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Long non-coding RNA *HOTTIP* promotes tumor growth and inhibits cell apoptosis in lung cancer

H-P. Deng, L. Chen, T. Fan, B. Zhang, Y. Xu and Q. Geng✍

Department of Cardiothoracic Surgery, Renmin Hospital of Wuhan University, Wuhan, Hubei, China

Corresponding author: Qing Geng, Department of Cardiothoracic Surgery, Remin Hospital of Wuhan University, 238 Jie Fang Road, Wu han, 430060, PR China. E-mail: szgqing@126.com

Abstract

Lung cancer is one of the leading causes of cancer-related deaths worldwide. Early diagnosis is the best defense against this threat and is therefore of vital importance. In this study, we investigated the role of long non-coding RNA *HOTTIP* in the tumor growth of lung cancer. Initially, we found that expression of HOTTIP was significantly elevated in 20 cases of lung cancer. *HOTTIP* was also differentially expressed in a consecutive of lung cancer cell lines. Furthermore, specific shRNA against *HOTTIP* was employed to deplete expression of *HOTTIP* in A549 cells and NCI-H446 cells. After successfully depletion of HOTTIP, cell proliferation and colony formation were significantly inhibited in vitro. Tumor growth in vivo was also suppressed after depletion of *HOTTIP* in a mouse model of lung cancer. Moreover, depletion of *HOTTIP* caused cell cycle arrest in G0/G1 phase and induced significant cell apoptosis. Cell cycle regulators Cdc25C, Cyclin B1 and Cyclin D1 were decreased upon depletion of *HOTTIP*. Pro-apoptotic factor Bad was up-regulated, whereas anti-apoptotic factors Bcl-2 and Bcl-xL were down-regulated after *HOTTIP* ablation. These data suggest that lncRNA *HOTTIP* contributes to tumor growth in vivo and in vitro and inhibits cell apoptosis in lung cancer.

Key words: lncRNA *HOTTIP*, tumor growth; apoptosis, cell cycle, lung cancer.

Introduction

Lung cancer is one of the leading causes of cancer-related deaths around the world (1). Among all the lung cancers, non-small cell lung cancer (NSCLC) currently accounts for approximately 80% of all cases (2, 3), of which more than 65% present with locally advanced or metastatic disease. Lack of effective biomarkers and imageology for early detection are the mainly contributors to the poor prognosis of lung cancer. Newly emerged molecular targeted agents have undoubtedly achieved longer survival in some subtypes of this disease. However, conventional cytotoxic chemotherapy has limited the application of these agents in the treatment of advanced stage of lung cancer (4). Development of chemotherapy resistance even frustrates physicians which accounts for high recurrence rates. Similarly, while transcriptional factor Specificity protein 1 (Sp1) was reported to be required for lung tumor growth and suppress metastasis, it was only accumulated strongly in early stage and declined in late stage(5), as similar with that in breast cancer(6), indicating that inhibition of Sp1 was only seemingly effective in early stage of these malignancies. These findings greatly necessitate our looking for novel developments of effective biomarkers for lung cancer progression.

Recently, the emergence of long non-coding RNAs (lncRNAs) has greatly advanced our understanding of human tumorigenesis. LncRNAs are defined as transcripts with over 200 nucleotides and are typically transcribed by RNA polymerase II (7). Functionally, lncRNAs work as molecular scaffolds for promoting protein-DNA and protein-protein interactions; they also serve as decoys that stabilize interactions between distal and proximal macromolecules (8-11). Mounting reports have documented that aberrant expression of lncRNAs is widely involved in human tumorigenesis and controls the oncogenic activities of cancer cell, such as proliferation, survival and migration/invasion (10, 12). Among the discovered lncRNAs, HOX-associated lncRNAs are of great biological importance. For instance, HOX transcript antisense RNA (*HOTAIR*) and HOXA transcript at the distal tip (*HOTTIP*) are two mostly studied lncRNAs in this subgroup, of which HOTTAIR is the most documented one. *HOTTAIR* is a 2.2 kb lncRNA located in the mammalian *HOXC* locus which serves as sequence-specific scaffold for at least two complexes related to histone modifications, namely polycomb repressive complex (PRC2) and the LSD1/CoREST/REST complex (10-12). Current studies have widely reported that *HOTAIR* is a crucial pro-oncogenic factor that predicts tumor recurrence in hepatocellular carcinoma (HCC) (13). *HOTAIR* is also a negative prognostic factor in various types of cancer, including colorectal cancers (14, 15), pancreatic cancer (16), and oesophageal squamous cell carcinoma (17).

As another HOX-associated lncRNA, *HOTTIP* is also associated with the PRC2 and WDR5/MLL1 chromatin modifying complexes and directly binds WDR5 (18). *HOTTIP* primarily coordinates the activation of 5'HOXA genes in fibroblasts (18) , and recent studies showed a close association between *HOTTIP* and HOXA13 in HCCs (19) and pancreatic cancer (20). *HOTTIP* promoted cell proliferation, invasion, and chemoresistance by regulating HOXA13 in pancreatic cancer (20). However, beadchip array argued that *HOTTIP* did not regulate HOXA13 in pancreatic cancer cells, but played a role in regulating several other HOX genes including HOXA1, HOXA9, HOXA10, HOXA11 and HOXB2 (21). These findings indicated that *HOTTIP* might have the similar pro-oncogenic functions as the HOX-associated lncRNA *HOTAIR*, but each lncRNA

might have distinct sets of target genes in human tumors.

Despite the reports in HCC and pancreatic cancer, the role of lncRNA *HOTTIP* in human lung cancer remains unclear. Herein, the present study aims to determine whether *HOTTIP* plays critical roles in lung cancer progression in vitro and in vivo. Expression of *HOTTIP* in lung cancer tissues would be initially assessed, and effects of *HOTTIP* knockdown in lung cancer cell survival will also be explored.

Materials and methods

Cells and reagents

Human lung cancer cell lines SK-LU-1, NCI-H1975, NCI-H157, A549, NCI-H446 and Calu-3 were all purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Cells were maintained in Dulbecco's modified eagle medium (DMEM) media (Gibco, Los Angeles, CA, USA) supplied with 10% fetal bovine serum (FBS, Gibco) in a humidified incubator containing 5% CO_2 at 37°C. Cell culture media was refreshed every two days. For cell proliferation detection, CCK-8 kit was purchased from Dojindo (Japan). All the primary and secondary antibodies were purchased from santa cruz biotechnology (CA, USA).

Human tissues

A total of twenty cases of lung cancer patients who were admitted to Remin Hospital of Wuhan University were collected. These cases were diagnosed with lung cancer in different stage. Their adjacent non-cancerous tissues were also obtained. All patients were informed of our study protocol and showed their full consent to participate into the present study. Permission to use human tissue sections for research purposes was obtained and approved by an institutional review board at Remin Hospital of Wuhan University.

Construction of HOTTIP-knockdown lentivirus

For knockdown of *HOTTIP*, specific *HOTTIP* shRNA (sh*HOTTIP*) and scramble control shRNA (shCtr) were inserted into the pLVX-tdTomato-Puro lentiviral vector (Open Biosystems, Rockford, IL). sh*HOTTIP* and shCtr sequences were described previously (20) with sh*HOTTIP* target sequence as 5'-GCACAGAGAUAAUGGCAAAUU-3'.

shRNA lentivirus was used to generate stable *HOTTIP*-knockdown cells. Our procedure was in accordance with a previous report (20). After construction of *HOTTIP*-knockdown cells, lentivirus -delivered shRNAs were subject to infect lung cancer cell lines. Briefly, cells were seeded into a six-well plate at a concentration of 8×10^4 cells/well and infected with the lentivirus at the multiplicity of infection (MOI) of 50. After 96h of co-incubation, infection efficiency was determined by quantitative real-time PCR (qRT-PCR) and under fluorescence microscopy by counting the percentage of GFP positive cells.

qRT-PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's instructions. Extracted total RNAs were then immediately converted into cDNA by inverse transcription using oligodT primers and SuperScript II reverse transcriptase (Invitrogen). For qRT-PCR, each sample was amplified in triple in a 20-μL reaction system containing SYBR Green reaction mix (Qiagen,Germany) and 0.5 mM of primers. Samples were analyzed using a Roche Light-Cycler (Roche, Basel, Switzerland). *GAPDH* was used as an internal control. Primers for *HOTTIP* were as follows:
Forward, 5′-CCTAAAGCCACGCTTCTTTG-3'
Reverse, 5′-TGCAGGCTGGAGATCCTAGT-3′;
Sequences for GAPDH primers were as follows:
Forward, 5'- GTGGACATCCGCAAAGAC-3'
Reverse, 5'-AAAGGGTGTAACGCAACTA-3'
Relative gene expression was calculated using the formula $2^{-\Delta CT}$ (CT, cycle threshold) whereΔCT=CT value of target gene−CT value of GAPDH.

Western blot analysis

Total proteins from cells were extracted and subjected to one-dimonsional SDS-PAGE electrophoresis. After that, proteins were transferred to a polyinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) by using a transfer apparatus. Membranes were later blocked by 5% skim milk in TBS buffer containing 0.05% Tween-20 (TBST) for 1h at room temperature. Subsequently, membranes were washed with TBST three times, and probed with corresponding primary antibodies overnight at 4°C. The secondary antibody was incubated with the membrane at the presence of blocking agent for 1h at room temperature. After three washes with TBST, immune complexes were developed using enhanced chemoluminescent autoradiography (ECL) kit (Amersham, Pittsburgh, PA, USA). GAPDH was synchronously developed as loading control.

Cell viability assay

For detection of cell viability, control and *HOTTIP*-depleted A549 cells and NCI-H446 cells were incubated into 96-well plates (5000 cells/well). Cell viability was monitored for a consecutive of 6 days. On each monitored day, 10μl of CCK-8 solution was added to each well. After incubated for another 2h at 37°C, the absorbance value for each well was determined using a synergy 2 multi-mode microplate reader (Bio Tek Instruments, Winooski, VT, USA) at 570 nm.

Colony formation assay

Control and *HOTTIP*-depleted lung cancer cells in the logarithmic growth phase were initially digested with a trypsin solution (Gibco, USA), and then 2ml of the single-cell suspension was seeded onto 10cm plates (800 cells/well) and allowed to form natural colonies. After 12 days of incubation, both groups of colony were stained with crystals violet, followed by washing twice with PBS. Subsequently, cells were fixed by paraformaldehyde and washed again with PBS solution, which was later photographed with an inverted microscope. Colonies with over 50 cells were manually counted.

Flow cytometry

A549 cells and NCI-H446 cells which were depleted of *HOTTIP* (sh*HOTTIP* group) or not (shCtr) were harvested for cell cycle analysis with flow cytometry. Cells were initially fixed in 70% pre-cold ethanol and

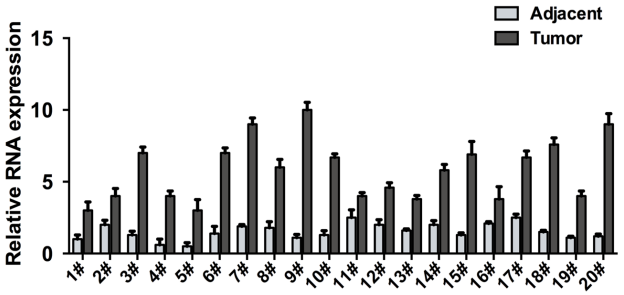

Figure 1. lncRNA HOTTIP is highly expressed in human lung cancer tissues. qRT-PCR was performed in a set of 20 cases of lung cancer. Expression of HOTTIP was significantly higher in the cancer tissues than the adjacent ones, with the fold changes up to 10 in the No.9 case.

incubated at 4°C overnight. After that, cells were centrifuged and re-suspended in staining buffer (Beyotime, Nanjing, China), which was followed by staining with propidium iodide (PI) at 37°C for 30min. DNA contents were measured using the FC500 flow cytometer (Beckman, USA) and quantification of cell percentages in G0/G1, S and G2/M phases respectively were performed by using Modfit 2.0 software.

Hoechst 33342 staining

Both control and *HOTTIP*-depleted cells were washed with PBS and fixed with a mixture containing methanol and acetic acid (3:1, v/v) for 16min at room temperature. After fixation, cells were washed three times with PBS and stained with 5μg/mL of Hoechst 33342 stain for 10min at room temperature. Morphological changes in the nuclei of cells after staining with Hoechst 33342 were observed under a fluorescence microscope (Leica, Germany). Apoptosis was also quantified by counting the number of apoptotic cells.

Xenograft model of lung cancer

Twelve male BALB/c-nude mice were purchased from SLRC Laboratory Animal Co. (Shanghai, China). Mice were all six-week old and randomly divided into two groups (n=6 per group), namely sh*HOTTIP* group and shCtr group. Mice from each group were housed with ad libitum access to water and food in temperature and light-controlled environment. An equal amount of A549 cells, either depleted of *HOTTIP* or not, were injected into the right flank of the corresponding group of mice. Tumor dimensions were measured once a week and tumor volume was calculated as described previously (22). Five weeks after the injection, all mice were sacrificed and tumors were dissected for weighing. The protocol of animal experiment was approved by the Institutional Animal Care of Remin Hospital of Wuhan University. All efforts were made to minimize sufferings.

Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Differences between groups were analyzed with the Student's *t*-test using SPSS version 16.0 software. A p-value of less than 0.05 was considered significant.

Results

lncRNA HOTTIP is highly expressed in human lung cancer tissues

Initially, expression of *HOTTIP* in clinical lung cancer was determined. A total of 20 cases of lung cancer were collected. qRT-PCR showed that expression of *HOTTIP* was significantly higher in the tumor tissues than that in the paired adjacent non-cancerous tissues. The fold changes within these cases ranged from 3 to 10 (Figure 1). These data suggest that *HOTTIP* is overexpressed in clinical lung cancer tissues.

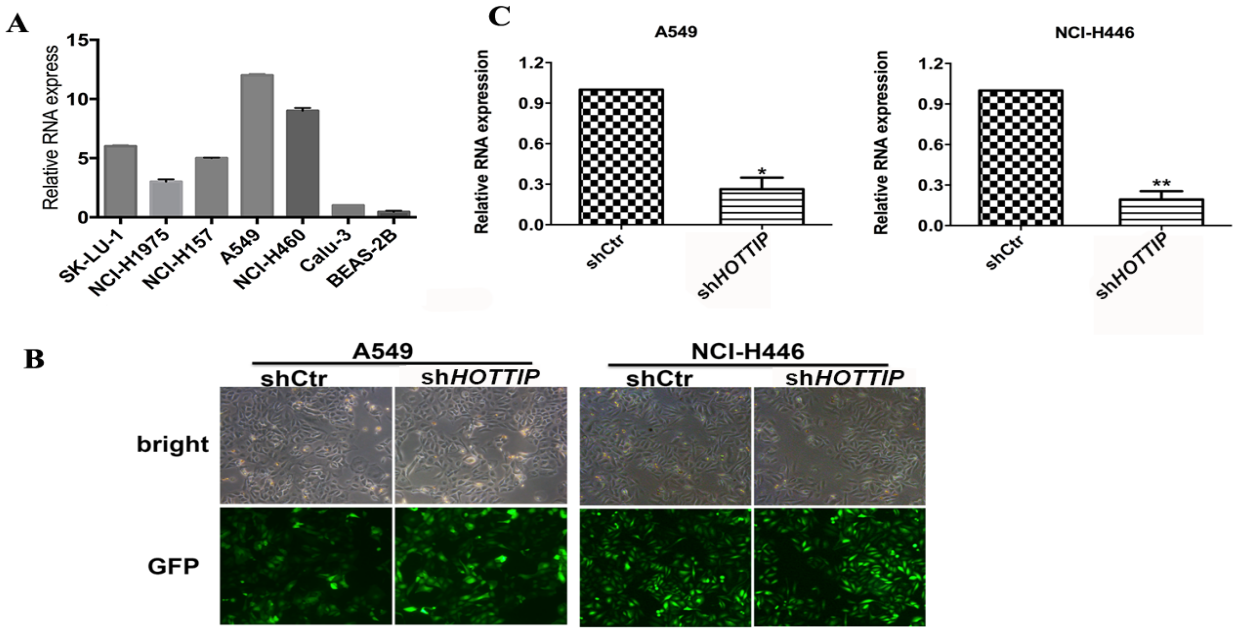

Figure 2. Construction of lentivirus-delivered shRNA against HOTTIP in lung cancer A549 cells and NCI-H446 cells. (A) Expression of HOTTIP was differentially expressed in the 6 lung cancer cell lines. Among these cell lines, A549 cells and NCI-H446 cells exhibited the highest expression. (B) Fluorescent microscope revealed the high efficiency of infection with the specific shHOTTIP or shCtr in both A549 cells and NCI-H446 cells. (C) qRT-PCR showed that infection with shHOTTIP instead of shCtr significantly decreased expression of HOTTIP in A549 cells and NCI-H446 cells. *, $p<0.05$, **, $p<0.01$.

Successful construction of lentivirus-delivered shRNA against HOTTIP in lung cancer cell lines

In view of the high expression of *HOTTIP* in lung cancer tissues, we further determined the expression of *HOTTIP* in a set of lung cancer cell lines, including SK-LU-1, NCI-H1975, NCI-H157, A549, NCI-H446 and Calu-3. Our data showed that *HOTTIP* was differentially expressed in these cell lines. A549 cells and NCI-H446 cells exhibited the highest expression of *HOTTIP* (Figure 2A), making these two cell lines optimal for subsequent analyses. We used A549 and NCI-H446 cell lines as models of lung cancer for construction of lentivirus-delivered shRNA against *HOTTIP* (sh*HOTTIP*). After construction, fluorescent microscope showed that both cell lines were highly infected with lentivirus (Figure 2B). Furthermore, qRT-PCR showed that infection with sh*HOTTIP* instead of shCtr significantly decreased expression of *HOTTIP* in A549 cells and NCI-H446 cells (Figure 2C). These data suggest the successful construction of lentivirus-delivered shRNA against *HOTTIP* in lung cancer A549 cells and NCI-H446 cells.

Depletion of HOTTIP slowed down tumor growth in vitro

To investigate effects of *HOTTIP* knockdown on lung cancer progression, we assessed cell viability and colony formation abilities. Cell viability was monitored in a consecutive of 6 days. We found that cell proliferation rates between sh*HOTTIP* and shCtr groups began to differ since day 3 in both cell lines. By day 6, cell proliferation rate in sh*HOTTIP* group was significantly lower, accounting for only approximately 50% of the control A549 cells, and about 60% of control NCI-H446 cells (Figure 3A). Consistently, colony formation was also inhibited in both cell lines. Remarkable colonies were observed in the control cells, whereas colonies were rarely present in the sh*HOTTIP* groups. Quantification of the colonies revealed that the number of colony was decreased by up to 57.1% in sh*HOTTIP*-infected A549 cells and even by 66.7% in sh*HOTTIP*-infected NCI-H446 cells (Figure 3C). These observations suggest that depletion of *HOTTIP* significantly inhibited tumor growth in lung cancer in vitro.

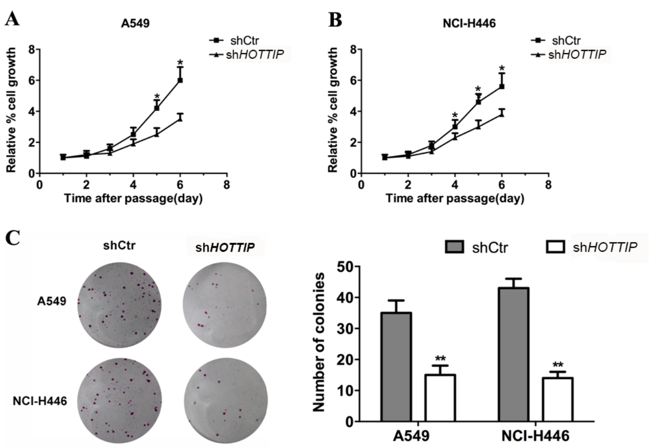

Figure 3. Depletion of HOTTIP slowed down tumor growth in vitro. (A-B) effects of HOTTIP knockdown on A549 cells (A) and NCI-H446 cells (B) viability. On the monitored day 6, cell proliferation rate in shHOTTIP group was significantly lower, accounting for only approximately 50% of the control A549 cells and about 60% of control NCI-H446 cells. (C) Colony formation assay showed that depletion of HOTTIP significantly decreased the number of colony in both cell lines. *, p<0.05, **, p<0.01.

Depletion of HOTTIP arrested cell cycle and induced cell apoptosis

Furthermore, we examined the effects of *HOTTIP* knockdown on cell cycle progression and cell fate. Strikingly, flow cytometry showed that cell cycle was disturbed in both cell lines after depletion of *HOTTIP* (Figure 4A). Cell proportions in G0/G1 phase were significantly higher than control cells when *HOTTIP* was depleted. On the contrary, cell proportions in S phase and G2/M phase were consistently decreased in *HOTTIP*-depleted A549 cells and NCI-H446 cells (Figure 4B). Morphological changes were also revealed by the Hoechst 33342 staining. In the control cells, the nuclei were stained as homogeneously blue, whereas in the sh*HOTTIP* groups, nuclear fragmentation and bright chromatin condensation were observed (Figure 4C). Apoptotic cells were counted. It was shown that numbers of apoptotic cells were significantly increased by depletion of *HOTTIP* in both A549 cells and NCI-H446 cells (Figure 4D). These findings suggest that depletion of *HOTTIP* arrested cell cycle and induced cell apoptosis.

Depletion of *HOTTIP* inhibited tumor growth in vivo

Moreover, we established a xenograft model of lung cancer. Tumor dimensions were monitored periodically. Tumor volume was calculated accordingly. On week 2, we found that *HOTTIP*-depleted group exhibited smaller size of tumor than the control mice. By the fifth week, average tumor size in sh*HOTTIP* group was approximately 200mm^3, whilst it was over 600 mm^3 in the control mice (Figure 5A). All mice were sacrificed on the fifth week. Tumors were dissected and weighed. It was measured that the average tumor weight in sh*HOT*-

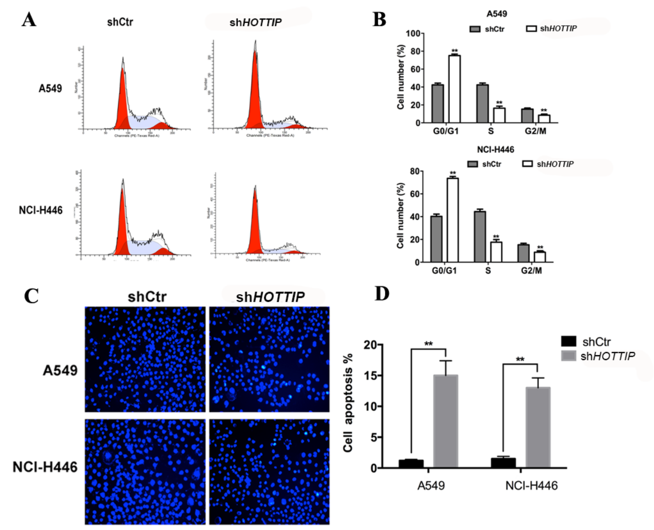

Figure 4. Depletion of HOTTIP arrested cell cycle and induced cell apoptosis. (A) Flow cytometry showed that cell cycle was disturbed in both cell lines after depletion of HOTTIP. (B) Cells accumulated in G0/G1 phase were significantly higher, whereas cells in S phase and G2/M phase were remarkably decreased in HOTTIP-depleted cells. (C) Hoechst 33342 staining showed that depletion of HOTTIP induced significant nuclear fragmentation and chromatin condensation. (D) Apoptotic cells were quantified. It was shown that apoptosis was significantly higher in HOTTIP-depleted A549 cells and NCI-H446 cells. **,p<0.01.

TIP was significantly decreased as compared with control group (Figure 5B). Consistently, tumor sizes in macroscopic view were generally smaller in sh*HOTTIP* group relative to control group (Figure 5C). Moreover, immunohistochemistry staining showed that PCNA, a nuclear marker indicating cell proliferation, was accordingly less stained in *HOTTIP*-depleted group. These data strongly suggest that depletion of *HOTTIP* significantly slowed down tumor growth in the mouse model. In addition, cleaved-caspase-3 which initiates the apoptosis process was strongly stained and up-regulated after depletion of *HOTTIP*, reinforcing the notion that depletion of *HOTTIP* induced cell apoptosis.

Knockdown of HOTTIP interrupted expression of cell cycle regulators and apoptosis-related factors

Furthermore, we analyzed expression of cell cycle regulators and apoptosis-related factors in response to knockdown of *HOTTIP* in A549 cells and NCI-H446 cells. as shown in Figure 6, knockdown of *HOTTIP* decreased the key cell cycle regulators Cyclin B1, Cyclin D1 and Cdc25C. Bcl-2 and Bcl-xL which are representatives of anti-apoptosis were also decreased in response to *HOTTIP* knockdown. However, Bad which represents the pro-apoptotic factor was increased after knockdown of *HOTTIP*. These data were in consistence with above observations that depletion of *HOTTIP* arrested cell cycle and induced apoptotic death in lung cancer cells.

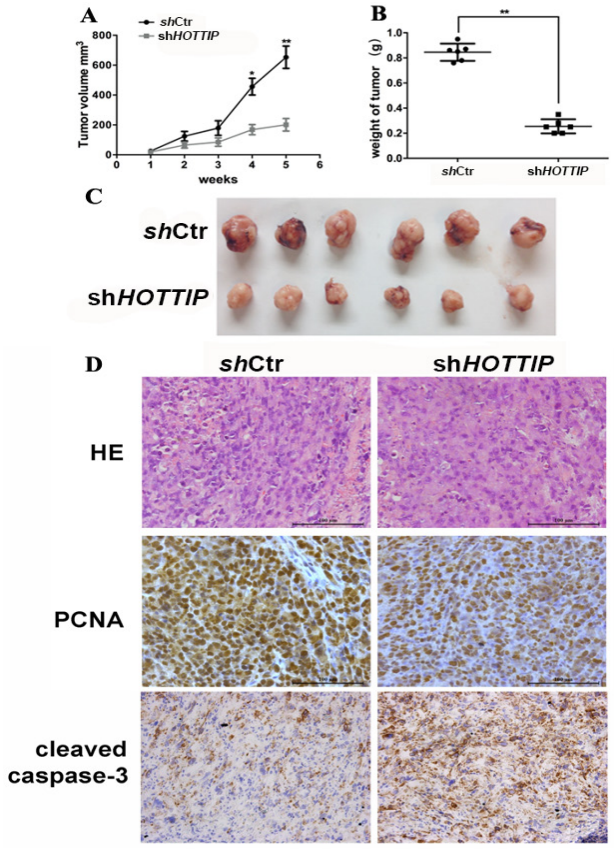

Figure 5. Depletion of HOTTIP inhibited tumor growth in vivo. (A) In the established mouse model, tumor volume was monitored once every one week. Tumor volume in shHOTTIP group was smaller than the control group since 2rd week. By the fifth week, tumor volume in shHOTTIP group was approximately 33% of the control group. (B) Tumors were dissected and weighed on the fifth week. Tumor weights in shHOTTIP group were significantly decreased than the control group. (C) Dissected tumors were displayed together. Macroscopic examination revealed that tumors were less grown in the shHOTTIP group. (D) Histology analysis showed that the proliferating cell nuclear antigen (PCNA) was decreased, whereas apoptosis initiator cleaved-caspase-3 was increased by depletion of HOTTIP.

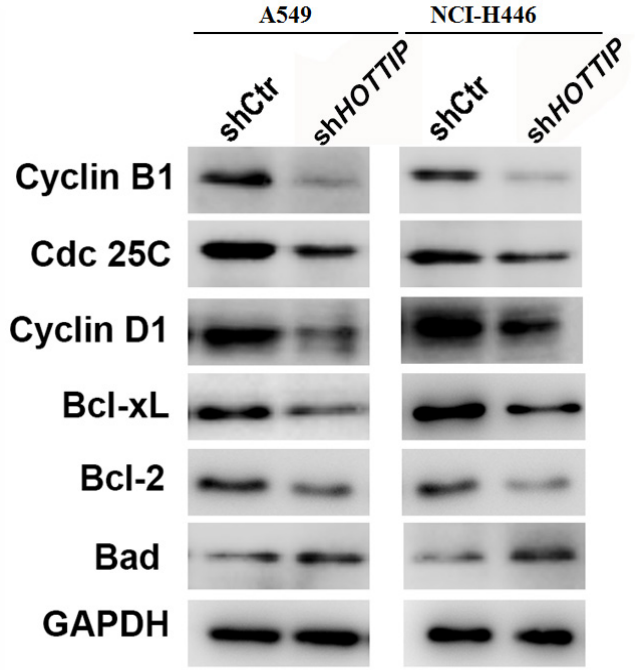

Figure 6. Knockdown of HOTTIP interrupted expression of cell cycle regulators and apoptosis-related factors. After knockdown of HOTTIP, key cell cycle regulators Cycling B1, Cyclin D1 and Cdc25C were decreased. Anti-apoptotic factors Bcl-2 and Bcl-xL were also decreased. However, pro-apoptotic factor Bad was increased after knockdown of HOTTIP in both cell lines.

Discussion

Lung cancer is one of the most prevalent malignancies worldwide, ranking as the first and second leading causes of cancer mortality in males and females, respectively. Lung adenocarcinoma (LAD) is histologically the most common subtype of NSCLC (23). Unfortunately, affected patients have only limited access to early diagnosis and timely interference. Hence, early detection is the best defense against lung cancer and is therefore of vital importance (24).

Currently, lncRNAs have emerged as key regulators of tumor development and progression. The present study proposed that lncRNA *HOTTIP* played critical roles in lung cancer growth in vitro and in vivo. In the collected 20 cases of lung cancer, expression of *HOTTIP* in cancer tissues was consistently higher than that in the adjacent non-cancerous tissues. Knockdown of *HOTTIP* by specific shRNA caused significant inhibition of cell proliferation and colony formation, as well as the tumor growth in a mouse model. While knockdown of *HOTTIP* slowed down tumor growth, it arrested cell cycle at G0/G1 phase, and induced significant cell apoptosis. This was also supported by our immunoblot analysis. Knockdown of *HOTTIP* consistently decreased the expression of cell cycle regulators Cyclin B1, Cyclin D1 and Cdc25C and also suppressed expression of Bcl-2 and Bcl-xL, whereas it increased expression of Bad. Cyclin B1, Cyclin D1 and Cdc25C are critical cell

cycle regulators which promote cell cycle progression (25). Decreased expression of these proteins supported the notion that depletion of *HOTTIP* induced cell cycle arrest. Furthermore, Bad is a pro-apoptotic factor, while Bcl-2 and Bcl-xL are classic anti-apoptotic factors (26). Inhibition of anti-apoptotic factors with the promotion of pro-apoptotic factor Bad strongly suggested that depletion of *HOTTIP* led to the eventual cell apoptosis after inducing the cell cycle arrest. Taken together, our conclusive data suggest that *HOTTIP* is a critical mediator of lung cancer growth in vivo and in vitro. *HOTTIP* could promote cell cycle progression and inhibit the apoptotic death in lung cancer cells.

This is the first report to our knowledge to investigate the role of *HOTTIP* in human lung cancer. Previous studies have widely reported that *HOTAIR*, a HOX-associated lncRNA, serves as a critical regulator which is involved in multiple cancers, including HCC, colorectal cancer and pancreatic cancer (10, 13, 14, 17). Similarly, *HOTTIP* was also reported to be a critical mediator of cancer progression in HCC and pancreatic cancer (19, 20). The significance of our findings would be twofolds: first, our data suggest that *HOTTIP* also plays critical roles in lung cancer growth. These findings not only extend the list of cancers that *HOTTIP* is involved in, but also suggest the oncogenic activities of *HOTTIP* in various human tumors; Second, *HOTTIP* is also a HOX-associated lncRNA as *HOTAIR*. Our findings may suggest that *HOTTIP* and *HOTAIR* have the similar pro-oncogenic functions in human tumors since they derive from the same subtype of lncRNAs.

One interesting question would be the detailed mechanisms of how *HOTTIP* regulates lung cancer progression. Current studies have showed that *HOTTIP* is closely associated with HOXA13. The levels of *HOTTIP* and HOXA13 were both up-regulated and associated with HCC patients' clinical progression and predicted disease outcome in HCC. However, a recent study by beadchip array analysis argued that *HOTTIP* did not regulate HOXA13 in pancreatic cancer cells, but played a role in regulating several other HOX genes including HOXA1, HOXA9, HOXA10, HOXA11 and HOXB2 (21). Hence, current evidences are still controversial. One hypothesis would be that *HOTTIP* may exhibit distinct mechanisms in pancreatic cancer and liver cancer. However, another report on pancreatic ductal adenocarcinoma showed that knockdown of *HOTTIP* decreased expression of HOXA13, and HOXA13 partially mediated the effect of *HOTTIP* (20), leading us to speculate that *HOTTIP* may regulate strikingly different sets of genes in distinct types of cancers and even at different stages. However, more work needs to be done.

In all, we studied the roles of lncRNA *HOTTIP* in lung cancer growth in vitro and in vivo. Depletion of *HOTTIP* significantly slowed down tumor growth and induced cell apoptosis in lung cancer. *HOTTIP* promotes lung cancer growth possibly by inhibition of cell apoptotic death. The detailed mechanisms remain to be elucidated. Our findings may serve as strong evidence for development of novel therapeutic strategies against lung cancer.

Acknowledgments

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