PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 March 21; 13(11): 1659-1665
World Journal of Gastroenterology ISSN 1007-9327

Sonic hedgehog-Gli1 pathway in colorectal adenocarcinomas

Yue-Hong Bian, Shu-Hong Huang, Ling Yang, Xiao-Li Ma, Jing-Wu Xie, Hong-Wei Zhang

Yue-Hong Bian, Shu-Hong Huang, Ling Yang, Xiao-Li Ma, Hong-Wei Zhang, Institute of Developmental Biology, College of Life Science, Shandong University, Jinan 250100, Shandong Province, China
Jing-Wu Xie, Sealy Centers for Cancer Cell Biology, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555-1048, United States
Supported by the grants from National Natural Science Foundation of China, No. 30228031, No. 30671072
Correspondence to: Dr. Hong-Wei Zhang, Institute of Developmental Biology, College of Life Science, Shandong University, Jinan 250010, Shandong Province, China. zhw@sdu.edu.cn
Telephone: +86-531-88364935 Fax: +86-531-88565610
Received: 2006-10-09 Accepted: 2007-01-30

Abstract

AIM: To determine the role of Sonic hedgehog (Shh) pathway in colorectal adenocarcinomas through analysis of the expression of Shh pathway-related molecules, Shh, Ptch1, hedgehog-interacting protein (Hip), Gli1, Gli3 and PDGFRα.

METHODS: Expression of Shh in 25 colorectal adenocarcinomas was detected by RT-PCR, *in situ* hybridization and immunohistochemistry. Expression of Ptch1 was observed by *in situ* hybridization and immunohistochemistry. Expression of Hip, Gli1, Gli3 and PDGFRα was analyzed by *in situ* hybridization.

RESULTS: Expression of cytokeratin AE1/AE3 was observed in the cytoplasm of colorectal crypts. Members of the Hh signaling pathway were expressed in colorectal epithelium. Shh was expressed in cytoplasm of dysplastic epithelial cells, while expression of Ptch1, Hip and Gli1 were mainly detected in the malignant crypts of adenocarcinomas. In contrast, PDGFRα was expressed highly in aberrant crypts and moderately in the stroma. Expression of Gli3 could not be detected in colorectal adenocarcinomas.

CONCLUSION: These data suggest that Shh-Ptch1-Gli1 signaling pathway may play a role in the progression of colorectal tumor.

Key words: Sonic hedgehog pathway; Sonic hedgehog; Ptch1; Hedgehog-interacting protein; Gli1; Gli3; PDGFRα; Colorectal adenocarcinomas

Bian YH, Huang SH, Yang L, Ma XL, Xie JW, Zhang HW. Sonic hedgehog-Gli1 pathway in colorectal adenocarcinomas. *World J Gastroenterol* 2007; 13(11): 1659-1665

http://www.wjgnet.com/1007-9327/13/1659.asp

INTRODUCTION

Hedgehog (Hh) signaling plays key roles in embryonic organ patterning, cell differentiation and cell proliferation during embryonic development. Hh signaling is essential for the development of the limb, lung, brain and foregut. Single hedgehog gene in *Drosophila* and three hedgehog genes, Sonic (Shh), Indian (Ihh) and Desert (Dhh) in vertebrates have been identified. All three Hh proteins in vertebrates appear to be processed by the same mechanism, use the same receptors and can elicit similar biological responses, but their relative potencies differ in an assay-dependent manner[1-3]. Hhs bind to receptor Patched (Ptch), a 12-transmembrane protein. Ptch acts to inhibit the 7-transmembrane protein Smoothened (Smo), rendering the pathway inactive in the absence of Hh ligand. After binding of Hh to Ptch, the repression of Smo is released and the target genes (e.g. Ptch1, Gli, PDGFRα, Wnts, CyclinD1 and Bmp) are activated[4,5]. The Hh signaling is negatively regulated by a hedgehog-interacting protein (Hip), which is also inducible by hedgehog and is lost in hedgehog mutant[6].

In the mouse, both Shh and Ihh are expressed in the gut endoderm and have been shown to participate in radial axis patterning of the gut[7]. Recent studies have shown that Shh is expressed in the normal adult human gut[8]. Aberrant activation of Hh signaling pathway has been shown to cause the occurrence of basal-cell carcinoma (BCC) and medulloblastoma, and mutations of Hh pathway components have been found both in familial and sporadic cases[9-12]. More recently, small-cell lung cancer (SCLC), prostate cancer and pancreatic adenocarcinoma have been linked to Hh signaling pathway, providing a molecular mechanism for these aggressive diseases[13-15]. Dysregulation of Hh signaling pathway may also play a role in gastrointestinal (GI) tumors[16,17]. To better understand the mechanism of colorectal adenocarcinoma, a common cancer worldwide, we examined here the expression of several key molecules functioned in the Hh signaling pathway at protein and mRNA levels.

MATERIALS AND METHODS

Meterials

Twenty five tissue specimens of colorectal adenocarcinomas were obtained from Shandong Qilu Hospital, Jinan, China with approval from the local ethics research committee. Five of the 25 samples also included non-cancerous colorectal tissue as normal control. All of the samples were fixed in 4% paraformaldehyde with PBS at 4℃ overnight. The samples were dehydrated in gradated ethanol and stored in 70% ethanol at -20℃. The samples were embedded in paraffin and sectioned (6 μm). The sections were stained with H&E. Each sample was reviewed and confirmed by pathologists based on the clinical characteristics.

Immunohistochemistry

Immunohistochemistry was performed according to the protocol described by Ma *et al*[16,17]. Antigen unmasking was achieved by boiling sections in 0.01 mol/L sodium citrate; pH 6.0, for 10 min. Tissues were blocked in 10% sheep serum twice and incubated for 2 h at 37℃ with primary antibodies. Antibodies were applied at the following dilutions: Shh, and Ptch1 (1:100; Cat# 9024 for Shh and Cat# 6149 for Ptch1, Santa Cruz Biotechnology Inc, Santa Cruz, CA), PCNA (1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), cytokeratin AE1/AE3. (MAB 0049, Maixin Biotechnology, Inc., China). Negative controls were performed in all cases by omitting the first antibodies. All primary antibodies have been previously tested for immun ohistostaining[18].

In situ hybridization

The sense and antisense digoxigenin-labeled RNA probes were made using a DIG RNA labeling kit and following the manufacturer's instructions (Boehringer Mannherm). The *in situ* hybridization was performed as described previously[16,17]. In brief, rehydrated sections were treated with proteinase K (20 μg/mL) for 20 min and refixed in 4% paraformaldehyde with PBS for 5 min. Sections were washed with 50% formamide in 2 × SSC at 37℃ for 2 h and hybridization was performed at 42℃ overnight with 5 μg/mL of digoxigenin-labeled probe in the following hybridization buffer: 40% formamide, 10% dextran sulfate, 1 × Denhardt's solution, 1 mg/mL yeast RNA, 10 mmol/L Dithiothreit, and 4 × SSC. Washes were done in 4 × SSC, 2 × SSC, 20 μg/mL RNase A, 1 × SSC, 0.1 × SSC at 37℃, 3 × 15 min, and then in buffer 1 (100 mmol/L Tris-Cl, 150 mmol/L NaCl, pH 7.5). Sections were incubated for 30 min in blocking buffer -2% goat serum, 0.1% TritonX-100 in buffer 1 and left overnight at room temperature in alkaline phosphatase-conjugated anti-digoxigenin antibody (Roche) at 1:500 dilution in blocking buffer. Washes were done in buffer 2 (100 mmol/L Tris-Cl, 100 mmol/L NaCl, 50 mmol/L $MgCl_2$, pH 7.5) and 3 (100 mmol/L Tris-Cl, 100 mmol/L NaCl, 50 mmol/L $MgCl_2$, pH 9.5), 2 × 10 min. Color reaction was performed in the NBT/BCIP (Roche) solution containing 10 mmol/L levamisole in the dark. Blue staining indicated strong hybridization. Sense probes were used in all hybridization as negative controls and no positive signals were observed.

RT-PCR

RT-PCR was performed using RT-PCR system according to the manufacturer's protocol. Total RNA was isolated from paraffin-embedded tissue using an RNA isolation kit (Promega). Three micrograms of total RNA were reverse transcribed by using M-MLV reverse transcriptase (Promega) with a mixture of oligo $(dT)_{15}$ and random primers (Promega). One tenth of each RT reaction mixture was then subjected to PCR amplification using Taq DNA polymerase (TAKARA). The PCR primers for detecting specific transcripts were as follows: for Shh forward, 5'-ACCGAGGGCTGGGACGAAGA-3' and reverse, 5'-ATTTGGCCGCCACCGAGTT-3', respectively. Following denaturation at 94℃ for 10 min, 35 PCR cycles were performed at 94℃ for 60 s, at 52℃ for 50 s, and at 72℃ for 60 s.

For β-actin, forward: 5'-TCCTCCCTGGAGAAGAGC TA-3' reverse: 5'-TCAGGAGGAGCAATGATCTTG-3'. Following denaturation at 94℃ for 10 min, 35 PCR cycles were performed at 94℃ for 60 s, at 59℃ for 50 s, and at 72℃ for 60 s. The PCR products were analyzed by 0.7% agarose gel electrophoresis.

RESULTS

Cytokeratin AE1/AE3 expression in colorectal adenocarcinomas

To determine whether colorectal adenocarcinomas originate from epidermis, we analyzed the expression of an epidermic molecular marker, cytokeratin AE1/AE3. Expression of cytokeratin AE1/AE3 was observed in the cytoplasm of colorectal crypts (Figure 1A and B). In addition, we examined the expression of the proliferation marker, PCNA. The results showed nuclear staining in the base of crypts in both normal (Figure 1C) and colorectal adenocarcinoma tissue (Figure 1D). However, there were a great number of stained nuclei in malignant crypts in contrast to that in normal tissue, suggesting that malignant crypts had a high level proliferation compared with normal crypts. Taken together, these data indicate that primary colorectal adenocarcinomas originate in epidermis.

Shh expression and localization in primary colorectal adenocarcinomas

It has been shown that Shh is expressed in stomach and esophageal cancers[16,17]. To determine if Shh is expressed in colorectal adenocarcinomas, we examined the expression of Shh at mRNA and protein levels. The result of *in situ* hybridization revealed that Shh mRNA was well stained in the cytoplasm of epithelial cells throughout the section in 10 of 25 samples (Figure 2B). The sense probes gave no detectable signals (Figure 2C). This result was confirmed by immunohistochemistry and RT-PCR (Figure 2G, only in 9 of 25 samples). The pattern of staining of immunohistochemistry (13 out of 25 showed a positive reaction) was similar to that of *in situ* hybridization (Figure 2E). No staining was observed when secondary detection was performed in the absence of primary antibodies (Figure 3F). The cells except cancer cells showed a negative reaction, suggesting that the

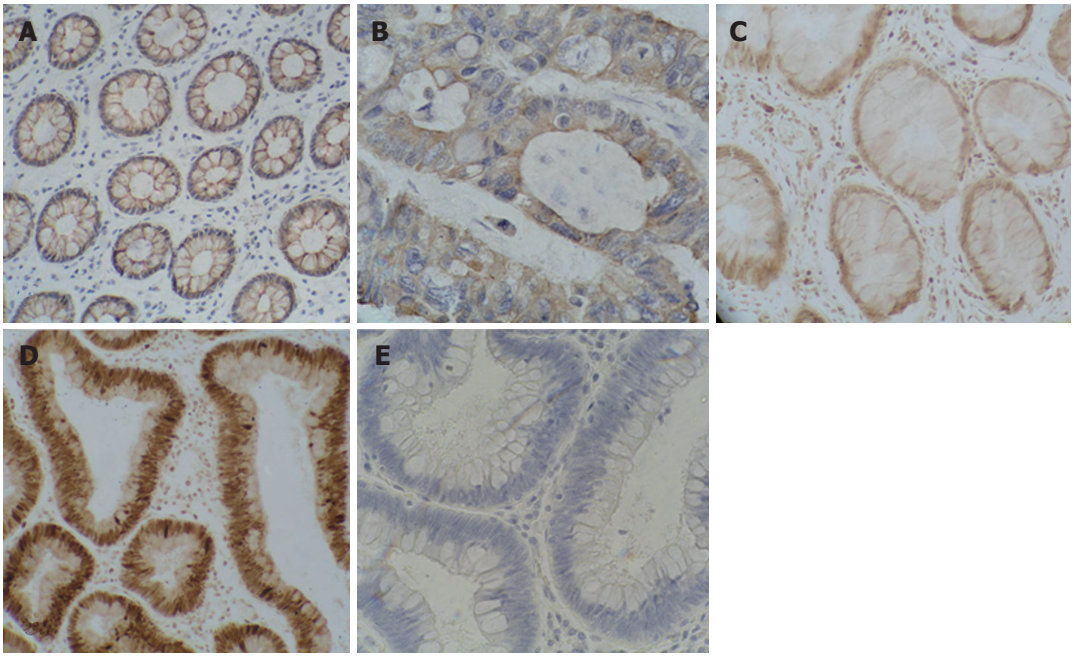

Figure 1 Expression of cytokeratin AE1/AE3 and PCNA in primary colorectal adenocarcinomas. We performed immunohistochemistry with cytokeratin AE1/AE3 antibodies showing positive staining in cytoplasm of colorectal normal crypts (**A**) and malignant crypts (**B**) (positive in brown). While the proliferation marker PCNA stained the nuclei of the normal (**C**) and tumor (**D**) tissues (positive in brown). **E** is the negative control.

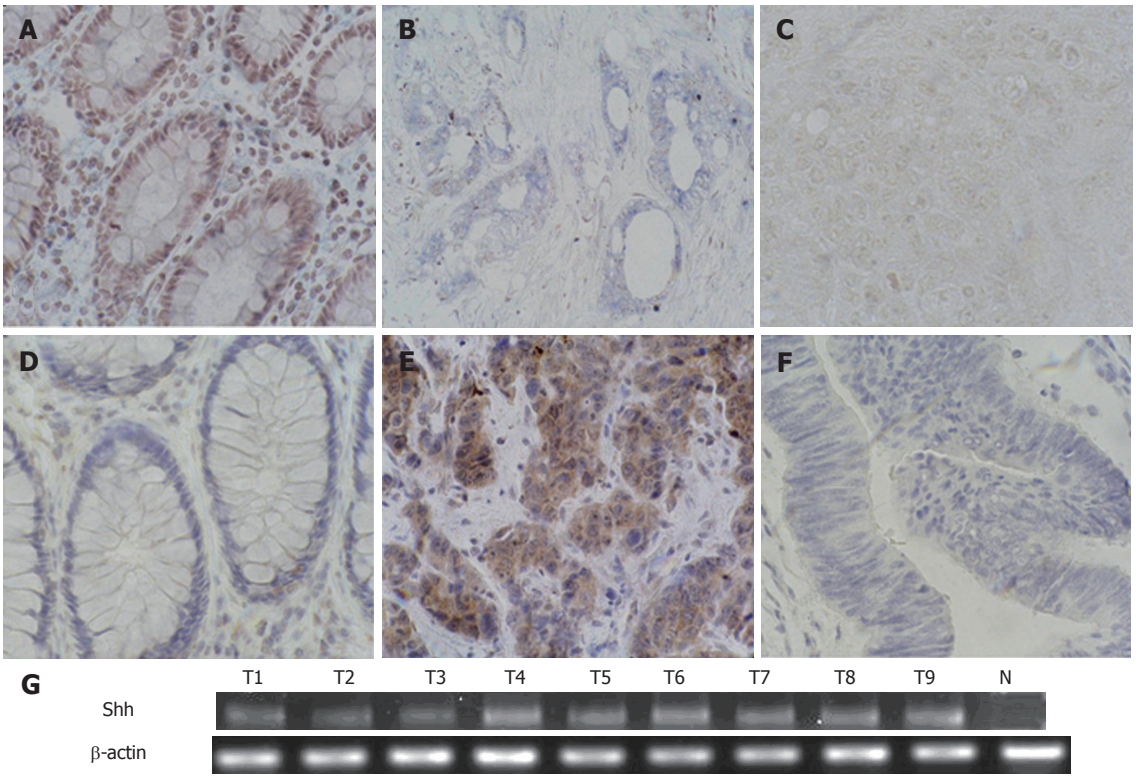

Figure 2 Expression of Shh in primary colorectal adenocarcinomas. *In situ* hybridization was performed to detect Shh transcript in normal (**A**) and cancerous (**B**) tissues (positive in blue), and the sense probe did not reveal any positive signals (**C** is the sense control of **B**). Results of *in situ* hybridization were confirmed by immunohistostaining in normal (**D**) and tumor (**E**) tissues (positive in brown, **F** is the negative control) and by RT-PCR (**G**).

expression of mRNA and protein was tumor specific. One normal tissue (the morphologically normal crypts close to the adenocarcinoma tissue) did not show the expression of Shh (Figure 2A and D). Our data demonstrated that, like other GI cancers, colorectal cancers expressed Shh in epithelial cells.

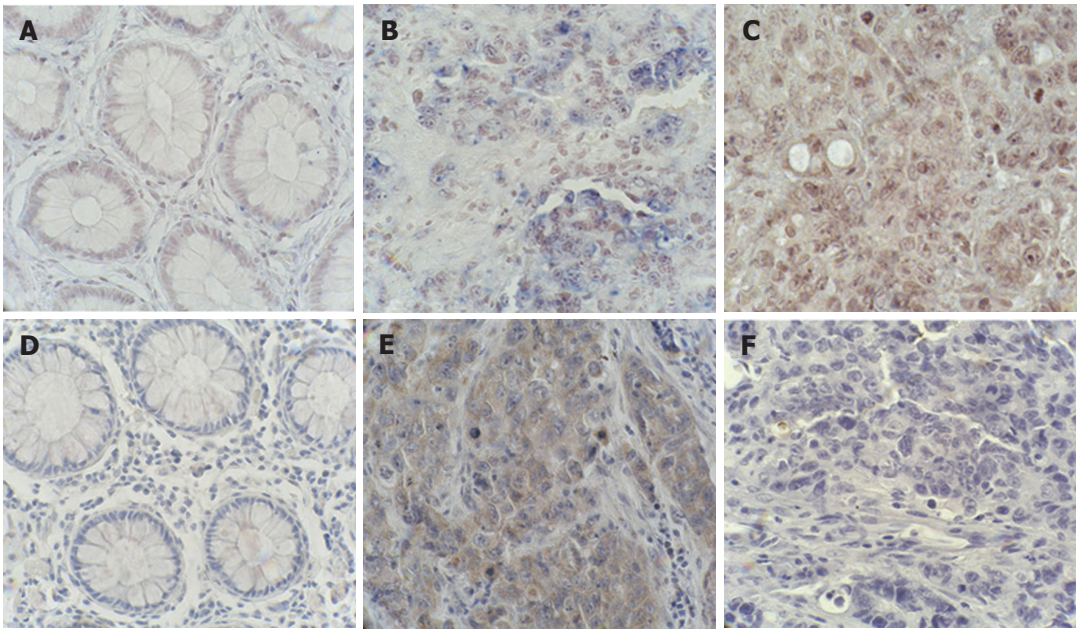

Figure 3 Expression of Ptch1 in primary colorectal adenocarcinomas. Ptch1 transcript (blue as positive) was detected by *in situ* hybridization in the normal control (**A**) and colorectal cancers (**B**). **C** is from the same tumor with **B** being derived from the Ptch1 sense probe. To confirm the *in situ* hybridization results, we performed immunohistochemistry with PTCH1 antibodies, showing positive staining of PTCH1 protein (positive in brown) in malignant crypts (**E**). **D** is the normal control and **F** is the negative control.

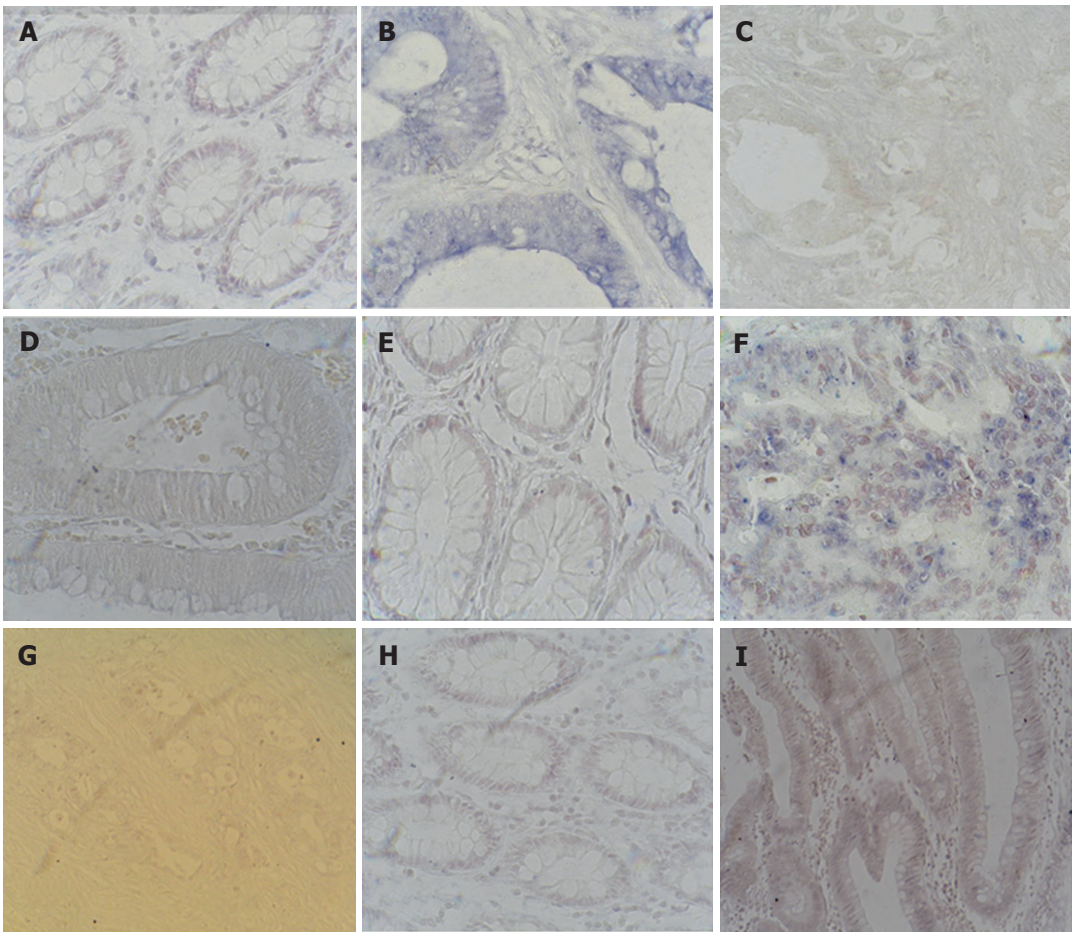

Figure 4 Expression of Gli1, Gli3 and Hip in primary colorectal adenocarcinomas. We detected expression patterns of Gli1, Gli3 and Hip transcripts by *in situ* hybridization (positive in blue). Gli1 (**A**) and Hip (**E**) were not expressed in normal control tissue. **B**: Gli1 was expressed in cytop-lasm of malignant crypt. The expression of Gli1 (**C**) and Hip (**D**) sense probe in tumor tissue. **F**: Hip was expressed in cytoplasm of tumor cells. **G**: Gli3 was not expressed in colorectal adenocarcinomas. **H** is the normal control of Gli3 and **I** is the sense control of Gli3.

Expression of the Hh signaling pathway components Ptch1, Gli1 and Hip, but not Gli3 in primary colorectal adenocarcinomas

The Hh signaling pathway can include Ptch receptor and Gli transcriptor, and it is also regulated by Hip. To determine if these genes are activated in colorectal cancer cells, the mRNAs of Ptch1, Gli1 and Hip were examined in colorectal adenocarcinoma tissue. The positive staining of Ptch1 (Figure 3A-C), Gli1 (Figure 4A-C) and Hip (Figure 4D-F) appeared in the majority of the malignant crypts in adenocarcinomas, but not in the stroma. The results (Table 1) indicate that there was no consistency in the expression of Ptch1, Hip and Gli1. Interestingly, we did not examine the expression of Gli3 by *in situ* hybridization

Table1 Colorectal adenocarcinoma specimens and summary of Shh, Ptch1, Smo Gli1, Sufu, PDGFRα and Hip expression

No	WHO Classification	Differentiation	Age	Gender	Shh -ish	SHH -ihc	Ptch1 -ish	PTCH1 -ihc	Gli1 -ish	PDGFRα -ish	Hip -ish
1	RAC	PD	50	M	+	+	-	+	+	+	-
2	RAC	WD	69	F	-	-	-	-	+-	-	-
3	RAC	PD	60	M	+	+	+	+	+	-	-
4	RAC	MD	69	F	+-	+	-	-	-	-	-
5	CAC	MD	50	F	-	-	-	-	-	-	-
6	RAC	WD	72	M	-	-	+-	+	+-	-	-
7	CAC	MD	60	F	-	-	-	-	-	-	-
8	RAC	MD	27	F	-	-	+-	+	-	-	-
9	RAC	MD	63	M	-	-	-	-	-	-	-
10	CAC	WD	55	M	-	-	-	-	+-	-	-
11	RAC	MD	66	F	-	+	-	-	+	-	-
12	CAC	PD	79	F	+	+	-	-	+	-	-
13	RAC	PD	54	M	-	-	-	-	-	-	-
14	RAC	WD	73	F	-	-	-	-	-	-	-
15	RAC	MD	68	F	+	+	+	+	-	-	-
16	RAC	MD	70	F	-	-	-	-	+-	+	-
17	RAC	MD	68	M	-	-	-	-	+	+	-
18	RAC	MD	67	F	-	-	-	-	-	-	-
19	CAC	PD	68	M	+	+	+	+	+	+	+
20	CAC	PD	48	F	-	+	+-	+	+-	-	-
21	RAC	PD	73	M	+	+	-	+	-	-	-
22	RAC	MD	51	M	-	-	-	-	+	-	-
23	RAC	MD	70	M	+	+	+	+	+	-	+
24	RAC	PD	37	F	+	+	+	+	-	-	+
25	RAC	PD	79	M	+	+	+	+	-	-	+

These data were derived from *in situ* hybridization (ish) and immunohistochemistry (ihc). + means positive result (ratio of positive cells to cancer cells ⩾ 25%), - means negative result (no positive cell or ratio of positive cells to cancer cells ⩽ 10%), +- means weakly positive result (10% < ratio of positive cells to cancer cells < 25%); WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated; CAC: Colonic adenocarcinoma; RAC: Rectal adenocarcinoma.

in any tissue (Figure 4G-I). To confirm the results of *in situ* hybridization, we detected Ptch1 protein by immunohistochemistry. The results were in agreement with the *in situ* hybridization data (Figure 3D-G). These findings indicate that the Shh-Ptch-Gli1 pathway was likely involved in colorectal adenocarcinomas. However, it should be noted that upregulation of Shh expression may not be the sole cause of enhanced Ptch1 or Gli1 expression, because, in some cases, upregulation of Ptch1 or Gli1 expression was not in accordance with the expression of Shh (Table 1). This suggests that the hedgehog signaling pathway might be activated by other regulatory mechanisms in colorectal carcinoma.

Expression of PDGFRα in primary colorectal adenocarcinomas

The platelet-derived growth factors (PDGFs) are a pleotrophic family of peptide growth factors that signal through cell surface tyrosine kinase receptors (PDGFR) and stimulate various cellular functions including growth, proliferation, and differentiation. In the past few years, the role of PDGF signaling has been demonstrated in cancer cell proliferation and tumor angiogenesis[19-22]. Only recently Xie *et al*[19] found that in the hedgehog-responsive cell line C3H10T1/2, Gli1 activates PDGFRα expression and enhances its activity. In the murine BCC cell line ASZ001 (Ptch1 null), DNA synthesis and cell proliferation can be slowed by re-expression of PTCH1 possibly through down-regulating PDGFRα expression, or by downstream inhibition with PDGFRα-neutralizing antibodies[19]. To test whether Shh signaling pathway in colorectal carcinomas is activated through expression of PDGFα, we analyzed mRNA of PDGFRα using *in situ* hybridization. PDGFRα was detected strongly in aberrant crypts and moderately in stroma of colorectal cancers (Figure 5B) that expressed Gli1. Thus, it may imply that Shh-Gli1 pathway in colorectal cancers is activated through increased expression of PDGFRα.

DISCUSSION

Hh signaling drives cell proliferation, promotes cell survival, and directs cell differentiation in the developing embryo. Abortive regulation of the pathway during development has been associated with significant human birth defects, including holoprosencephaly, basal cell nevus syndrome, and polydactyly[9,10,23-25]. Recent studies have shown that aberrant signaling of this pathway is involved in a variety of human cancers, such as basal cell carcinomas, medulloblastomas and small-cell lung cancer[9-12,15].

Onisu *et al*[18] have studied the expression of Shh, Patch1 and Smo in colonic neoplasia by immunohistochemistry. In the current study, we showed that overexpression of Shh at both mRNA and protein levels were similar in colonic adenocarcinoma and rectal adenocarcinoma tissue. To the best of our knowledge, this is the first report on the expression of Gli3, and Hip in colorectal cancer tissue. Since overexpression of Shh, Ptch1, Hip, Gli1 and PDGFRα occurred in colorectal

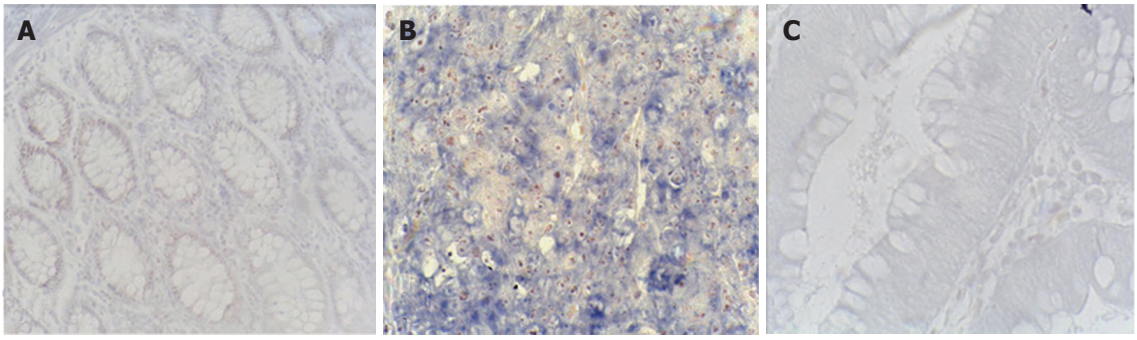

Figure 5 Expression of PDGFRα in primary colorectal adenocarcinomas. **A**: We analyzed expression pattern of PDGFRα transcript by *in situ* hybridization (blueas positive); **B**: Expression of PDGFRα in normal control was high in aberrant crypts and moderate in the stroma; **C**: The expression of PDGFR sense probe in tumor tissue.

cancers, whereas no positive signal of Gli3 was observed at mRNA or protein levels, it may imply that the Shh-Gli1 pathway is activated in colorectal cancers. This supports the view that activation of the hedgehog pathway may play a role in the development of colorectal carcinoma.

We showed in this study that upregulation of Ptch1 or Gli1 expression, in some cases, was not consistent with the expression pattern of Shh (Table 1). This suggests that upregulation of Shh expression may not be the sole cause of activated Ptch1 or Gli1 expression, and that the hedgehog signaling pathway might be activated by other regulatory mechanisms in colorectal carcinoma. Furthermore, we unexpectedly found that the negative regulator, Hip, was expressed in several samples. This may support a model in which Hip, like Ptch1, modulates the responses to Hh signaling via a negative regulatory feedback loop.

The link between the hedgehog pathway, PDGFRα and human cancer was first revealed in Gorlin's syndrome, a disease with a high risk of basal cell carcinoma[9,10,19,26]. Those previous studies have shown that PDGFRα can be regulated by Gli1 and that PDGFRα mediates Gli1-induced Ras/Erk activation[9,10,19,26]. In our studies, we also detected the expression of PDGFRα in colorectal adenocarcinomas which expressed Gli1. Therefore, up-regulation of PDGFRα appears to be one of the mechanisms by which Sonic hedgehog-Gli1 signaling induces colorectal cancers.

Taken together, our findings suggest that activation of the Shh-Gli1 pathway may be involved in colorectal cancer progression.

ACKNOWLEDGMENTS

We appreciate the help from Dr. Shaojun Du, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, in revising the manuscript.

REFERENCES

1 **Hammerschmidt M**, Bitgood MJ, McMahon AP. Protein kinase A is a common negative regulator of Hedgehog signaling in the vertebrate embryo. *Genes Dev* 1996; **10**: 647-658
2 **Johnson RL**, Scott MP. New players and puzzles in the Hedgehog signaling pathway. *Curr Opin Genet Dev* 1998; **8**: 450-456
3 **Pathi S**, Pagan-Westphal S, Baker DP, Garber EA, Rayhorn P, Bumcrot D, Tabin CJ, Blake Pepinsky R, Williams KP. Comparative biological responses to human Sonic, Indian, and Desert hedgehog. *Mech Dev* 2001; **106**: 107-117
4 **Denef N**, Neubüser D, Perez L, Cohen SM. Hedgehog induces opposite changes in turnover and subcellular localization of patched and smoothened. *Cell* 2000; **102**: 521-531
5 **Taipale J**, Cooper MK, Maiti T, Beachy PA. Patched acts catalytically to suppress the activity of Smoothened. *Nature* 2002; **418**: 892-897
6 **Chuang PT**, McMahon AP. Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. *Nature* 1999; **397**: 617-621
7 **van den Brink GR**, Hardwick JC, Nielsen C, Xu C, ten Kate FJ, Glickman J, van Deventer SJ, Roberts DJ, Peppelenbosch MP. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. *Gut* 2002; **51**: 628-633
8 **Nielsen CM**, Williams J, van den Brink GR, Lauwers GY, Roberts DJ. Hh pathway expression in human gut tissues and in inflammatory gut diseases. *Lab Invest* 2004; **84**: 1631-1642
9 **Johnson RL**, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein EH, Scott MP. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 1996; **272**: 1668-1671
10 **Hahn H**, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996; **85**: 841-851
11 **Raffel C**, Jenkins RB, Frederick L, Hebrink D, Alderete B, Fults DW, James CD. Sporadic medulloblastomas contain PTCH mutations. *Cancer Res* 1997; **57**: 842-845
12 **Berman DM**, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK, Cooper MK, Taipale J, Olson JM, Beachy PA. Medulloblastoma growth inhibition by hedgehog pathway blockade. *Science* 2002; **297**: 1559-1561
13 **Thayer SP**, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernández-del Castillo C, Yajnik V, Antoniu B, McMahon M, Warshaw AL, Hebrok M. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* 2003; **425**: 851-856
14 **Sheng T**, Li C, Zhang X, Chi S, He N, Chen K, McCormick F, Gatalica Z, Xie J. Activation of the hedgehog pathway in advanced prostate cancer. *Mol Cancer* 2004; **3**: 29
15 **Watkins DN**, Berman DM, Burkholder SG, Wang B, Beachy PA, Baylin SB. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 2003; **422**: 313-317
16 **Ma X**, Sheng T, Zhang Y, Zhang X, He J, Huang S, Chen K,

Sultz J, Adegboyega PA, Zhang H, Xie J. Hedgehog signaling is activated in subsets of esophageal cancers. *Int J Cancer* 2006; **118**: 139-148

17 **Ma X**, Chen K, Huang S, Zhang X, Adegboyega PA, Evers BM, Zhang H, Xie J. Frequent activation of the hedgehog pathway in advanced gastric adenocarcinomas. *Carcinogenesis* 2005; **26**: 1698-1705

18 **Oniscu A**, James RM, Morris RG, Bader S, Malcomson RD, Harrison DJ. Expression of Sonic hedgehog pathway genes is altered in colonic neoplasia. *J Pathol* 2004; **203**: 909-917

19 **Xie J**, Aszterbaum M, Zhang X, Bonifas JM, Zachary C, Epstein E, McCormick F. A role of PDGFRalpha in basal cell carcinoma proliferation. *Proc Natl Acad Sci USA* 2001; **98**: 9255-9259

20 **Clarke ID**, Dirks PB. A human brain tumor-derived PDGFR-alpha deletion mutant is transforming. *Oncogene* 2003; **22**: 722-733

21 **Uhrbom L**, Hesselager G, Nistér M, Westermark B. Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. *Cancer Res* 1998; **58**: 5275-5279

22 **Heinrich MC**, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299**: 708-710

23 **Roessler E**, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. *Nat Genet* 1996; **14**: 357-360

24 **Weed M**, Mundlos S, Olsen BR. The role of sonic hedgehog in vertebrate development. *Matrix Biol* 1997; **16**: 53-58

25 **Hill RE**, Heaney SJ, Lettice LA. Sonic hedgehog: restricted expression and limb dysmorphologies. *J Anat* 2003; **202**: 13-20

26 **Xie J**, Quinn A, Zhang X, Bare J, Rothman A, Collins C, Cutone S, Rutter M, McCormick MK, Epstein E. Physical mapping of the 5 Mb D9S196-D9S180 interval harboring the basal cell nevus syndrome gene and localization of six genes in this region. *Genes Chromosomes Cancer* 1997; **18**: 305-309

S- Editor Wang J **L- Editor** Zhu LH **E- Editor** Liu Y