Hedgehog signaling pathway regulates human pancreatic cancer cell proliferation and metastasis

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Abstract. Pancreatic cancer is one of the most aggressive and devastating malignancies. The Hedgehog (Hh) pathway has been reported to play an important role in pancreatic cancer development and progression. The aim of this study was to examine the activation of the Hh pathway in human pancreatic cancer tissue samples and pancreatic cancer cell lines, and the molecular mechanisms involved in the Hh pathway mediated effects on pancreatic cancer cell proliferation and invasion. The expression levels of Hh molecules in human pancreatic cancer tissue samples and pancreatic cancer cell lines were evaluated using RT-PCR. The role of the Hh pathway in cell proliferation and invasion was evaluated using flow cytometry, MTT, colony formation assays and Transwell invasion assays, and the expression of cancer stem cell markers and epithelial-mesenchymal transition (EMT) were evaluated using flow cytometry and RT-PCR. Tumorigenicity assays were used to further investigate the role of the Hh pathway in vivo. Hh molecules were highly expressed in human pancreatic cancer tissue samples and pancreatic cancer cell lines. Inhibition of the Hh pathway notably decreased cell proliferation and induced apoptosis through inhibition of the PI3K/AKT pathway and cancer stem cells. Furthermore, inhibition of the Hh signaling pathway significantly inhibited EMT by suppressing the activation of transcription factors Snail and Slug, which are correlated with significantly reduced pancreatic cancer cell invasion, suggesting that the Hh signaling pathway is involved in early metastasis. These results indicate that activation of the Hh pathway is a common event. Inhibition of the Hh pathway may be a potential molecular target of new therapeutic strategies for pancreatic cancer.

Introduction

Pancreatic cancer is one of the most devastating malignancies worldwide, and the fourth leading cause of cancer-related death in the United States. It was estimated that in 2011, 44,030 new cases would be diagnosed with pancreatic cancer and 37,660 individuals would die from this disease (1). Pancreatic cancer has the worst prognosis of all human tumors. Only approximately 20% of pancreatic cancer patients are eligible for surgical resection; most pancreatic cancers are not resectable at the time of diagnosis. In addition, there are limited treatment options available. Chemotherapies and radiotherapies are largely ineffective, and metastatic disease frequently recurs even after surgical resection of primary lesions (2-4). Recently, a number of risk factors have been identified, including age, cigarette smoking, high dietary intake of meat and fat, low serum folate levels, obesity, long-standing diabetes mellitus, chronic pancreatitis and family history (5), but exactly how these risk factors contribute to carcinogenesis remains largely unknown. Therefore, novel strategies for the prevention of tumor progression and metastasis are urgently needed.

The cancer stem cell (CSC) hypothesis provides novel molecular targets and strategies for prevention of pancreatic cancer. CSCs may be responsible for tumor onset, maintenance, mutation accumulation and metastasis due to their ability to express anti-apoptotic and multidrug resistance-associated proteins, thus sustaining tumor growth (6-9). Conventional therapeutic approaches merely kill the majority of differentiated tumor cells, except for CSCs, which have intrinsic detoxifying mechanisms and can easily elude these therapies. Recently, CSCs and epithelial-mesenchymal transition (EMT)-type cells, which share molecular characteristics with CSCs, have been proposed to play critical roles in chemoresistance and metastasis as demonstrated in several malignancies including pancreatic cancer. Thus, it has become increasingly important to increase our understanding, at the molecular level, of the features of CSCs and EMT in pancreatic cancer. Such knowledge is likely to be helpful in the discovery of novel molecular targets for the prevention of this disease.

The Hedgehog (Hh) signaling pathway functions post-embryonically in the development and homeostasis of many organs and tissues through its effects on stem or progenitor cells. Aberrant activation of the Hh signaling pathway correlates with a variety of human tumors where the pathway is implicated in tumorigenesis, malignancy, metastasis and...
cancer stem cells (10-13). Our previous study identified that activation of this pathway was a key event in the histogenesis of pancreatic cancer (14). In a transgenic mouse model of pancreatic cancer, inhibition of the Hh signaling pathway was found to reduce tumor-associated stromal tissues and to ameliorate gemcitabine uptake in tumor cells (15). Recent studies found that activation of the Shh signaling pathway was involved in the regulation of CSC self-renewal, differentiation and tumorigenic potential, suggesting that the Shh signaling pathway may be a novel therapeutic approach for the treatment of pancreatic cancer (16-19). In the present study, the expression of Hh molecules in human pancreatic cancer tissue samples and pancreatic cancer cell lines was detected. In addition, the effects of Hh signaling pathway on pancreatic cancer cell proliferation and invasion were evaluated. Finally, the molecular mechanisms by which inhibition of the Hh signaling pathway decreased cell proliferation, invasion and induced apoptosis were assessed.

Materials and methods

Tissue samples and cell lines. A total of 54 human pancreatic cancer tissue samples were collected from the Peking University First Hospital. Informed consent of the patients was obtained before surgical resection, and this study was approved by the Research Ethics Committee of Peking University First Hospital. Pancreatic cancer cell lines (PANC-1, ASPC-1 and Mia PaCa-2) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO, USA), penicillin (100 U/ml) and streptomycin (100 µg/ml).

Establishment of pancreatic cancer cell clones expressing SMO RNAi. The RNAi targeting the human SMO gene (GenBank accession no. NM_005631) and the negative control sequence were designed and constructed by GeneChem (Shanghai, China). The sequence of the SMO RNAi was GACTCTGTCCTGCATCAT, and that of the negative control was TTCTCCGAACGTGTCACGT. Briefly, the shRNAs were inserted into pFU-GW-RNAi lentivirus vectors containing the HpaI and XhoI enzyme sites (Fig. 1), and all constructs were confirmed by sequence analysis. PANC-1 cells were transfected with the recombinant lentiviral vectors targeting the SMO gene (PANC-1-si) or the negative control vectors (PANC-1-nc). The constructs were stably transfected into cells to generate knockdown clones. The cells were cultured for 48 h, and the transduction efficiency was assessed using FACS analysis. The transfected cells were harvested and prepared for subsequent studies.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from pancreatic cancer cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Aliquots (1 µg) of RNA were DNase-treated and processed for first-strand cDNA synthesis using the RT-PCR kit. Real-time RT-PCR was carried out on an Applied Biosystems 7300 Fast Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using real-time PCR Master Mix (Toyobo). Quantification of gene expression was calculated by the 2^-∆∆Ct method. GAPDH was used as an internal control. The primer sequences for real-time RT-PCR are shown in Table I. Three independent experiments were performed.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). Total RNA was extracted from human pancreatic cancer tissue samples using TRIzol reagent (Invitrogen). Aliquots (1 µg) of RNA were DNase-treated and processed for first-strand cDNA synthesis using the RT-PCR kit. Real-time RT-PCR was carried out on an Applied Biosystems 7300 Fast Real-time PCR System (Applied Biosystems, Foster City, CA, USA) using real-time PCR Master Mix (Toyobo). Quantification of gene expression was calculated by the 2^-∆∆Ct method. GAPDH was used as an internal control. The primer sequences for real-time RT-PCR are shown in Table I.

Western blot analysis. Pancreatic cancer cells were seeded in 10-cm cell culture plates in complete medium and allowed to reach 80% confluency. Then, cells were lysed and the proteins were obtained using the Total Protein Extraction kit (KeyGen, Nanjing, China). Total protein (70 µg/sample) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After being transferred to nitrocellulose membranes, blots were incubated with the SMO antibody (Chemicon International, Temecula, CA, USA) and β-actin antibody (Zsbio, Beijing, China), and the washed blots were then incubated with secondary antibodies conjugated with horseradish peroxidase. To detect the activation of the PI3K/AKT pathway, pancreatic cancer cells (PANC-1-si and PANC-1-nc) were treated with Shh (1 µg/ml, Abcam, Cambridge, UK), and then western blot analysis was performed with the phospho-AKT antibody (Cell Signaling Technology, Beverly, MA, USA). Three independent experiments were performed.

MTT assays. Pancreatic cancer cells were propagated in 96-well plates at a density of 5.0x10^4 cells/well for 72 h prior to the proliferation assays. MTT (20 µl) (5 mg/ml, Sigma) was
added to each well and incubated for 4 h at 37°C. Then culture medium was removed, 200 µl dimethyl sulfoxide (DMSO) was added and thoroughly mixed for 15 min to resolve the cellular formazan. Optical density was measured at 570 nm (OD\textsubscript{570}) with an ELISA plate reader (Bio-Rad Laboratories, Hercules, CA, USA). The cell growth inhibition rate (GIR) was calculated as follows: GIR = (1 - OD\textsubscript{570} of treated cells/OD\textsubscript{570} of untreated cells) x 100%. Three independent experiments were performed.

**Colony formation assays.** Cells (3.0x10\textsuperscript{3} cells/plate) were seeded into 60-mm culture dishes and allowed to grow for 14 days. Colonies were fixed in methanol and visible colonies containing ~50 or more cells were counted. Three independent experiments were performed.

**Flow cytometric analysis.** An Annexin V-PE Apoptosis Detection kit (BD Biosciences, Franklin Lakes, NJ, USA) was used to measure the apoptosis of pancreatic cancer cells (wild-type, PANC-1-si and PANC-1-nc). The cells were seeded in 6-well plates and incubated until 70% confluency. The cells were then harvested, washed twice with cold phosphate-buffered saline (PBS), and resuspended in 1X binding buffer at a concentration of 1.0x10\textsuperscript{5} cells/ml. Solution (100 µl) (1.0x10\textsuperscript{5} cells) was transferred to a 5-ml culture tube. A total of 5 µl of Annexin V-PE and 5 µl of 7-AAD were added, and the mixture was incubated for 15 min at room temperature in the dark. The labeled cells (1.0x10\textsuperscript{5} cells/sample) were analyzed using FACSscan flow cytometer (BD Biosciences) in conjunction with CellQuest software. To detect the expression of cancer stem cell marker CD133, pancreatic cancer cells (PANC-1-si and PANC-1-nc) were collected, washed with PBS and suspended in PBS at a concentration of 1.0x10\textsuperscript{5} cells/ml, then labeled with CD133-PE (Miltenyi Biotec, Germany) and analyzed using FACSscan flow cytometer. Three independent experiments were performed.

**Transwell invasion assays.** Cells (1.0x10\textsuperscript{5} cells/well) were seeded into the upper chamber of a Transwell insert (Costar, Cambridge, MA, USA), which was precoated with the extracellular matrix (ECM) substitute Matrigel (BD Biosciences) in phenol red-free medium. In the lower chamber, phenol red-free DMEM supplemented with 5% of steroid-depleted fetal bovine serum (Biochrom, Berlin, Germany) acted as the chemoattractant. The cells were then cultured for an additional 48 h. Non-migrating cells were removed from the upper chamber by scraping, and cells remaining on the lower surface of the insert were stained using 0.1% crystal violet, and the cell number was counted under a microscope. Three independent experiments were performed.

**Nude mouse xenograft model.** Six-week-old nude mice (BALB/c AnN CrI-Br) were maintained in a barrier facility on high efficiency particulate air-filtered racks and fed by

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**Table I. Primer sequences.**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sense sequences</th>
<th>Antisense sequences</th>
</tr>
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<tbody>
<tr>
<td>SHH</td>
<td>Sense 5'-CCAATTACACCCCCGACATC-3'</td>
<td>Antisense 5'-CAGTTTTCACCTCTGGCCACT-3'</td>
</tr>
<tr>
<td>PTCH1</td>
<td>Sense 5'-TGGGATTTAAAACGACAGGAAC-3'</td>
<td>Antisense 5'-TCTCCAATTTCTTGCCGAGT-3'</td>
</tr>
<tr>
<td>SMO</td>
<td>Sense 5'-TGCTCATCTGTGAGGTCTACTTT-3'</td>
<td>Antisense 5'-ATCTTGTGTCGACGCCCTCTCAC-3'</td>
</tr>
<tr>
<td>GLI1</td>
<td>Sense 5'-TATGGACCTGCTTTGGA-3'</td>
<td>Antisense 5'-CCCTTGTAGACCAAGAAC-3'</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Sense 5'-GGCTCTCTGAAAGAGAGTGGAGG-3'</td>
<td>Antisense 5'-TGCGAGTGTCTCCAATCCGG-3'</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>Sense 5'-TCGCTTCGTAGGGCTCCATGTGCGG-3'</td>
<td>Antisense 5'-AAGGGTCACCTGAAGCTCAC-3'</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Sense 5'-AGGCACAAGCGAGTCTCACTGCA-3'</td>
<td>Antisense 5'-ATCTGGGCTTCCAGGCCTAC-3'</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Sense 5'-ACAACACCGAGTGACTGAGAC-3'</td>
<td>Antisense 5'-GGCACACAAGGTCTTCCTGAG-3'</td>
</tr>
<tr>
<td>Snail</td>
<td>Sense 5'-TGCCCCTCAAGATGCACATCCGA-3'</td>
<td>Antisense 5'-GGGACAGAGGAAGGCTTCTC-3'</td>
</tr>
<tr>
<td>Slug</td>
<td>Sense 5'-ATCTCGGCAAAGCGTTCATTCCA-3'</td>
<td>Antisense 5'-GAGCCCTCAAGATTTGAGCTGC-3'</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense 5'-ACGATTGTTGCTCTATGGG-3'</td>
<td>Antisense 5'-TGATTGTGGGAGGATCTCGC-3'</td>
</tr>
</tbody>
</table>
breeders from the Department of Laboratory Animal Science, Peking University Health Science Center. Pancreatic cancer cells (wild-type, PANC-1-si and PANC-1-nc) were subcutaneously injected into the right axilla of nude mice. Mice were monitored for tumor formation every 3 days. Tumor size was measured in two dimensions, and the volume was determined by the equation $V = l \times w^2 \times 0.5$ (where $V$ is the volume; $l$, length; $w$, width). Four weeks later, all mice were sacrificed, and tumors were excised. All animal studies were reviewed and approved by the Ethics Committee for Animal Studies at the Peking University, China.

Statistical analysis. Results are presented as the means ± SD. Statistical analyses were undertaken using SPSS version 13.0. Student's t-test (for two groups) and one-way analysis of variance (ANOVA) test (for multiple groups) followed by the Student-Newman-Keuls (SNK) test were used to assess the differences. P<0.05 was considered to indicate a statistically significant result.

Results

Activation of the Hh signaling pathway in pancreatic cancer tissue samples and cell lines. We detected SHH, PTCH1, SMO and GLI1 expression in 54 human pancreatic cancer tissue samples for the purpose of evaluating the activation of the Hh signaling pathway. Expression levels of Hh molecules in cancer tissues and matched adjacent non-tumor tissues were determined by real-time RT-PCR. As shown in Fig. 2, a significant difference in expression of Hh molecules was noted between cancer tissues and matched adjacent non-tumor tissues (P<0.05, respectively). In addition, to confirm the activation of the Hh signaling pathway in pancreatic cancer, we used RT-PCR to detect the expression of SHH, PTCH1, SMO and GLI1 in three pancreatic cancer cell lines (PANC-1, ASPC-1 and Mia PaCa-2). As shown in Fig. 3, varied expression of Hh molecules confirmed the general presence of an active Hh signaling pathway. High GLI1 expression was observed in the PANC-1 cell line, which is characterized by poor differentiation and better invasiveness. In addition, GLI1 is the only reliable marker of Hh signaling pathway activation. Therefore, the PANC-1 cell line was selected for subsequent experiments.

Lentivirus-mediated shRNA targeting SMO decreases cell proliferation and enhances cell apoptosis. PANC-1 cells were transfected with recombinant lentiviral vectors targeting the SMO gene (PANC-1-si) or negative control vectors (PANC-1-nc). The effects of the lentivirus-mediated shRNA on mRNA and protein expression of SMO were examined.
by RT-PCR and western blot analysis, and the result revealed that SMO expression was significantly downregulated in PANC-1-si cells (Fig. 4). The effect of lentivirus-mediated shRNA targeting SMO on cell proliferation was measured by MTT and colony formation assays. Pancreatic cancer cells were propagated in 96-well plates for 72 h prior to the proliferation assays. Inhibition of the Hh signaling pathway significantly decreased the cell proliferation. The average inhibitory rate was 35.7±1.34% for PANC-1-si compared with the negative control (P<0.05) (Fig. 5). In addition, cells were seeded and allowed to grow for 14 days. Visible colonies containing ~50 or more cells were counted. The results indicated that the number of PANC-1-si colonies was much lower than that of wild-type or negative control cells (Fig. 6). Moreover, SMO suppression caused a moderate increase in the apoptotic rate. As shown in Fig. 7, apoptosis of PANC-1-si cells was markedly increased to 11.69±1.372%, significantly higher than that of wild-type or negative control cells (P<0.05). These findings imply that inhibition of the Hh signaling pathway significantly decreases proliferation of PANC-1 cells. To investigate the possible
mechanisms, we analyzed activation of the PI3K/AKT pathway in pancreatic cancer cells. Pancreatic cancer cells (PANC-1-si and PANC-1-nc) were treated with Shh prior to Western blot assays. As shown in Fig. 8, downregulation of SMO caused a notable decrease in phosphorylated AKT.

**Lentivirus-mediated shRNA targeting SMO inhibits cell invasion.** To further study the potential influence of the Hh signaling pathway on cancer cell invasion, we performed Transwell invasion assays. As shown in Fig. 9, the number of PANC-1-si cells that migrated through the upper chamber of the Costar wells was less than that of wild-type or negative control cells. Moreover, to test the possibility that the Hh signaling pathway directly drives EMT and metastatic behavior in human pancreatic cancer cells, we analyzed the EMT-related genes by real-time RT-PCR. As shown in Fig. 10, downregulation of SMO caused a notable increase in E-cadherin and a notable decrease in N-cadherin, vimentin and fibronectin. The expression of the transcription factors Snail and Slug also displayed a reduction. Collectively, these
studies demonstrate that the Hh signaling pathway could play a role in the invasion of pancreatic cancer.

**Lentivirus-mediated shRNA targeting SMO decreases the expression of a cancer stem cell marker.** The involvement of the Hh signaling pathway in proliferation and invasion suggests a possible role in the behavior of pancreatic cancer stem cells. To test cancer stem cell behavior in vitro, we detected the expression levels of cancer stem cell marker CD133 in pancreatic cancer cells. As shown in Fig. 11, our data showed that SMO suppression resulted in downregulation of the CD133^+ subfraction in PANC-1-si.

**Lentivirus-mediated shRNA targeting SMO decreases xenograft tumor growth.** To investigate the antitumor effect in vivo, we established xenograft models with PANC-1, PANC-1-nc or PANC-1-si cells. Four weeks later, all mice were sacrificed, and tumors were excised. As shown in Fig. 12, the growth of PANC-1-si xenograft tumors was significantly inhibited when compared with the control groups.

**Discussion**

The Hedgehog (Hh) signaling pathway was originally identified through the genetic analysis of segmentation in *Drosophila* and is highly conserved from insects to vertebrates (20,21). In mammals, there are three Hh ligand proteins: Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH). They exert their molecular and cellular effects through two transmembrane protein receptors: Patched (PTCH) and Smoothened (SMO), two key regulators of the Hh signaling pathway. PTCH is a 12-transmembrane protein that acts as a negative regulator of SMO, which is a 7-transmembrane protein. When this pathway is activated, Hh ligand proteins activate SMO, complex with coactivator or corepressor proteins, and bind to transcriptional factor GLI in the regulatory regions of target genes to regulate the transcription of GLI-responsive genes (22,23).

Aberrant activation of the Hh signaling pathway has been demonstrated during the progression of human pancreatic cancer. Berman et al (24) analyzed the expression of PTCH mRNA in freshly resected pancreatic tumors by quantitative RT-PCR, and found that PTCH mRNA levels were 69-5,044 times higher in pancreatic tumors (mean, 448; n=15) than in adjacent normal tissue. Kayed et al (25) examined the expression of Hh molecules (IHH, PTCH and SMO) in pancreatic cancer tissues by quantitative RT-PCR, revealing that IHH, PTCH and SMO mRNA levels were increased 35-, 1.2- and 1.6-fold, respectively, compared to normal pancreatic tissues.

In the present study, we evaluated the expression levels of Hh molecules in human pancreatic cancer tissue samples and pancreatic cancer cell lines. Consistent with recent published data (25-28), our results revealed that expression of Hh signaling pathway-related molecules were at a higher level in the cancer tissues than in the matched adjacent non-tumor tissues and existed in all pancreatic cancer cell lines. Although pancreatic cancer tissue samples and cell lines expressed detectable levels of the mRNA of the molecules, the expression levels varied. Little is understood about the exact mechanism of Hh molecules in pancreatic cancer cell proliferation and invasion. Further analysis showed that the therapeutic effects of SMO suppression were correlated with the induction of apoptosis in pancreatic cancer cells. The mechanism of this effect has not yet been fully elucidated. Our results showed that the phosphorylation levels of AKT were markedly reduced in PANC-1-si cells after Shh stimulation compared to control groups (Fig. 8). The results suggest that the suppression of PI3K/AKT activity might be responsible for the apoptosis-inducing effect of SMO suppression. As reported by several authors, the PI3K/AKT pathway has also been reported to play

![Figure 11. The CD133^+ subfraction in pancreatic cancer cells detected by FACS analysis. The lower right quadrants represent the CD133^+ subfractions. The percentage of the CD133^+ subfractions of PANC-1-nc and PANC-1-si cells were 1.68±0.121 and 0.81±0.178%.](image1)

![Figure 12. Effect of SMO suppression on the growth of xenograft tumors. Pancreatic cancer cells (PANC-1, PANC-1-nc or PANC-1-si) were subcutaneously injected into the right axilla of nude mice. The average tumor volume of PANC-1-si was notably smaller than that of the control groups.](image2)
important roles in the Hh signaling pathway-mediated metastasis and chemoresistance (29-31).

CSCs have been identified in many different types of solid tumors (32-37). Recent evidence indicates that the Hh signaling pathway is recruited to stimulate CSC growth. It is one of the key pathways that regulates stem cells in the adult body (38,39). Several studies have also shown that the Hh signaling pathway plays an important role in maintaining the biological characteristics of pancreatic cancer stem cells. Hermann and colleagues (40) successfully isolated a different highly tumorigenic subpopulation of pancreatic cancer cells. They found that cells expressing CD133 have the characteristics that defined CSCs, and compared to CD133+ pancreatic cancer cells, CD133+ cells showed significantly enhanced resistance to gemcitabine. Dembinski and Krauss (41) found that compared with pancreatic cancer cells, SHH was increased nearly 4-fold in BxPC-3 CSCs and 2-fold in Panc03.27 CSCs, and the zinc finger transcription factor GLI1 was upregulated 2.5-fold in BxPC-3 and 1.5-fold in the Panc03.27 CSC populations. Furthermore, Feldmann et al (42) inhibited the Hh signaling pathway by means of a small-molecule SMO inhibitor, cyclo-pamine, and found that the treatment preferentially reduced aldehyde dehydrogenase (ALDH)-expressing populations in pancreatic cancer cells; ALDH overexpression is characteristic for stem cells.

In the present study, we found that the Hh signaling pathway may play a role in the regulation of self-renewal in pancreatic cancer stem cells. CD133 expression was analyzed using FACScan flow cytometry in pancreatic cancer cells, which is reported to be a putative marker of CSCs in pancreatic cancer. We demonstrated that the percentage of CD133+ cells significantly differed between PANC-1-si and PANC-1-nc cells. The results revealed that inhibition of the Hh signaling pathway by knockdown of SMO affects the pancreatic CSC population.

Moreover, recent evidence indicates that the Hh signaling pathway is recruited to orchestrate the reprogramming of cancer cells via EMT in various types of cancer (42-44). EMT is an embryonic program in which epithelial cells lose their characteristics and gain mesenchymal features. Accumulating evidence suggests that EMT plays an important role during malignant tumor progression. Transformed epithelial cells can activate embryonic programs of epithelial plasticity and switch from a sessile, epithelial phenotype to a motile, mesenchymal phenotype. Induction of EMT can, therefore, lead to switch from a sessile, epithelial phenotype to a motile, mesenchymal phenotype. Induction of EMT can, therefore, lead to metastasis and chemoresistance (29-31).

In conclusion, our data demonstrated that the Hh signaling pathway is frequently activated in human pancreatic cancer tissue samples and pancreatic cancer cells, and plays a pivotal role in maintaining pancreatic cancer cell proliferation and invasion. Inhibition of the Hh signaling pathway by SMO suppression represents an effective method to decrease cell proliferation and induce apoptosis through inhibition of PI3K/AKT pathways and CSCs. Further studies found that inhibition of the Hh signaling pathway significantly inhibited EMT, suggesting blockade of signaling involved in early metastasis. These data suggest that targeted therapy against the Hh signaling pathway may be beneficial in the treatment of pancreatic cancer.

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References


