaling network (6-9). Deregulation of the stem cell signaling network due to the accumulation of germline mutation, SNP, chronic inflammation, epigenetic change, and genetic alteration leads to carcinogenesis (10).

Epithelial cells undergo fibroblastoid morphological changes associated with increased motility or invasiveness due to decreased cell-cell adhesion (11-14). Fibroblastoid morphological changes of epithelial cells are known as epithelial-to-mesenchymal transition (EMT).

Regulatory mechanisms of EMT are hot issues in life science, especially in the fields of developmental biology and oncology. Herein recent advances in the Hedgehog research will be reviewed with the emphasis on EMT and microRNA (miRNA).

## 2. Hedgehog signaling pathway

Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH) are mammalian Hedgehog family ligands,

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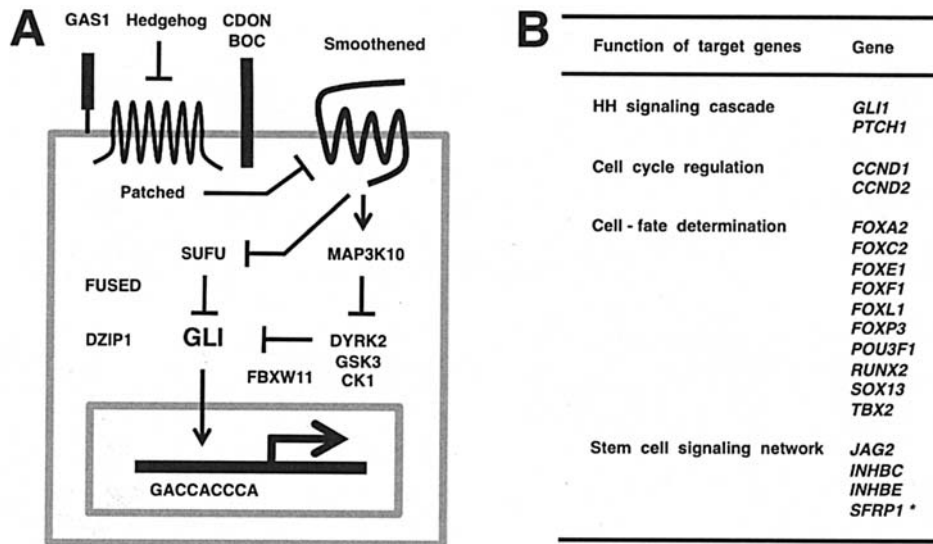


Figure 1. Hedgehog signaling pathway. (A) Schematic representation of Hedgehog signaling cascade. Patched family members are Hedgehog receptors, while CDON, BOC and GAS1 are Hedgehog co-receptors. In the absence of Hedgehog signaling, GLI1 is transcriptionally repressed, GLI2 is phosphorylated by GSK3 and CK1 for the FBXW11-mediated degradation, and GLI3 is processed to a cleaved repressor. In the presence of Hedgehog signaling, Smoothened is relieved from Patched-mediated suppression to induce MAP3K10 activation and SUFU inactivation. GLI activators then bind to the GACCACCCA motif for the transcriptional upregulation of target genes. (B) List of representative Hedgehog target genes.

consisting of N-terminal signal peptide, Hedgehog core domain, and C-terminal processing domain (15-17). Hedgehog precursors are autoprocessed to cut off the C-terminal processing domain for cholesteroylation, and then further processed by Hedgehog acyltransferase (HHAT) to cut off the N-terminal signal peptide for palmitoylation (18,19). Mature Hedgehog proteins with lipid modifications are then transported to the cell surface for packaging into lipoprotein particles depending on Dispatched 1 (DISP1), or for multimerization via lipophilic tails (19,20). Mature Hedgehog proteins secreted from producing cells induce concentration-dependent effects on target cells expressing Hedgehog receptors.

Patched family members, PTCH1 and PTCH2, are Hedgehog receptors distantly related to Dispatch family members with multi-transmembrane domains and a sterol-sensing domain (21,22). PTCH1 and PTCH2 do not directly transduce Hedgehog signals to the intracellular signaling cascade, but indirectly through the Smoothened seven-transmembrane-type receptor (23). Patched family members, inhibiting Smoothened function, are rapidly internalized upon Hedgehog-binding (24). Due to the release of Smoothened from Patched-dependent suppression, Hedgehog-binding to Patched family receptors indirectly activates the Smoothened-GLI signaling cascade (Fig. 1A).

CDON and BOC are transmembrane proteins with extracellular immunoglobulin-like (Ig-like) and fibronectin type III (FNIII) domains, which enhance Hedgehog signaling activity as co-receptors (25). GAS1 is a GPI-anchored cell surface protein binding to Hedgehog ligands for the potentiation of Hedgehog signaling (26). On the other hand, HHIP1/HHIP is a Hedgehog-binding protein to compete Patched receptors for Hedgehog-binding (27,28).

*GLI1* gene was initially cloned as an oncogene amplified in malignant glioma, and then characterized as a transcription factor functioning as a Hedgehog signaling effector (29,30).

GLI1, GLI2, and GLI3 are human homologs of *Drosophila* Cubitus interruptus. In the absence of Hedgehog signaling, GLI1 is transcriptionally repressed, GLI2 is phosphorylated by GSK3 and CK1 for the FBXW11 ( $\beta$ TRCP2)-mediated degradation, and GLI3 is processed to a cleaved repressor (30,31). In the presence of Hedgehog signaling, Smoothened induces MAP3K10 (MST) activation and SUFU inactivation for the stabilization and nuclear accumulation of GLI family members, respectively (32,33). GLI1 functions as transcriptional activator of Hedgehog target genes, while GLI2 and GLI3 as transcriptional activator or repressor in a context-dependent manner (30).

Hedgehog signaling activation leads to transcriptional activation of target genes through GLI-binding to the GACCACCCA motif (34-36). *GLI1*, *PTCH1* and *HHIP1* are upregulated by Hedgehog signaling, but *CDON*, *BOC* and *GAS1* are downregulated. Hedgehog-dependent *GLI1* upregulation constitutes a positive feedback loop, while Hedgehog-dependent regulation of *PTCH1*, *HHIP1*, *CDON*, *BOC*, and *GAS1* constitutes a negative feedback network. Hedgehog signals induce transient upregulation of target genes through the combination of positive and negative feedback mechanisms.

Hedgehog signals upregulate *CCND1* and *CCND2* for cell cycle acceleration, *FOXA2*, *FOXC2*, *FOXE1*, *FOXF1*, *FOXL1*, *FOXP3*, *POU3F1*, *RUNX2*, *SOX13*, and *TBX2* for cell fate determination. Hedgehog signals also upregulate *JAG2*, and *INHBC/E* to regulate Notch, and Activin signaling cascades, respectively. In addition, Hedgehog signals upregulate *SFRP1* at least in mesenchymal cells without its promoter CpG hypermethylation to inhibit canonical WNT signaling cascade in epithelial cells (Fig. 1B).

### 3. Epithelial-to-mesenchymal transition (EMT)

Epithelial cells are tightly held together with uniform neighboring cells to move as a sheet en block, while mesen-

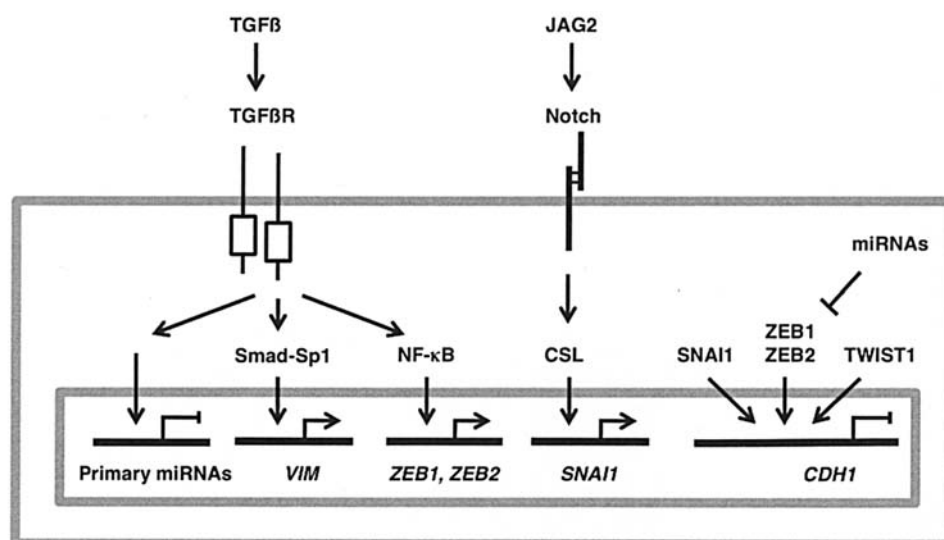


Figure 2. Hedgehog and EMT. Hedgehog signals induce JAG2 upregulation for Notch-CSL-mediated *SNAI1* upregulation, and also induce TGFβ1 secretion for *ZEB1* and *ZEB2* upregulation via TGFβ receptor and NF-κB. TGFβ-mediated downregulation of miR-141, miR-200a, miR-200b, miR-200c, miR-205, and miR-429 results in upregulation of ZEB1 and ZEB2 proteins. Hedgehog signaling activation indirectly leads to EMT through Notch, TGFβ signaling cascades, and miRNA regulatory networks.

chymal cells are loosely connected with diverse neighboring cells to move individually. EMT, allowing cells to dissociate from epithelial tissue, is necessary for gastrulation movements and neural crest formation during embryogenesis, and also for invasion and metastasis during carcinogenesis (11-14).

E-cadherin, occludin and cytokeratin are downregulated during EMT, while N-cadherin, vimentin, fibronectin, SNAI1/SAIL, SNAI2/SLUG, ZEB2/SIP1, and TWIST1 are upregulated (14). E-cadherin and N-cadherin are representative adhesion molecules expressed on epithelial cells and mesenchymal cells, respectively. E-cadherin at the adherens junction is implicated in the stable cell-cell contact of epithelial cells, while N-cadherin in the weak intercellular contact of mesenchymal cells (37,38). Class switch from E-cadherin to N-cadherin results in the loss of epithelial phenotype and the acquisition of mesenchymal phenotype. Transcriptional repression of *CDH1* gene or functional repression of E-cadherin protein is the critical step for EMT.

Zinc-finger domain proteins SNAI1, SNAI2, SNAI3, ZEB1, ZEB2, KLF8 as well as basic helix-loop-helix (bHLH) domain proteins TWIST1 and TWIST2, bind to the proximal promoter region of the *CDH1* gene for the EMT induction through E-cadherin repression (11-14,39-42). We previously reported *SNAI1* expression in neuroblastoma, diffuse type gastric cancer, and *SNAI2* expression in embryonic stem cells, leiomyosarcoma, neuroblastoma, and glioblastoma (41). Rosivatz *et al* reported preferential upregulation of *ZEB2* in intestinal type gastric cancer, and those of *SNAI1* and *TWIST1* in diffuse type gastric cancer (39). Alves *et al* reported co-expression of *ZEB2* and *SNAI2* in intestinal type gastric cancer, and that of *SNAI1* and *SNAI2* in diffuse type gastric cancer (42). Upregulation of EMT regulators is associated with more malignant phenotypes in a variety of human cancer, such as gastric cancer, pancreatic cancer, breast cancer, and ovarian cancer.

#### 4. Hedgehog and EMT

Hedgehog signaling cascade cross-talks with WNT, EGF/FGF, and TGFβ/Activin/Nodal/BMP signaling cascades, which are implicated in EMT through E-cadherin repression (6-14). In this section, direct and indirect mechanisms of EMT regulation by the Hedgehog signaling cascade will be reviewed (Fig. 2).

Upregulation of *SNAI1* and *PTCH1* mRNAs is induced 3 h after GLI1 expression in RK3E cells by using the 'tet-on' system, and that of Snai1 protein 12 h after GLI1 expression (43). Although these facts indicate that the Hedgehog signaling cascade induces the SNAI1 upregulation, there is no evidence for the direct transcriptional activation of *SNAI1* by the Hedgehog signaling cascade.

On the other hand, Hedgehog signals induce *JAG2* upregulation (Fig. 1B), and TGFβ1 secretion to promote motility and invasiveness of cancer cells (44). *JAG2* signal induces processing of Notch receptor to Notch intracellular domain (NICD). NICD is then associated with CSL transcription factor in the nucleus to induce *SNAI1* upregulation (45). TGFβ1 signal activates TGFβ receptor for the NF-κB-mediated transcriptional upregulation of *ZEB1* and *ZEB2* (46), and also for the SMAD-Sp1-mediated transcriptional upregulation of mesenchymal markers, such as Vimentin (VIM). Together these facts indicate that the Hedgehog signals indirectly induce EMT through the upregulation of multiple EMT regulators via Notch and TGFβ signaling cascades (Fig. 2).

#### 5. MicroRNA (miRNA)

Primary miRNAs are processed by Drosha/DGCR8 complex to give rise to precursor miRNAs, which are then processed by Dicer to produce mature miRNAs. Most target mRNAs with partial complementarity to miRNA are repressed

through translational downregulation and deadenylation, while several target mRNAs are activated (47-50). Mechanisms of miRNA-induced translational or transcriptional regulation as well as clinical application of miRNA are the frontier of medical science in the post-genome era.

Zebrafish miR-214 binds to the 3'-UTR of *Sufu* to downregulate *Sufu* (51). Because *Sufu* is implicated in the nuclear trafficking of Gli activator and repressor, miR-214-induced downregulation of *Sufu* results in maximal activation of Gli in the presence of Hedgehog and complete repression of in the absence of Hedgehog. It is noteworthy that human miR-214, up-regulated in ovarian cancer, binds to the 3'-UTR of PTEN to downregulate PTEN for the activation of PI3K-AKT signaling cascade (52), which is also able to induce EMT. Effects of human ortholog of zebrafish miR-214 on the Hedgehog-Gli signaling cascade as well as EMT remain to be elucidated.

TGF $\beta$  downregulates the expression of human miR-141, miR-200a, miR-200b, miR-200c, miR-205, and miR-429, which are targeted to *ZEB1* and *ZEB2* mRNAs (53). TGF $\beta$ -induced downregulation of miRNAs mentioned above synergizes with NF- $\kappa$ B signaling to induce upregulation of *ZEB1* and *ZEB2* (Fig. 2).

SNAI, ZEB and TWIST family members repress the *CDHI* gene to induce EMT, but also regulate the transcription of other target genes. TWIST1 is upregulated in human breast cancer, gastric cancer, esophageal cancer, and prostate cancer. TWIST1 directly activates the transcription of miR-10b primary miRNA, located within the *HOXD* gene cluster (54). *HOXD10* mRNA is the target of miR-10b in human breast cancer, and *HOXD10* is implicated in the suppression of RhoC-mediated cell motility. TWIST1 promotes invasion and metastasis through miR-10b-induced *HOXD10* repression.

## 6. Perspectives

KAAD-cyclopamine, SANT1-4, and Cur61414 are small-molecule Hedgehog signaling inhibitors targeting Smoothed (55). Other therapeutic devices targeted to the Hedgehog signaling cascade and EMT regulators will be described in the last section.

miRNAs targeted to mRNAs, encoding stem cell signaling components or EMT regulators, are potent drug targets. miRNAs inducing proliferative, anti-apoptotic, pro-angiogenic, or pro-metastatic effects on tumor cells could be down-regulated for cancer therapy, while those inducing pro-apoptotic, anti-angiogenic, or anti-metastatic effects could be applied for synthetic miRNA (48,49). Because the off-target effects are a serious problem associated with miRNA and siRNA technologies, great care should be taken before clinical application of these technologies.

RNA aptamer is a short RNA oligonucleotide with a stable three-dimensional structure (49). RNA aptamers binding to extracellular region of PTCH1 could be utilized for drug delivery to cancer cells with Hedgehog signaling activation. RNA aptamers binding to cytoplasmic region of SMO, and those binding to Fused or GLI1 could be utilized as Hedgehog signaling inhibitors.

Peptide mimetics, resembling WNT and FGF family members, have been developed (56,57). Because WNT5A

transduces signals through ROR1 or ROR2 to activate the non-canonical signaling cascade for the induction of EMT partly through SNAI1 upregulation (58-61), WNT5A mimetic is able to suppress invasion and metastasis of cancer cells. Peptide mimetic, resembling core region of mature Hedgehog signaling domain, could be developed as a novel Hedgehog antagonist.

## References

1. Beachy PA, Karhadkar SS and Berman DM: Tissue repair and stem cell renewal in carcinogenesis. *Nature* 432: 324-331, 2004.
2. Hooper JF and Scott MP: Communicating with Hedgehogs. *Nature Rev Mol Cell Biol* 6: 306-317, 2005.
3. 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.
4. Katoh Y and Katoh M: Hedgehog signaling in gastric cancer. *Cancer Biol Ther* 4: 1050-1054, 2005.
5. Katoh Y and Katoh M: Hedgehog signaling pathway and gastrointestinal stem cell signaling network. *Int J Mol Med* 18: 1019-1023, 2006.
6. 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.
7. Garciadiego-Cazares D, Rosales C, Katoh M and Chimal-Monroy J: Coordination of chondrocyte differentiation and joint formation by  $\alpha 5\beta 1$  integrin in the developing appendicular skeleton. *Development* 131: 4735-4742, 2004.
8. Bailey J, Singh PK and Hollingsworth MA: Cancer metastasis facilitated by developmental pathways: Sonic hedgehog, Notch, and bone morphogenetic proteins. *J Cell Biochem* 102: 829-839, 2007.
9. Katoh M: Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. *Stem Cell Rev* 3: 30-38, 2007.
10. Katoh M: Dysregulation of stem cell signaling network due to germline mutation, SNP, *Helicobacter pylori* infection, epigenetic change, and genetic alteration in gastric cancer. *Cancer Biol Ther* 6: 832-839, 2007.
11. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. *Nature Rev Cancer* 2: 442-454, 2002.
12. Barrallo-Gimeno A and Nieto MA: The *Snail* genes as inducers of cell movement and survival: implications in development and cancer. *Development* 132: 3151-3161, 2005.
13. Katoh M: Epithelial-mesenchymal transition in gastric cancer. *Int J Oncol* 27: 1677-1683, 2005.
14. Lee JM, Dedhar S, Kalluri R and Thompson EW: The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 172: 973-981, 2006.
15. Marigo V, Roberts DJ, Lee SM, *et al*: Cloning, expression, and chromosomal location of *SHH* and *IHH*. *Genomics* 28: 44-51, 1995.
16. 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.
17. Katoh Y and Katoh M: Comparative genomics on Sonic hedgehog orthologs. *Oncol Rep* 14: 1087-1090, 2005.
18. 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.
19. Breitling R: Greased hedgehogs: new links between hedgehog signaling and cholesterol metabolism. *Bioessays* 29: 1085-1094, 2007.
20. 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.
21. 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.
22. Katoh Y and Katoh M: Identification and characterization of *DISP3* gene *in silico*. *Int J Oncol* 26: 551-556, 2005.
23. Van den Heuvel M and Ingham PW: Smoothed encodes a receptor-like serpentine protein required for hedgehog signalling. *Nature* 382: 547-551, 1996.

24. Gallet A and Therond PP: Temporal modulation of the Hedgehog morphogen gradient by a Patched-dependent targeting to lysosomal compartment. *Dev Biol* 277: 51-62, 2005.
25. Tenzen T, Allen BL, Cole F, *et al*: The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. *Dev Cell* 10: 647-656, 2006.
26. Allen BL, Tenzen T and McMahon AP: The Hedgehog-binding proteins Gas1 and Cdo cooperate to positively regulate Shh signaling during mouse development. *Genes Dev* 21: 1244-1257, 2007.
27. Chuang PT and McMahon AP: Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. *Nature* 397: 617-621, 1999.
28. Katoh Y and Katoh M: Comparative genomics on HHIP family orthologs. *Int J Mol Med* 17: 391-395, 2006.
29. Kinzler KW, Bigner SH, Bigner DD, *et al*: Identification of an amplified, highly expressed gene in a human glioma. *Science* 236: 70-73, 1987.
30. Ruiz i Altaba A, Mas C and Stecca B: The Gli code: an information nexus regulating cell fate, stemness and cancer. *Trends Cell Biol* 17: 438-447, 2007.
31. Bhatia N, Thiagarajan S, Elcheva I, *et al*: Gli2 is targeted for ubiquitination and degradation by  $\beta$ -TrCP ubiquitin ligase. *J Biol Chem* 281: 19320-19326, 2006.
32. Katoh M, Hirai M, Sugimura T and Terada M: Cloning and characterization of MST1, a novel serine/threonine kinase with SH3 domain. *Oncogene* 10: 1447-1451, 1995.
33. Varjosalo M, Björklund M, Cheng F, *et al*: Application of active and kinase-deficient kinome collection for identification of kinases regulating hedgehog signaling. *Cell* 133: 537-548, 2008.
34. 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.
35. Kasper M, Schnidar H, Neill GW, *et al*: Selective modulation of Hedgehog/GLI target gene expression by EGF signaling in human keratinocytes. *Mol Cell Biol* 26: 6283-6299, 2006.
36. Katoh Y and Katoh M: WNT antagonist, SFRP1, is Hedgehog signaling target. *Int J Mol Med* 17: 171-175, 2006.
37. Takeichi M: The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development* 102: 639-655, 1988.
38. Hazan RB, Phillips GR, Qiao RF, *et al*: Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion and metastasis. *J Cell Biol* 148: 779-790, 2000.
39. Rosivatz E, Becker I, Specht K, *et al*: Differential expression of the EMT regulators Snail, SIP1, and Twist in gastric cancer. *Am J Pathol* 161: 1881-1891, 2002.
40. Katoh M and Katoh M: Identification and characterization of human *SNAI3* (*SNAI3*) gene *in silico*. *Int J Mol Med* 11: 383-388, 2003.
41. Katoh M and Katoh M: Comparative genomics on *SNAI1*, *SNAI2*, and *SNAI3* orthologs. *Oncol Rep* 14: 1083-1086, 2005.
42. Alves CC, Rosivatz E, Schott C, *et al*: Slug is overexpressed in gastric carcinomas and may act synergistically with SIP1 and Snail in the down-regulation of E-cadherin. *J Pathol* 211: 507-515, 2007.
43. Li X, Deng W, Nail CD, *et al*: Snail induction is an early response to Gli1 that determines the efficiency of epithelial transformation. *Oncogene* 25: 609-621, 2006.
44. Yoo YA, Kang MH, Kim JS and Oh SC: SHH signaling promotes motility and invasiveness of gastric cancer cells through TGF $\beta$ -mediated activation of the ALK5-Smad3 pathway. *Carcinogenesis* 29: 480-490, 2008.
45. Sahlgren C, Gustafsson MV, Jin S, Poellinger L and Lendahl U: Notch signaling mediates hypoxia-induced tumor cell migration and invasion. *Proc Natl Acad Sci USA* 105: 6392-6397, 2008.
46. Chua HL, Bhat-Nakshatri P, Clare SE, *et al*: NF- $\kappa$ B represses E-cadherin expression and enhances EMT of mammary epithelial cells: potential involvement of ZEB1 and ZEB2. *Oncogene* 26: 711-724, 2007.
47. Vasudevan S, Tong Y and Steitz JA: Switching from repression to activation: microRNAs can up-regulate translation. *Science* 318: 1931-1934, 2007.
48. Marquez RT and McCaffrey AP: Advances in microRNAs: implication for gene therapists. *Hum Gene Ther* 19: 27-38, 2008.
49. Katoh M: RNA technology targeted to the WNT signaling pathway. *Cancer Biol Ther* 7: 275-277, 2008.
50. Grosshans H and Filipowicz W: The expanding world of small RNAs. *Nature* 451: 414-416, 2008.
51. Flynt AS, Li N, Thatcher EJ, Solnica-Krezel L and Patton JG: Zebrafish miR-214 modulates Hedgehog signaling to specify muscle cell fate. *Nat Genet* 39: 259-263, 2007.
52. Yang H, Kong W, He L, *et al*: MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res* 68: 425-433, 2008.
53. Gregory PA, Bert AG, Paterson EL, *et al*: The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting *ZEB1* and *SIP1*. *Nat Cell Biol* 10: 593-601, 2008.
54. Ma L, Teruya-Feldstein J and Weinberg RA: Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449: 682-689, 2007.
55. 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.
56. Safholm A, Leandersson K, Dejmeck J, *et al*: A formylated hexapeptide ligand mimics the ability of Wnt5a to impair migration of human breast epithelial cells. *J Biol Chem* 281: 2740-2749, 2006.
57. Li S, Christensen C, Kiselyov VV, *et al*: FGF-derived peptides: functional agonists of FGF receptor. *J Neurochem* 104: 667-682, 2008.
58. Katoh M and Katoh M: Comparative genomics on *ROR1* and *ROR2* orthologs. *Oncol Rep* 14: 1381-1384, 2005.
59. Fukuda T, Chen L, Endo T, *et al*: Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. *Proc Natl Acad Sci USA* 105: 3047-3052, 2008.
60. Katoh M and Katoh M: STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer. *Int J Mol Med* 19: 273-278, 2007.
61. Dissanayake SK, Wade M, Johnson CE, *et al*: Wnt5a/PKC pathway mediates motility in melanoma cells via inhibition of metastasis suppressors and initiation of EMT. *J Biol Chem* 282: 17259-17271, 2007.