# HOTTIP and HOXA13 are oncogenes associated with gastric cancer progression

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**Abstract.** A long non-coding RNA named HOTTIP (HOXA transcript at the distal tip) coordinates the activation of various 5' *HOXA* genes which encode master regulators of development through targeting the WDR5/MLL complex. HOTTIP acts as an oncogene in several types of cancers, whereas its biological function in gastric cancer has never been studied. In the present study, we investigated the role of HOTTIP in gastric cancer. We found that HOTTIP was upregulated in gastric cancer cell lines. Knockdown of HOTTIP in gastric cancer cells inhibited cell proliferation, migration and invasion. Moreover, downregulation of HOTTIP led to decreased expression of homeobox protein Hox-A13 (HOXA13) in gastric cancer cell lines. HOXA13 was involved in HOTTIP-induced malignant phenotypes of gastric cancer cells. Our data showed that the levels of HOTTIP and HOXA13 were both markedly upregulated in gastric cancer tissues compared with their counterparts in non-tumorous tissues. Furthermore, the expression levels of HOTTIP and HOXA13 were both higher in gastric cancer which was poorly differentiated, at advanced TNM stages and exhibited lymph node-metastasis. Spearman analyses indicated that HOTTIP and HOXA13 had a highly positive correlation both in non-tumor mucosae and cancer lesions. Collectively, these findings suggest that HOTTIP and HOXA13 play important roles in gastric cancer progression and provide a new insight into therapeutic treatment for the disease.

## Introduction

Gastric cancer is the second leading cause of cancer-related death worldwide (1). For most patients, gastric cancer is only diagnosed at the advanced stages with poor prognosis (2,3). Gastric carcinogenesis is known as a multistep process involving a series of epigenetic and genetic alterations (4-11). The mechanism of gastric carcinogenesis has not yet been fully elucidated and more studies are needed to search for novel molecules which are involved in the process.

Long non-coding RNAs (lncRNAs) are RNA transcripts which are >200 bp in length and do not encode for a protein (12,13). Studies suggest that lncRNAs constitute an important component of tumor biology (14-18). Most lncRNAs play a functional role in gene expression by targeting either genomically local or distant genes (19-21). Evidence suggests that lncRNAs play essential roles in tumorigenesis (14,22-24) and cancer progression (15,25-27) by acting as either oncogenes or tumor suppressors.

The regulation of *HOX* genes by lncRNAs is gaining great interest in developmental biology research. *HOX* genes are highly conserved at the genomic level. The proteins which *HOX* genes encode are master regulators of embryonic development and continue to be expressed throughout adulthood in various tissues. HOXA transcript at the distal tip (HOTTIP) is at the 5' end of the *HOXA* cluster and upregulates the expression of 5' *HOXA* genes by binding the adaptor protein WDR5 and targeting the WDR5/MLL complex (28). Evidence suggests that HOTTIP and homeobox protein Hox-A13 (HOXA13) are both upregulated and associated with progression and poor survival of hepatocellular carcinoma (29). Moreover, the expression of HOTTIP and HOXA13 showed a high correlation in hepatocellular carcinoma (29). The role of HOTTIP has also been investigated in pancreatic (30,31) and lung cancer, and tongue squamous cell carcinoma (32,33). For example, HOTTIP promoted disease progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer (30). In addition, HOTTIP promoted tumor growth and inhibited cell apoptosis in lung cancer (32). In addition, HOTTIP was found to be highly expressed and correlated with the progression of tongue squamous cell carcinoma (33). However, the role of HOTTIP in gastric cancer has never been reported.

In the present study, we investigated the expression of HOTTIP in gastric tissues and the function of HOTTIP in gastric cancer cells, with the aim of elucidating the mechanisms of gastric carcinogenesis and progression.

## Materials and methods

*Cell culture.* Human immortal gastric epithelial cell line GES-1 and human gastric cancer cell lines SGC7901, MKN28,

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*Key words:* gastric cancer, HOTTIP, HOXA13, lncRNA, oncogene

Table I. The sequences of siRNAs used in the present study.

| siRNAs | Sense (5'-3') | Antisense (5'-3') |
|---|---|---|
| NC | UUCUCCGAACGUGUCACGUTT | ACGUGACACGUUCGGAGAATT |
| siHOTTIP #1 | GCUUUAGAGCCACAUACUUTT | AAGUAUGUGGCUCUAAAGCTT |
| siHOTTIP #2 | GAGACAGAGUAGGGUUCUATT | UAGAACCCUACUCUGUCUCTT |
| siHOTTIP #3 | GGCACUUUAUAUGCUGUAATT | UUACAGCAUAUAAAGUGCCTT |
| siHOXA13 #1 | GCCACGAAUAAAUUCAUUATT | UAAUGAAUUUAUUCGUGGCTT |
| siHOXA13 #2 | GCGGACAAGUACAUGGAUATT | UAUCCAUGUACUUGUCCGCTT |
| siHOXA13 #3 | GACGAGCUCAACAAGAACATT | UGUUCUUGUUGAGCUCGUCTT |

Table II. qRT-PCR primers used in the present study.

| Genes | Forward primer (5'-3') | Reverse primer (5'-3') |
|---|---|---|
| GAPDH | GACTCATGACCACAGTCCATGC | AGAGGCAGGGATGATGTTCTG |
| HOTTIP | CCTAAAGCCACGCTTCTTTG | TGCAGGCTGGAGATCCTACT |
| HOXA13 | TGGAACGGCCAAATGTACTG | TGGCGTATTCCCGTTCAAGT |
| HOXA11 | GTACTTACTACGTCTCGGGTCCAG | AGTCTCTGTGCACGAGCTCCT |
| HOXA10 | GGGGACTTCTCTTCCAGTTTC | GGGAGAATTGTGGTGTGCTT |
| HOXA9 | CCACGCTTGACACTCACACT | AGTTGGCTGCTGGGTTATTG |

MKN45 and MGC803 were obtained from the Cell Resource Center, Shanghai Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (both from Gibco, Carlsbad, CA, USA) at 37˚C in a humidified incubator containing 5% carbon dioxide.

*Small interfering RNA (siRNA) transfection*. The siRNA oligonucleotides targeting HOTTIP, HOXA13 and the negative control were obtained from GenePharma Co., Ltd. (Shanghai, China). Transfection of the oligonucleotides was conducted with X-tremeGENE siRNA transfection reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturer's instructions. The sequences of siRNAs used in the present study are listed in Table I.

*RNA extraction and quantitative real-time PCR*. Total RNA was extracted from cells or tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA). Quantitative real-time PCR was performed using a SYBR Premix Ex *Taq*™ II (Takara Biotechnology Co., Ltd., Dalian, China) on a Bio-Rad CFX-96 Real-Time PCR system. GAPDH was used as an internal control. The sequences of the primers are listed in Table II. All qRT-PCR reactions were performed in triplicate.

*Cell proliferation and colony formation assays*. Cell proliferation was measured by the Cell Counting Kit-8 (CCK-8) assay (7Sea Biotech Co., Ltd., Shanghai, China). Cells transfected with siRNA were seeded and cultured into 96-well plates ($3x10^3$ cells/well) in 100 $\mu$l medium. At different time points indicated in the figures, 10 $\mu$l CCK-8 solution was added into the medium and further incubated with the cells for 3 h. The optical density (OD) was measured using a microplate reader at 450 nm. The CCK-8 assays were performed in triplicate.

For colony formation assay, cells transfected with different siRNAs were seeded into 6-well plates at 300 cells/well. After 14 days of incubation, cells were fixed with methyl alcohol and stained with 0.5% crystal violet. The number of colonies (≥50 cells/colony) was counted. Each experiment was performed in triplicate.

*Cell migration and invasion assays*. For migration assays, $5x10^4$ cells were plated in the top chamber with a non-coated membrane (24-well insert; pore size, 8-$\mu$m; Corning, Corning, NY, USA). For invasion assays, $1.5x10^5$ cells were plated in the top chamber with a Matrigel-coated membrane (24-well insert; pore size, 8-$\mu$m; Corning). Medium without serum was used in the top chamber in both assays. Medium with 10% FBS was added to the lower chamber. After incubation for 24 h (migration assay) or 48 h (invasion assay), respectively, the cells that did not migrate or invade through the pores were removed using a cotton swab. Cells on the lower surface of the membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The number of migrated or invaded cells was counted. Each experiment was performed in triplicate.

*Western blot analysis*. Cells were lysed with RIPA buffer containing complete protease inhibitor mixture (Roche Molecular Biochemicals). Proteins were separated by dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and

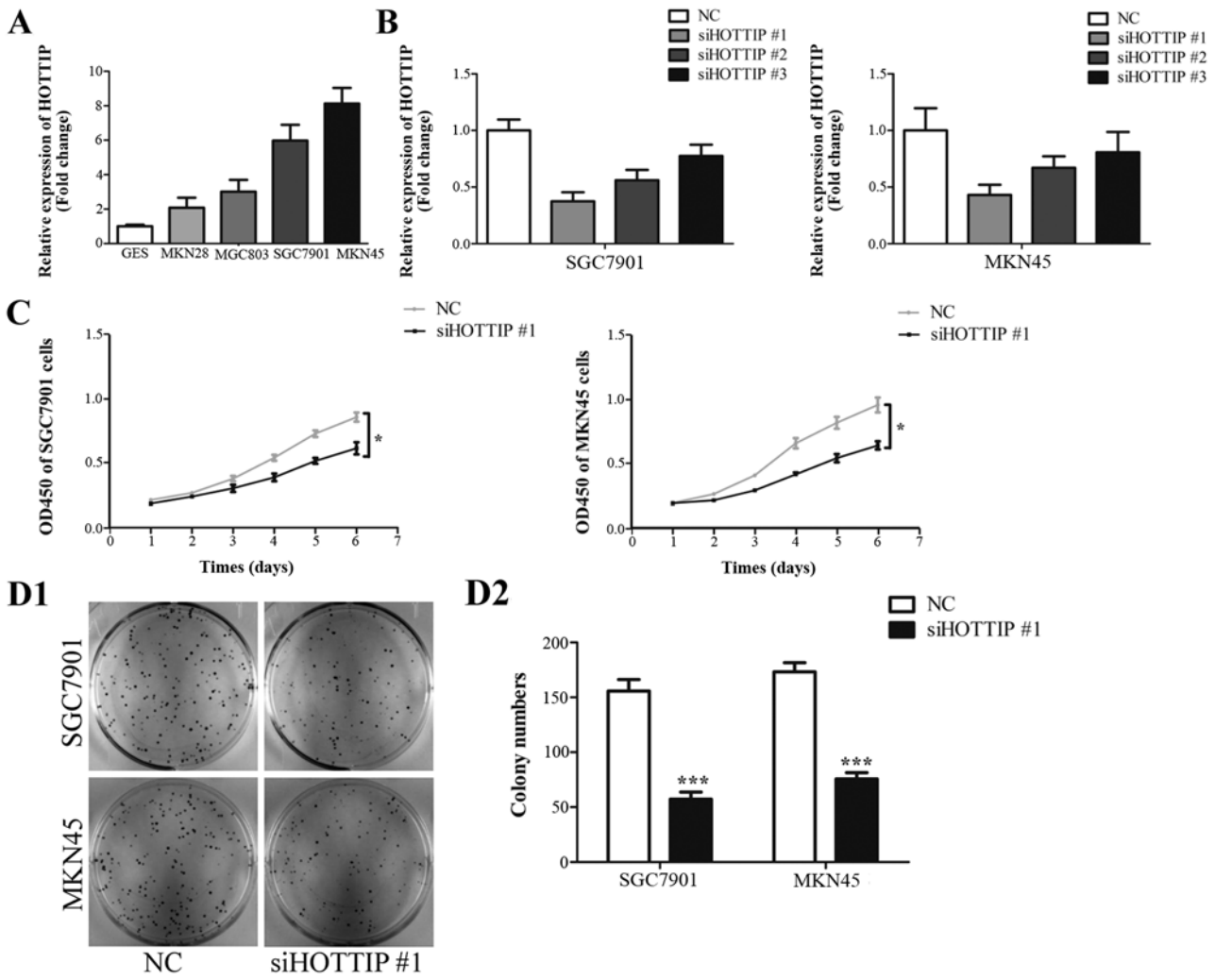


Figure 1. HOTTIP is upregulated in gastric cancer cells and downregulation of HOTTIP inhibits cell growth in SGC7901 and MKN45 cells. (A) Expression of HOTTIP was investigated in the GES-1, MKN28, MGC803, SGC7901 and MKN45 cell lines by qRT-PCR. (B) Efficiency of HOTTIP knockdown was investigated by qRT-PCR 48 h after siRNA treatment in the SGC7901 and MKN45 cells. (C) HOTTIP knockdown significantly inhibited cell proliferation in the SGC7901 and MKN45 cells. (D1 and D2) The effect of HOTTIP knockdown on cell growth was further investigated by colony formation assay. A representative experiment (D1) and quantitative analysis (D2) are shown. $^{*}$P<0.05; $^{**}$P<0.01; $^{***}$P<0.001.

transferred to nitrocellulose membranes (Pall Life Sciences, Ann Arbor, MI, USA). The membranes were blocked in 5% non-fat milk and blotted with antibodies against GAPDH (1:2,000) and HOXA13 (1:200) (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), respectively. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies and visualized with an enhanced chemiluminescence reagent.

*Tissue samples*. A total of 50 paired gastric tissue samples (cancer lesions and adjacent non-tumor mucosae) of gastric cancer patients were obtained from the Department of General Surgery, The First Affiliated Hospital of Xi'an Jiaotong University between June 2013 and February 2014. All patients did not receive chemotherapy or radiotherapy prior to surgery. All samples were collected in the same manner. The samples were immediately frozen in liquid nitrogen and stored at -80˚C until they were used. Informed consent was obtained from each patient before the surgery. The present study was approved by the Research Ethics Committee of Xi'an Jiaotong University.

*Statistical analysis*. Statistical analysis was performed using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA). Student's t-test for parametric variables was used. Spearman test was used to establish the correlation between HOTTIP and HOXA13. Data are presented as mean ± SEM unless otherwise indicated. All P-values were determined from two-sided tests, and statistical significance was determined based on a P-value of 0.05.

## Results

*HOTTIP is upregulated in gastric cancer cells and downregulation of HOTTIP inhibits cancer cell growth*. To determine the role of HOTTIP in gastric cancer, we first investigated the expression of HOTTIP in the GES-1, MKN28, MGC803, SGC7901 and MKN45 cell lines. In addition, we found that HOTTIP was upregulated in gastric cancer cell lines compared with that noted in the GES-1 cells (Fig. 1A). Then, we investigated the effect of HOTTIP on cell growth by downregulating HOTTIP expression in the SGC7901 and MKN45

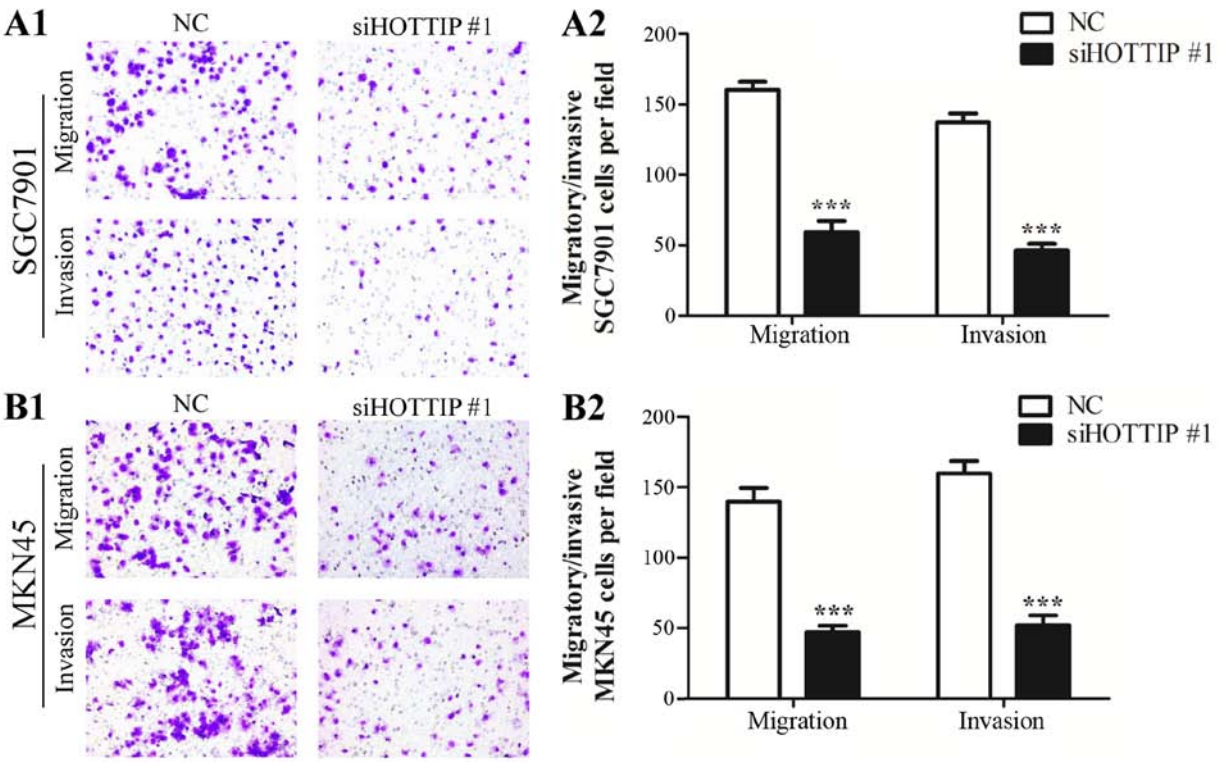


Figure 2. Inhibition of cell migration and invasion by HOTTIP knockdown in gastric cancer cells. (A1 and A2) The effect of HOTTIP knockdown on SGC7901 cells using Transwell migration and invasion assays. (B1 and B2) The effect of HOTTIP knockdown on MKN45 cells using Transwell migration and invasion assays; $^{***}P<0.001$.

cells. Efficiency of HOTTIP knockdown in the SGC7901 and MKN45 cells by three specific siRNAs was confirmed by qRT-PCR and siHOTTIP #1 was used in the following experiments (Fig. 1B). Knockdown of HOTTIP inhibited cell proliferation in the SGC7901 and MKN45 cells (Fig. 1C). The inhibition of cell growth by HOTTIP knockdown was further confirmed by colony formation assay. Downregulation of HOTTIP decreased colony numbers in the SGC7901 and MKN45 cells (Fig. 1D1 and D2). These results suggest that HOTTIP plays a growth-promoting role in gastric cancer cells.

*Downregulation of HOTTIP inhibits cell migration and invasion in gastric cancer.* We next investigated the effect of HOTTIP on the migration and invasion of SGC7901 and MKN45 cells. Downregulation of HOTTIP led to a 2- to 3-fold reduction in the migratory and invasive capabilities of the SGC7901 cells (Fig. 2A1 and A2). Similar results were observed in the MKN45 cells with decreased expression of HOTTIP (Fig. 2B1 and B2). These results suggest that HOTTIP promotes both migration and invasion of gastric cancer cells.

*Downregulation of HOTTIP leads to decreased HOXA13 expression in gastric cancer cells.* HOTTIP knockdown was previously found to lead to a reduction in *HOXA* gene expression in primary human fibroblasts (28), hepatocellular carcinoma (29) and pancreatic cancer cells (30,31). To ascertain whether HOTTIP exhibits a similar function in gastric cancer cells, we measured the expression of several *HOXA* genes (HOXA13, HOXA11, HOXA10 and HOXA9) in the SGC7901 cells treated with siHOTTIP #1. Downregulation of HOTTIP led to different degrees of decrease in the expression levels of these genes, among which HOXA13 expression was decreased the most (Fig. 3A). Downregulation of HOXA13 expression was further confirmed in MKN45 cells by qRT-PCR (Fig. 3A). Knockdown of HOTTIP inhibited the HOXA13 protein level in the SGC7901 and MKN45 cells (Fig. 3B). These results suggest that HOTTIP regulates HOXA13 expression in gastric cancer cells.

*HOXA13 is involved in HOTTIP-induced malignant phenotypes of gastric cancer cells.* We investigated the expression of HOXA13 in the GES-1 and gastric cancer cell lines. HOXA13 was upregulated in the gastric cancer cell lines compared with GES-1, which was similar to the HOTTIP expression pattern (Fig. 4A). To investigate the role of HOXA13 in gastric cancer, three specific siRNAs against HOXA13 were used to inhibit HOXA13 mRNA expression in the SGC7901 and MKN45 cells. siHOXA13 #2 showed most significant knockdown efficiency and was used in the following experiments (Fig. 4B). siHOXA13 #2 led to a clear reduction in the protein level of HOXA13 (Fig. 4C).

Knockdown of HOXA13 also inhibited cell growth (Fig. 5A and B), migration and invasion (Fig. 5C) in the SGC7901 and MKN45 cells, which resembled the inhibitory effects of HOTTIP knockdown. These results indicate that HOXA13 was involved in HOTTIP-induced malignant phenotypes of gastric cancer cells.

*HOTTIP and HOXA13 are both upregulated in gastric cancer.* To further understand the relationship between HOTTIP and HOXA13 in gastric cancer, we investigated HOTTIP and

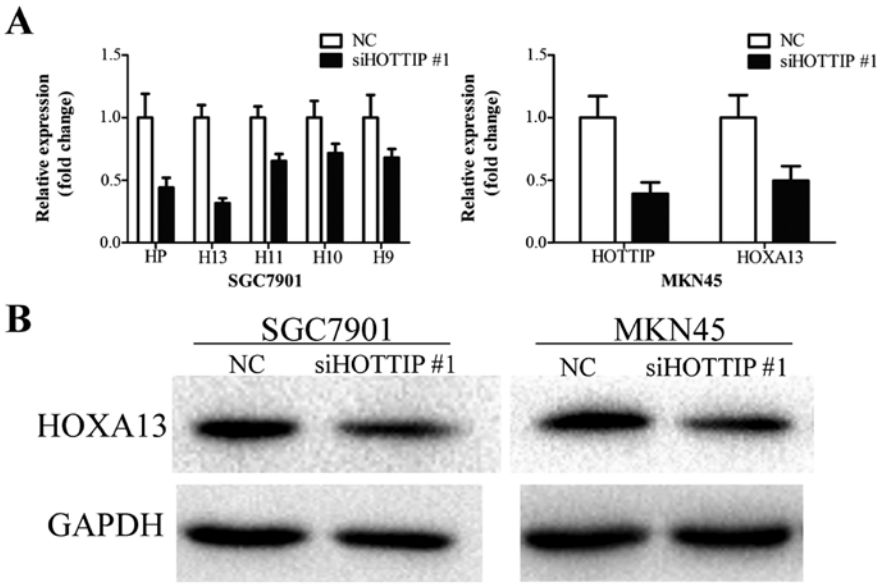


Figure 3. Inhibition of HOXA13 expression by HOTTIP knockdown in gastric cancer cells. (A) Knockdown of HOTTIP inhibited *HOXA* gene expression in the SGC7901 cells, and inhibition of HOXA13 was further confirmed in the MKN45 cells by qRT-PCR. HP, HOTTIP; H13, HOXA13; H11, HOXA11; H10, HOXA10; and H9, HOXA9. (B) Knockdown of HOTTIP inhibited HOXA13 protein levels in the SGC7901 and MKN45 cells.

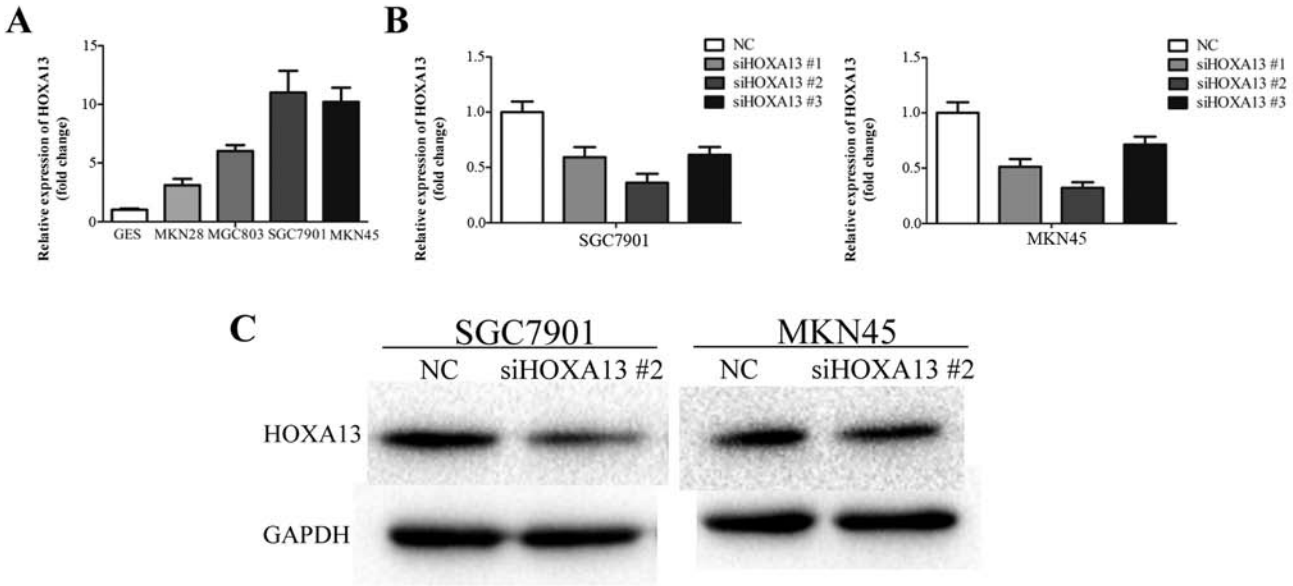


Figure 4. Overexpression of HOXA13 in gastric cancer cell lines and efficiency of HOXA13 knockdown in the SGC7901 and MKN45 cells. (A) Expression of HOXA13 mRNA in the GES and gastric cancer cell lines was investigated. (B) Efficiency of HOXA13 knockdown was investigated by qRT-PCR 48 h after siRNA treatment in the SGC7901 and MKN45 cells. (C) HOXA13 protein expression was inhibited by siHOXA13 #2 in the SGC7901 and MKN45 cells.

HOXA13 expression levels in 50 pairs of primary gastric cancer tissues and their counterpart non-tumorous tissues by qRT-PCR. The results showed that HOTTIP and HOXA13 were both markedly upregulated in the gastric cancer tissues when compared with these levels in the non-tumorous tissues (Fig. 6A and B), which were consistent with the expression patterns of HOTTIP and HOXA13 in the gastric cancer cells. Correlations between the HOTTIP or HOXA13 expression levels and clinicopathologic characteristics of gastric cancer are summarized in Tables III or IV, respectively. The data revealed that expression levels of HOTTIP and HOXA13 were both higher in gastric cancer which was poorly differentiated ($P<0.05$), at advanced TNM stages ($P<0.05$) and showed lymph node metastasis ($P<0.01$). Spearman analyses indicated that HOTTIP and HOXA13 had a positive correlation both in non-tumor mucosae (Fig. 6C) and cancer lesions (Fig. 6D). These results suggest that HOTTIP and HOXA13 are highly correlated and associated with gastric cancer progression.

## Discussion

In the present study, we found that both HOTTIP and HOXA13 were upregulated in gastric cancer tissues compared with levels in their counterpart non-tumorous tissues. In

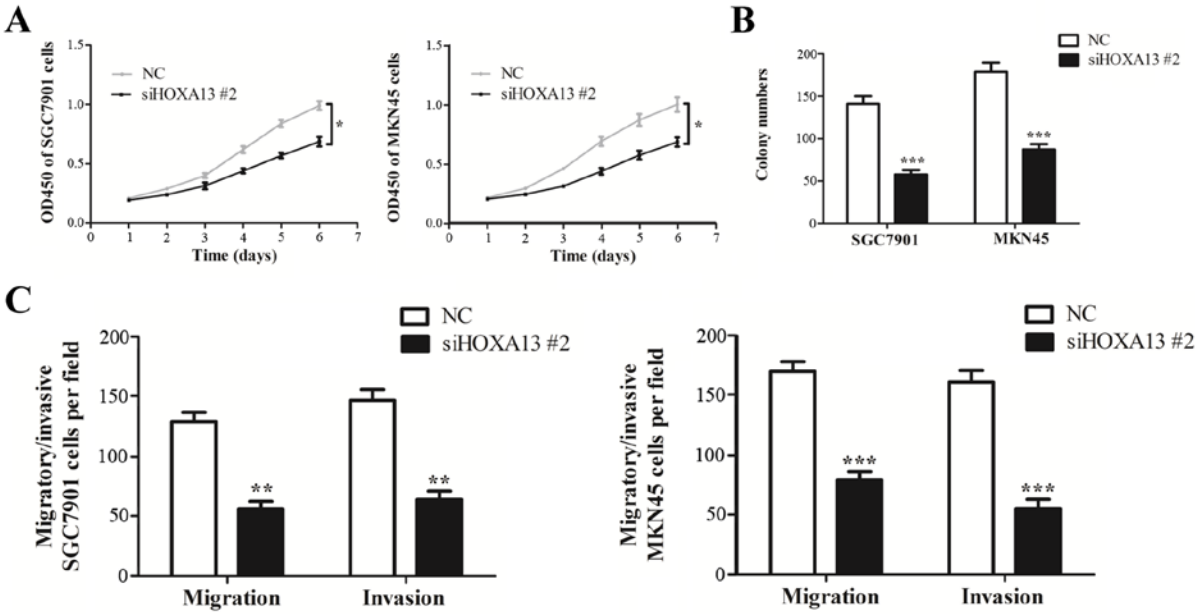


Figure 5. Inhibition of cell growth, migration and invasion by HOXA13 knockdown in gastric cancer cells. (A) HOXA13 knockdown significantly inhibited cell proliferation in the SGC7901 and MKN45 cells. (B) The effect of HOXA13 knockdown on cell growth was further investigated by colony formation assay. (C) The effect of HOXA13 knockdown on SGC7901 and MKN45 cells as assessed by Transwell migration and invasion assays. $^{*}$P<0.05; $^{**}$P<0.01; $^{***}$P<0.001.

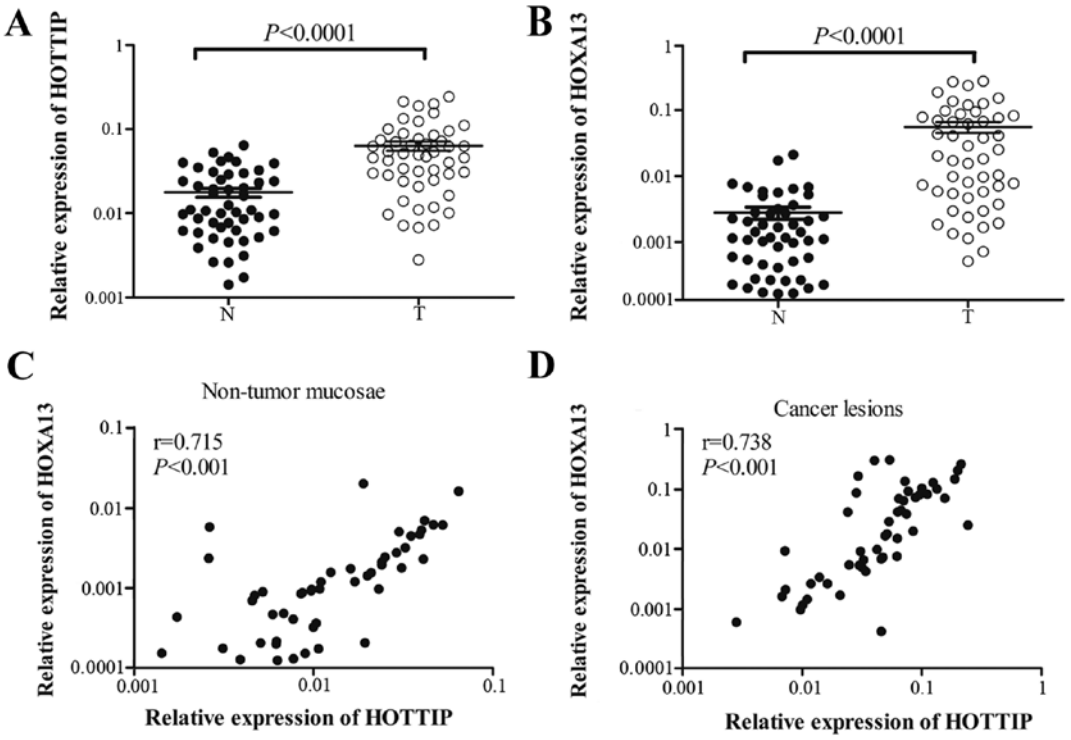


Figure 6. Comparison of HOTTIP and HOXA13 expression in tissue samples. (A and B) Expression levels of HOTTIP (A) and HOXA13 (B) were investigated by qRT-PCR in 50 paired human gastric cancer samples (T) and their counterpart non-tumorous samples (N). The expression levels of HOTTIP and HOXA13 were normalized to GAPDH using the $2^{-\Delta Ct}$ ($-\Delta Ct = Ct_{GAPDH} - Ct_{Gene}$) method. The y-axis ($log_{10}$ scale) was used to describe the relative levels of HOTTIP and HOXA13. (C and D) Correlation scatterplot of HOTTIP and HOXA13 expression in non-tumor mucosae (C) and cancer lesions (D).

addition, the expression levels of HOTTIP and HOXA13 were associated with poor differentiation, advanced TNM stages and lymph node metastasis. Moreover, HOTTIP and HOXA13 were highly correlated both in non-tumor mucosae and cancer lesions. Downregulation of HOTTIP inhibited cell growth and invasion. In addition, HOXA13 was involved in HOTTIP-induced malignant phenotypes of gastric cancer cells.

lncRNAs associated with human *HOX* gene loci have been widely studied in recent years (21,28,34-36). By character-

Table III. Relationship between HOTTIP expression and clinicopathological parameters in the primary gastric cancer cases.

| Variable | No. of cases | % | Relative expression of HOTTIP | P-value |
|---|---|---|---|---|
| Age (years) | | | | 0.226 |
| ≥60 | 29 | 58 | 0.0716±0.0116 | |
| <60 | 21 | 42 | 0.0518±0.00995 | |
| Gender | | | | 0.384 |
| Male | 38 | 76 | 0.0672±0.0103 | |
| Female | 12 | 24 | 0.0508±0.00652 | |
| Tumor size (cm) | | | | 0.362 |
| ≥5 | 28 | 56 | 0.0698±0.0111 | |
| <5 | 22 | 44 | 0.0550±0.0115 | |
| Degree of differentiation | | | | 0.0250 |
| Well/moderate | 27 | 54 | 0.0469±0.00888 | |
| Poor | 23 | 46 | 0.0825±0.0130 | |
| TNM stage | | | | 0.00240 |
| I/II | 15 | 30 | 0.0274±0.00230 | |
| III/IV | 35 | 70 | 0.0787±0.0104 | |
| Lymph node status | | | | 0.00950 |
| Metastasis | 38 | 76 | 0.0747±0.00971 | |
| No metastasis | 12 | 24 | 0.0272±0.00473 | |

Table IV. Relationship between HOXA13 expression and clinicopathological parameters in the primary gastric cancer cases.

| Variable | No. of cases | % | Relative expression of HOXA13 | P-value |
|---|---|---|---|---|
| Age (years) | | | | 0.520 |
| ≥60 | 29 | 58 | 0.0618±0.0152 | |
| <60 | 21 | 42 | 0.0476±0.0149 | |
| Gender | | | | 0.615 |
| Male | 38 | 76 | 0.0589±0.0131 | |
| Female | 12 | 24 | 0.0461±0.0175 | |
| Tumor size (cm) | | | | 0.368 |
| ≥5 | 28 | 56 | 0.0645±0.0169 | |
| <5 | 22 | 44 | 0.0448±0.0116 | |
| Degree of differentiation | | | | 0.0178 |
| Well/moderate | 27 | 54 | 0.0327±0.00832 | |
| Poor | 23 | 46 | 0.0831±0.0199 | |
| TNM stage | | | | 0.0192 |
| I/II | 15 | 30 | 0.0179±0.00674 | |
| III/IV | 35 | 70 | 0.0721±0.0143 | |
| Lymph node status | | | | 0.00550 |
| Metastasis | 38 | 76 | 0.0722±0.0131 | |
| No metastasis | 12 | 24 | 0.00409±0.000987 | |

izing the transcriptional landscape of the four human *HOX* loci, researchers have identified 231 *HOX* lncRNAs (21). HOTAIR, which was first described in fibroblasts, was found to be located in the *HOXC* cluster but regulated *HOXD* cluster genes (21). HOTAIR was also found to serve as a scaffold protein by binding polycomb repressive complex 2 (PRC) with its 5' domain and the LSD1/CoREST/REST complex with the 3' domain (37). Unlike HOTAIR, HOTTIP enhanced expression of neighboring *HOXA* genes particularly HOXA13 (28). Considering the vital role of *HOX* genes in development and differentiation and their dysregulation-caused tumorigenesis and tumor progression (38-42), it is important to understand the mechanism of HOTTIP in the regulation of *HOX* gene expression.

Upregulation of HOTTIP and HOXA13 has been reported in various studies. HOTTIP and HOXA13 were both upregulated and highly correlated in hepatocellular carcinoma (29) and pancreatic cancer (31,32). A previous study demonstrated that HOTTIP was upregulated not only in hepatocellular carcinoma tissues, but also in preneoplastic diseases. However, the HOXA13 expression level was specifically increased in hepatocellular carcinoma, indicating that upregulation of HOTTIP preceded that of HOXA13 in hepatocellular carcinogenesis during disease onset (29). HOTTIP was also found to be upregulated in lung cancer (32) and tongue squamous cell carcinoma (33), and involved in the tumor progression in pancreatic cancer (30). The expression level of HOXA13 was also increased and associated with tumor progression in hepatocellular carcinoma (29), pancreatic cancer (30), esophageal squamous cell carcinoma (43) and glioblastoma multiforme (44). A recent study found that HOXA13 expression was higher in cancerous tissues compared with that in their neighboring non-cancerous tissues. Moreover, a higher expression level of HOXA13 was significantly correlated with T and M stages, advanced UICC stage and histological differentiation in gastric cancer based on immunohistochemistry findings (45). In the present study, we also found that HOTTIP and HOXA13 were upregulated in gastric cancer tissues compared with level in their non-tumorous tissues. In addition, the increase in the expression level of these two genes was correlated with cancer tissue poor differentiation, advanced TNM stages and lymph node metastasis. HOTTIP and HOXA13 were positively associated in both non-tumor mucosae and cancer lesions. Our findings suggest that HOTTIP and HOXA13 are likely involved in the tumorigenesis and progression of gastric cancer.

Although we identified upregulation of HOTTIP and HOXA13 in gastric cancer, the roles of HOTTIP and HOXA13 in gastric cancer have never been fully understood. Downregulation of HOTTIP and HOXA13 has been reported to inhibit cell proliferation in liver cancer-derived cells (29). HOTTIP and HOXA13 promoted cell proliferation, migration and invasion in pancreatic cancer (30,31). Moreover, HOTTIP regulated the expression of HOXA13 in hepatocellular carcinoma (29) and pancreatic cancer (30). However, Cheng *et al* showed that HOTTIP regulated *HOX* genes

including HOXA10, HOXA11, HOXA9 and HOXA1, but not HOXA13 (31). HOXA13 was found to promote cell growth of esophageal squamous cancer cells *in vitro* and *in vivo* (43). HOXA13 also promoted cell invasion *in vitro* and tumor growth *in vivo* in glioblastoma multiforme (44). In the present study, we firstly identified that HOTTIP and HOXA13 both promoted cell growth and invasiveness in gastric cancer cells. In addition, downregulation of HOTTIP led to decreased HOXA13 expression in gastric cancer cells. The roles of HOTTIP and HOXA13 in gastric cancer cells *in vivo* warrant further investigation. Taken together, these data indicate that HOTTIP functions as an oncogene by regulating HOXA13 expression in gastric cancer.

In conclusion, our results showed that HOTTIP and HOXA13 were upregulated and associated with poor differentiation, advanced TNM stages and lymph node metastasis in gastric cancer. HOTTIP and HOXA13 were highly correlated in both non-tumor mucosae and cancer lesions. Downregulation of HOTTIP inhibited gastric cancer cell growth and invasiveness through the regulation of HOXA13. These results suggest that the molecular axis of HOTTIP and HOXA13 contributes to gastric cancer progression. Our finding provides a potential novel therapeutic target for gastric cancer treatment.

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