

lncRNA HIT000218960 enhances resistance to 5-fluorouracil by promoting HMGA2 and activating the AKT/mTOR/P70S6K pathway in gastric cancer cells

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Abstract. The aim of the present study was to investigate the effect of the long noncoding RNA HIT000218960 on gastric cancer cell resistance to 5-fluorouracil (5-FU) and to explore the underlying molecular mechanism. HIT000218960 expression was measured in gastric cancer tissues and cells lines using reverse transcription-quantitative PCR and western blotting. Gastric cancer cell lines with overexpressed or repressed HIT000218960 levels were generated to study its influence on apoptosis induced by 5-FU, which was analyzed using flow cytometry analysis. Compared with those in normal gastric mucosal tissues and non-cancerous gastric mucosal epithelial cells, HIT000218960 and high mobility group A2 (HMGA2) proteins were found to be upregulated in gastric cancer tissues and cells. Additionally, a positive correlation was found between the expression of HIT000218960 and HMGA2 in gastric cancer tissues. In patients with gastric cancer, HIT000218960 expression was revealed to associate negatively with the efficacy of chemotherapy and 3-year overall survival rate. Overexpression of HIT000218960 suppressed apoptosis in SNU-5 cells, whilst HIT000218960 knockdown increased the apoptosis of NCI-N87 cells following 5-FU treatment. Downstream, HIT000218960 was demonstrated to promote HMGA2 protein expression in gastric cancer cells. In these cells, knocking down HMGA2 expression significantly increased apoptosis in addition to reducing AKT, mTOR and P70S6 kinase (P70S6K) phosphorylation after 5-FU treatment. In conclusion, HIT000218960 is overexpressed in gastric

cancer tissues and cells, which is associated with the efficacy of chemotherapy. Mechanistically, this may be mediated by the upregulation HMGA2 expression and AKT/mTOR/P70S6K signaling.

Introduction

Gastric cancer is a common malignancy of the digestive system that has a high incidence rate in East Asia (1,2). In China, the incidence of gastric cancer ranks as the 2nd highest among all malignant cancers (3). Chemotherapy is a common strategy used for the treatment of gastric cancer (4). However, whilst chemotherapy can prolong disease-free survival and overall survival for certain patients with this disease, several limitations exist in terms of the efficacy of this therapy (5). Over time, tumor cells become resistant to therapies initially used for the treatment of the disease and to drugs with different chemical structures and mechanisms of action (6,7). Both primary and secondary resistance markedly limits the efficacy of chemotherapy in patients with gastric cancer (6,7).

5-fluorouracil (5-FU) is the most commonly applied chemotherapeutic agent for patients with gastric cancer that is frequently used in combination with cisplatin, oxaliplatin and calcium folinate as an adjuvant treatment (8,9). Unfortunately, numerous patients with gastric cancer become insensitive to 5-FU over time, which eventually diminishes the efficacy of chemotherapy (10). Therefore, studying gastric cancer cell resistance to 5-FU may aid in predicting the effectiveness of chemotherapy in patients. HIT000218960 is a recently discovered long noncoding RNA (lncRNA) that is highly expressed in gastric (11) and papillary thyroid cancer (12). Furthermore, the Sun *et al* (11) recently demonstrated that HIT000218960 promotes gastric cancer cell proliferation and migration through upregulation of the high mobility group A2 (HMGA2) protein.

The association between HIT000218960 and the effect of chemotherapy on gastric cancer remains poorly understood. Therefore, the present study analyzed the association between HIT000218960 expression and the efficacy of 5-FU chemotherapy in patients with gastric cancer. In addition, the molecular mechanism of the effects of HIT000218960 on gastric cancer responses to 5-FU was also explored *in vitro*.

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Materials and methods

Patients. A total of 37 normal gastric mucosal tissues from gastroscopy physical examination (17 females and 20 males; 38-69 years old; 60.3 ± 5.8 years old; between January 2015 and June 2016 in The 970th Hospital of the PLA Joint Logistics Support Force, Yantai, China) and 71 gastric cancer tissues from the surgical removal of tumor, extracted during biopsy were used in the present study. Patient data for the 71 gastric cancer cases (43 females and 28 males; 32-73 years old; 62.3 ± 5.8 years old; between January 2015 and June 2016 in The 970th Hospital of the PLA Joint Logistics Support Force) are provided in Table I. TNM classification for gastric cancer was based on 'Seventh edition of TNM classification for gastric cancer' (13).

The inclusion criteria for patients with gastric cancer included the following: i) Patients were pathologically diagnosed with only gastric cancer; ii) Patients were aware of the contents of this study; and iii) ≥ 18 years old. The inclusion criteria for volunteers for normal mucosal tissue included the following: i) Volunteers were determined to be free of any malignant tumors and were healthy; ii) volunteers were aware of the contents of this study; and iii) ≥ 18 years old.

Gastric patients exclusion criteria included the following: i) Diagnosis with another malignant tumor, severe cerebrocardiovascular disease or organ dysfunction; ii) lack of basic and 3-year follow-up records; iii) death from other illnesses or accident; iv) individuals who were pregnant, lactating or diagnosed with chronic viral (human immunodeficiency virus or Hepatitis B or C) or bacterial infections (*M. tuberculosis*); v) individuals who underwent chemotherapy, immunotherapy or targeted therapy prior to tissue acquisition; vi) individuals who received chemotherapy that differed from specified methodology. Exclusion criterion for individuals who donated 37 normal gastric mucosal tissues was those who were diagnosed with any malignant tumor or stomach disease.

The specified methodology consisted of 5 days of treatment with an intravenous infusion of 425-750 mg/m²/day 5-fluorouracil (cat. no. H31020593; Shanghai Xudonghaipu Pharmaceutical Co., Ltd.). On day 1 of treatment, an intravenous infusion of 60-80 mg/m² cisplatin (cat. no. H37021358; Qilu Pharmaceutical Co., Ltd.) was administered. Treatment was given every 3 weeks for a total of 8 treatments. After the chemotherapy treatment regimens ended, patient were followed up for 3 years and were evaluated according to the response evaluation criteria of solid tumors published in 2000 (14). Complete response (CR) is defined by the observation of the tumor disappearing completely. Partial response (PR) is defined by the tumor receding by $>50\%$, osteolytic lesion abbreviation and partial calcification. No response (NR) is defined by those who did not meet the evaluation criteria of CR and PR.

All patients with gastric cancer were treated at The 970th Hospital of the PLA Joint Logistics Support Force (Yantai, China) from January 2015 to June 2016. All patients were informed and signed a consent form. All experiments involving human tissues were approved and supervised by The Ethics Committee of The 970th Hospital of the PLA Joint Logistics Support Force.

Reverse transcription-quantitative PCR (RT-qPCR). Liquid nitrogen was used to homogenize normal gastric mucosal tissues and gastric cancer tissues prior to using RNAiso plus (cat. no. 9108; Takara Bio, Inc.) to extract total RNA according manufacturer's protocol. RNAiso plus was also used to extract total RNA from cells (GES-1, SNU-5, SNU-1, SNU-16, AGS and NCI-N87). cDNA was then synthesized using a PrimeScript™ RT reagent kit containing the gDNA eraser (cat. no. RR047A; Takara Bio Inc.) using the temperature protocol of 37°C for 15 min and 85°C for 5 sec. qPCR was then prepared using GoTaq® qPCR Master Mix (cat. no. A6001; Promega Corporation), according to the manufacturer's protocol. The thermocycling conditions were as follows: Initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. The sequences of the qPCR primers used in this assay were from a previous study (12): HIT000218960 forward, 5'-CGTGGAAACTCCTAAATGTGT-3' and reverse, 5'-TCTATTACATGATGTGTCAGGC-3' and β -actin forward, 5'-AGCACAATGAAGATCAAGATCAT-3' and reverse, 5'-ACTCGTCATACTCCTGCTTGC-3'. The relative expression of HIT000218960 was calculated using the $2^{-\Delta\Delta C_q}$ method (15) and β -actin was used as the reference gene. The 71 gastric cancer tissues were subsequently divided into the low (n=39) and high expression groups (n=32) in accordance with the mean HIT000218960 values, before the 3-year overall survival of the two groups of patients were compared.

Cell Culture. GES-1, SNU-5, SNU-1, SNU-16, AGS and NCI-N87 cell lines were purchased from American Type Culture Collection and cultured in DMEM (cat. no. 61870044; Thermo Fisher Scientific, Inc.) containing 10% FBS (cat. no. 10437028; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO₂. For treatment, 50 μ mol/l 5-FU (cat. no. 51-21-8; Sigma-Aldrich; Merck KGaA) were added into the cell culture medium to stimulate cells for 24 h at 37°C with 5% CO₂.

Apoptosis. An Annexin V FITC/propidium iodide kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used to analyze apoptosis rate in SNU-5, SNU-1, SNU-16, AGS and NCI-N87 cells after being stimulated with 50 μ mol/l 5-FU for 24 h at 37°C. In each staining reaction, 5 μ l Annexin FITC and 10 μ l PI staining solution were used to stain 5×10^5 cells for 15 min at room temperature. Beckman CytoFLEX flow cytometer (CytoFLEX; Beckman Coulter, Inc.) was used for apoptosis analyses and data were analyzed by FlowJo (X10.0.7; Stanford University). Early (Lower right quadrant) + late (upper right quadrant) apoptotic cells were analyzed.

Transfection. The SNU-5 and NCI-N87 cell lines overexpressing HIT000218960 was established by Genomeditech Co. and was verified using qPCR, with cells transfected with the empty plasmid (Lv-NC) as the negative control. Briefly, the sequence of HIT000218960 was obtained from the H-InvDB database (<https://www.h-invitational.jp/>). Jiman Biotechnology (Shanghai) Co., Ltd. then prepared a lentivirus (MOI=20) for overexpressing HIT000218960 according to these sequences. In addition, siDirect version 2.0 (<https://sidirect2.rnai.jp/>) was used to design small interfering (si-) RNA sequence based on the sequence of HIT000218960 from the H-InvDB

Table I. Basic clinical data of the 71 patients with gastric cancer.

Variable	N (%)
Sex	
Male	43 (60.56)
Female	28 (39.44)
Age (years)	
≥60	40 (56.34)
<60	31 (43.66)
Tumor diameter (cm)	
≥3	48 (67.61)
<3	23 (32.39)
TNM stage	
I	30 (42.25)
II	37 (52.11)
III	4 (5.64)
Histological grade	
I	18 (25.35)
II	42 (59.15)
III	11 (15.50)
Lymph node metastasis (n)	
0	9 (12.68)
1-2	14 (19.71)
3-6	16 (22.54)
≥7	32 (45.07)

Tumor staging was performed as previously described (13). TNM, tumor-node-metastasis grading system.

database. si-RNA sequence used for verification included si-HIT000218960 guide RNA oligo sequences, 5'-ACC GAACUUUCGCCGAUUCUG-3' and passenger RNA oligo sequences, 5'-CAACCUAUCCAACUUCUCAAUUG-3'; and si-HMGA2 guide RNA oligo sequences, 5'-UCUUUAGCU AUAUUUUCUGCA-3' and passenger RNA oligo sequences, 5'-CAGAAAUAUAGCUAAAGAGU-3' (Sangon Biotech, Co., Ltd.). A negative control siRNA was used (Si-NC; guide RNA oligo sequences, 5'-GUUCUCGAUUAUCCGUU CA-3' and passenger RNA oligo sequences, 5'-GAUCGC CAUAUGAUAAGAGAU-3'). Gastric cancer cells (2.5×10^6) were transfected with 50 nM siRNA using Lipofectamine® 2000, according to the manufacturer's protocol. Subsequent experiments were performed 72 h post-transfection.

Western blotting. Total protein was extracted from SUN-5 and NCI-N87 cells using a lysis buffer (20 mmol/l Tris-HCl; 2.5 mmol/l EDTA; 150 mmol/l NaCl; 0.5% NP-40; pH=8.0) (cat.no.P0013; Beyotime Institute of Biotechnology) containing PMSF (cat. no. ST605; Beyotime Institute of Biotechnology). After measuring total protein concentrations using a bicinchoninic acid protein assay kit (cat. no. P0012S; Beyotime Institute of Biotechnology), 40 μ g total protein/lane was separated using 10% SDS-PAGE. The protein was then transferred to

PVDF membranes. Membranes were blocked in 5% skimmed milk at room temperature for 1 h prior to being incubated with primary antibodies overnight at 4°C. The membranes were then incubated with secondary antibodies at room temperature for 2 h. Following washing with PBS-Tween-20 (0.05%) three times, ECL solution (cat. no. WBKLS0100; Beijing Xijiangke Biotechnologies Co., Ltd.) was added to membranes to visualize the protein bands. Image J software (v1.8.0; National Institutes of Health) was used to analyze the protein band gray value. All antibodies used in the present study were purchased from Abcam, and the information of antibodies were showed as follows: Phosphorylated (p-) AKT (cat. no. ab38449; 1:500), AKT (cat. no. ab8805; 1:1,000), p-mTOR (cat. no. ab109268; 1:1,000), mTOR (cat. no. ab32028; 1:1,500), p-P70S6K (cat. no. ab194521; 1:1,000), p70S6K (cat. no. ab32529; 1:2,000), HMGA2 (cat. no. ab97276 1:1000), β -actin (cat. no. ab8226; 1:3,000), HRP-conjugated Goat Anti-Mouse IgG H&L (cat. no. ab6789; 1:3,000) and HRP-conjugated Goat Anti-Rabbit IgG H&L (cat. no. ab6721; 1:3,000).

Statistical analysis. Data are presented as mean \pm standard deviation with three independent repeats and analysis was conducted using the SPSS software (version 22.0; IBM Corp.). Differences between two groups were analyzed by unpaired t-test or χ^2 tests. One-way ANOVA followed by Tukey's test was used to compare the differences between multiple groups. A Gehan-Breslow-Wilcoxon test was used to compare the survival of patients that exhibit differential expression levels of HIT000218960. Pearson correlation analysis was used to analyze the correlation between the relative expression levels of HIT000218960 and HMGA2 in the gastric cancer tissues. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

HIT000218960 expression levels predicts the prognosis of patients with gastric cancer following chemotherapy. The expression levels of HIT000218960 were measured using RT-qPCR in the 37 normal gastric mucosal and 71 gastric cancer tissues collected. The relative expression level of HIT000218960 in normal gastric mucosal tissues were found to be significantly lower compared with that in the gastric cancer tissues (Fig. 1A). Similarly, the relative expression levels of HIT000218960 in normal gastric mucosal epithelial cells (GES-1) were revealed to be significantly lower compared with those in the gastric cancer cell lines SNU-5, SNU-1, SNU-16, AGS and NCI-N87 (Fig. 1B).

Comparing the prognosis of chemotherapy-treated patients with gastric cancer between the low and high HIT000218960 groups showed that 87.18% (34/39) patients in the low expression group exhibited an effective response to chemotherapy, whilst only 59.38% (19/32) patients in the high expression group exhibited a response to chemotherapy (Fig. 1C). In addition, 12.82% (5/39) patients in the low HIT000218960 expression group exhibited no-response to chemotherapy, whilst only 40.62% (13/32) patients in the high HIT000218960 expression group no-response to chemotherapy (Fig. 1C). The 71 patients with gastric cancer were also followed up for 3 years and the results revealed that 74.36% (29/39) patients with low HIT000218960 expression levels survived compared with 53.13% (17/32) in the high HIT000218960 expression

Table II. Assessment of the association between HIT000218960 expression and the clinicopathological data of the 71 patients with gastric cancer.

Variable	N	HIT000218960 expression		χ^2	P-value
		Low (n=39)	High (n=32)		
Sex					
Male	28	16	12	0.337	0.561
Female	43	23	20		
Age (years)					
≥60	40	20	20	0.899	0.343
<60	31	19	12		
Tumor diameter (cm)					
≥3	48	22	26	4.956	0.026
<3	23	17	6		
TNM stage					
I	30	20	10	6.736	0.034
II	37	19	18		
III	4	0	4		
Histological grade					
I	18	10	8	0.485	0.785
II	42	24	18		
III	11	5	6		
Lymph node metastasis (n)					
0	9	7	2	3.388	0.336
1-2	14	9	5		
3-6	16	8	8		
≥7	32	15	17		

TNM, tumor-node-metastasis grading system.

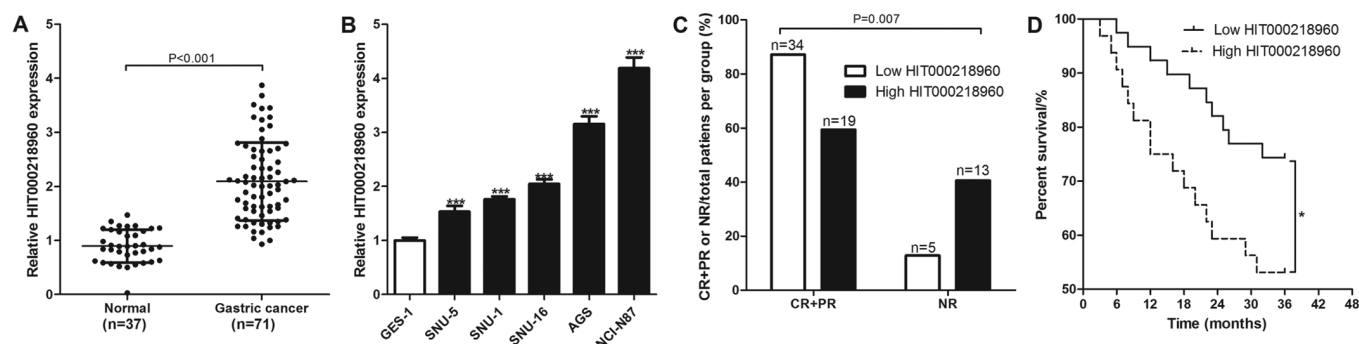


Figure 1. HIT000218960 expression is associated with the prognosis of patients with gastric cancer. (A) Comparison of HIT000218960 expression levels between normal gastric mucosal and gastric cancer tissues. (B) Comparison of HIT000218960 expression levels between normal gastric mucosal epithelial GES-1 cells and gastric cancer cell lines SNU-5, SNU-1, SNU-16, AGS and NCI-N87. ***P<0.001 vs. GES-1. (C) Comparison of chemotherapy results for patients with gastric cancer exhibiting high or low HIT000218960 expression. (D) Comparison of the 3-year survival of patients with gastric cancer exhibiting high or low HIT000218960 expression. *P<0.05. ***P<0.001. CR, complete response; PR, partial response; NR, no response.

group (Fig. 1D). HIT000218960 expression was found to associate significantly with tumor diameter and TNM stage in patients with gastric cancer (Table II).

HIT000218960 increases the resistance of gastric cancer cells to 5-FU. Following treatment with 50 μ mol/l 5-FU for 24 h,

gastric cancer cells exhibited a decreasing trend in the apoptosis in cells with increasing HIT000218960 expression levels (Fig. 2A). SNU-5 cells overexpressing HIT000218960 and NCI-N87 cells with HIT000218960 expression knocked down were then established (Fig. 2B and C), following which they were treated with 50 μ mol/l 5-FU for 24 h prior to apoptosis

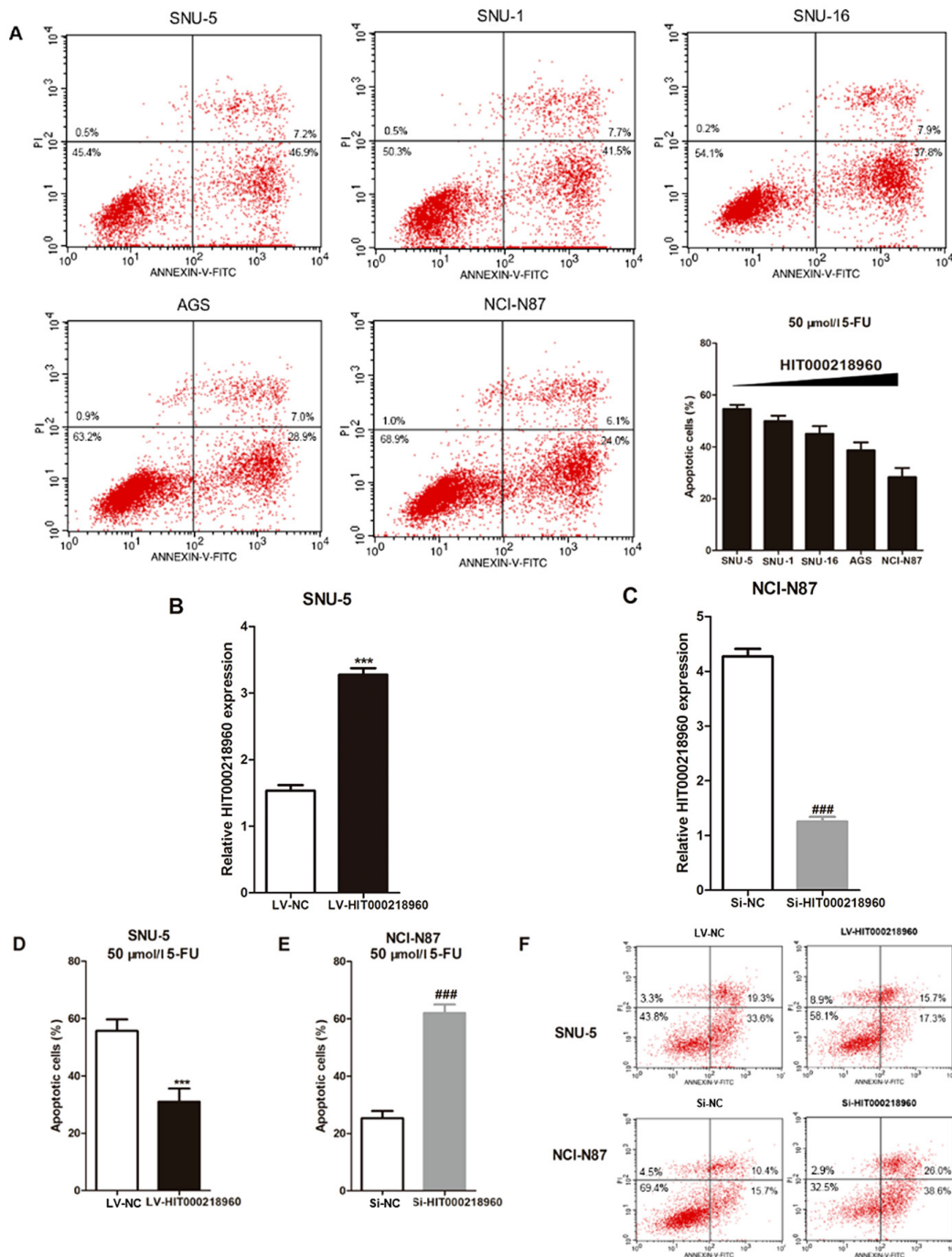


Figure 2. Effect of HIT000218960 expression on 5-FU-induced apoptosis in gastric cancer cells *in vitro*. (A) Flow cytometry was used to detect apoptosis of gastric cancer cells following treatment with 50 μ mol/l 5-FU. Detection of HIT000218960 expression in (B) SNU-5 cells and (C) NCI-N87 cells following over-expression and knockdown, respectively. using flow cytometry was used to detect apoptosis of (D) SNU-5 cells and (E) NCI-N87 cells following treatment with 50 μ mol/l 5-FU. (F) Flow cytometry images of (D) and (E) Experiments were repeated in triplicate. *** P <0.001 vs. LV-NC, ### P <0.001 vs. Si-HIT000218960. 5-FU, 5-fluorouracil; WT, wild-type; LV, lentivirus; si, small interfering; PI, propidium iodide.

analysis. HIT000218960 overexpression significantly reduced apoptosis in SNU-5 cells whereas HIT000218960 knockdown increased apoptosis in NCI-N87 cells after 5-FU treatment (Fig. 2D-F).

HIT000218960 upregulates the expression of HMGA2 in gastric cancer tissues. HMGA2 protein expression were measured in normal gastric mucosal and gastric cancer tissues. Relative HMGA2 protein expression levels in normal gastric

mucosal tissue were demonstrated to be significantly lower compared with those in the gastric cancer tissues (Fig. 3A). The relative expression level of HIT000218960 was found to be positively correlated with that of HMGA2 in the gastric cancer tissues (Fig. 3B). Subsequently, HIT000218960 overexpression resulted in significantly increased HMGA2 protein expression in SNU-5 cells, whilst HMGA2 knockdown significantly decreased HMGA2 levels in NCI-N87 and SNU-5 cells (Fig. 3C). Following the suppression of HMGA2 expression in

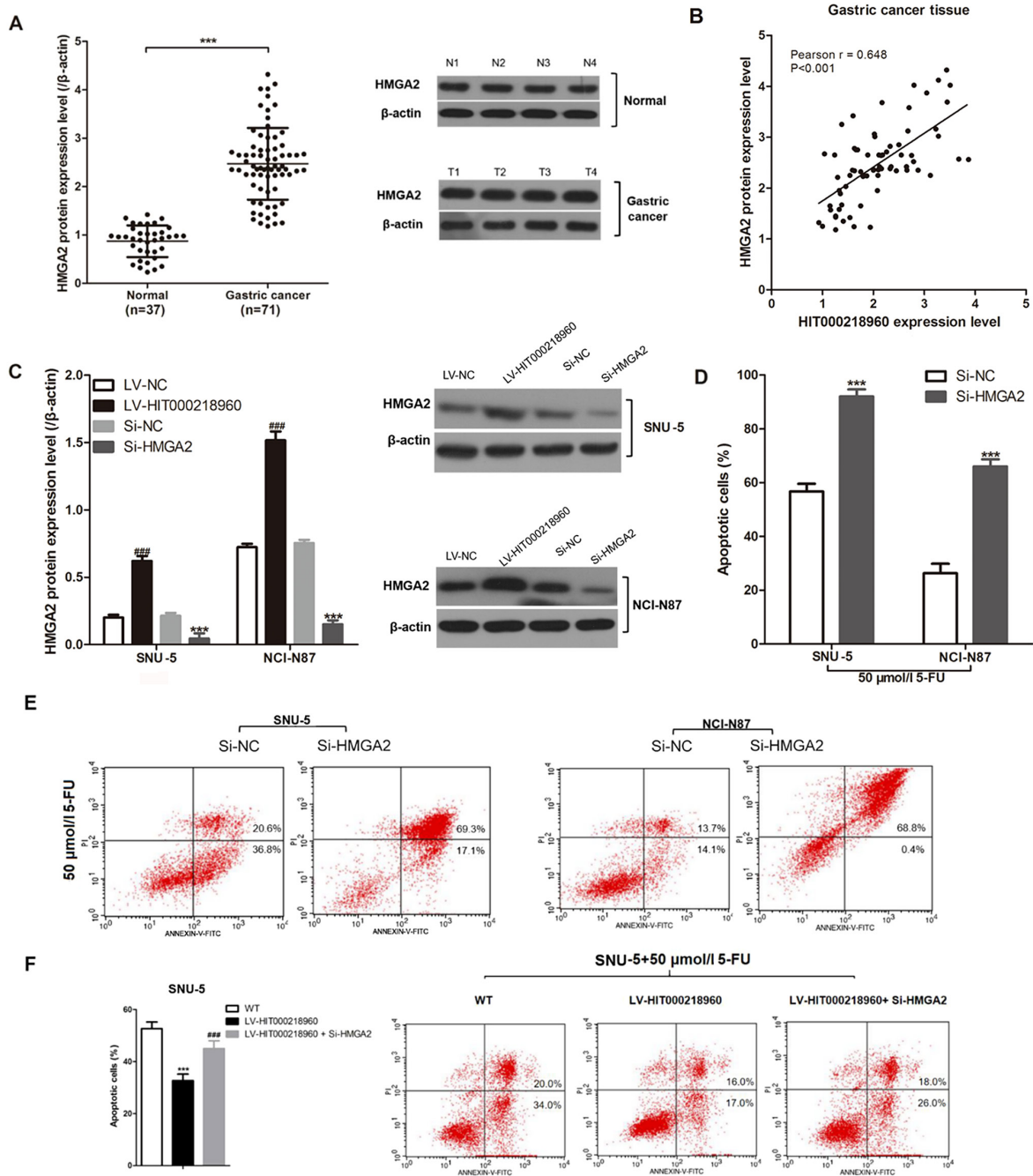


Figure 3. HIT000218960 inhibits 5-FU induced apoptosis by promoting HMGA2 expression. (A) HMGA2 protein expression is higher in gastric cancer tissues compared with that in normal gastric tissues. Representative western blotting images of (A) are presented on the right. *** $P < 0.001$ vs. normal. (B) HIT000218960 expression was positively correlated with that of HMGA2 in gastric cancer tissues. (C) Western blotting was used to detect the expression of HMGA2 protein in SNU-5 and NCI-N87 cells following transfection and representative protein bands are presented on the right. *** $P < 0.001$ vs. Si-NC and ### $P < 0.001$ vs. LV-NC. (D) HMGA2 knockdown promoted 5-FU-induced apoptosis in SNU-5 and NCI-N87 cells. (E) Representative flow cytometry images of (D) *** $P < 0.001$ vs. Si-NC group. (F) The inhibitive effects of HIT000218960 overexpression on 5-FU-induced apoptosis was partially abolished by the HMGA2 knockdown in SNU-5 cells. Experiments were repeated in triplicate. *** $P < 0.001$ vs. the WT. ### $P < 0.001$ vs. LV-HIT000218960. HMGA2, high mobility group A2; 5-FU, 5-fluorouracil; WT, wild-type, cell lines did not receive any transfection; LV, lentivirus; si, small interfering; PI, propidium iodide.

both SNU-5 and NCI-N87 cells (Fig. 3C), apoptosis was found to be significantly increased following treatment with 5-FU (Fig. 3D and E). By contrast, HIT000218960 overexpression significantly inhibited 5-FU-induced apoptosis, which was partially reversed by HMGA2 knockdown (Fig. 3F).

HIT000218960 activates the AKT/mTOR/P70S6 kinase (P70S6K) via HMGA2 upregulation. The AKT/mTOR/

P70S6K pathway has been previously reported to regulate the apoptosis downstream of HMGA2 in mammalian tumor cells (16-18). Therefore, changes in the expression levels of the AKT/mTOR/P70S6K signaling pathway components were analyzed in gastric cancer cells after HMGA2 knockdown following treatment with 50 μmol/l 5-FU for 24 h. AKT, mTOR and P70S6K phosphorylation were revealed to be significantly lower in the SNU-5 and NCI-N87 cells with HMGA2

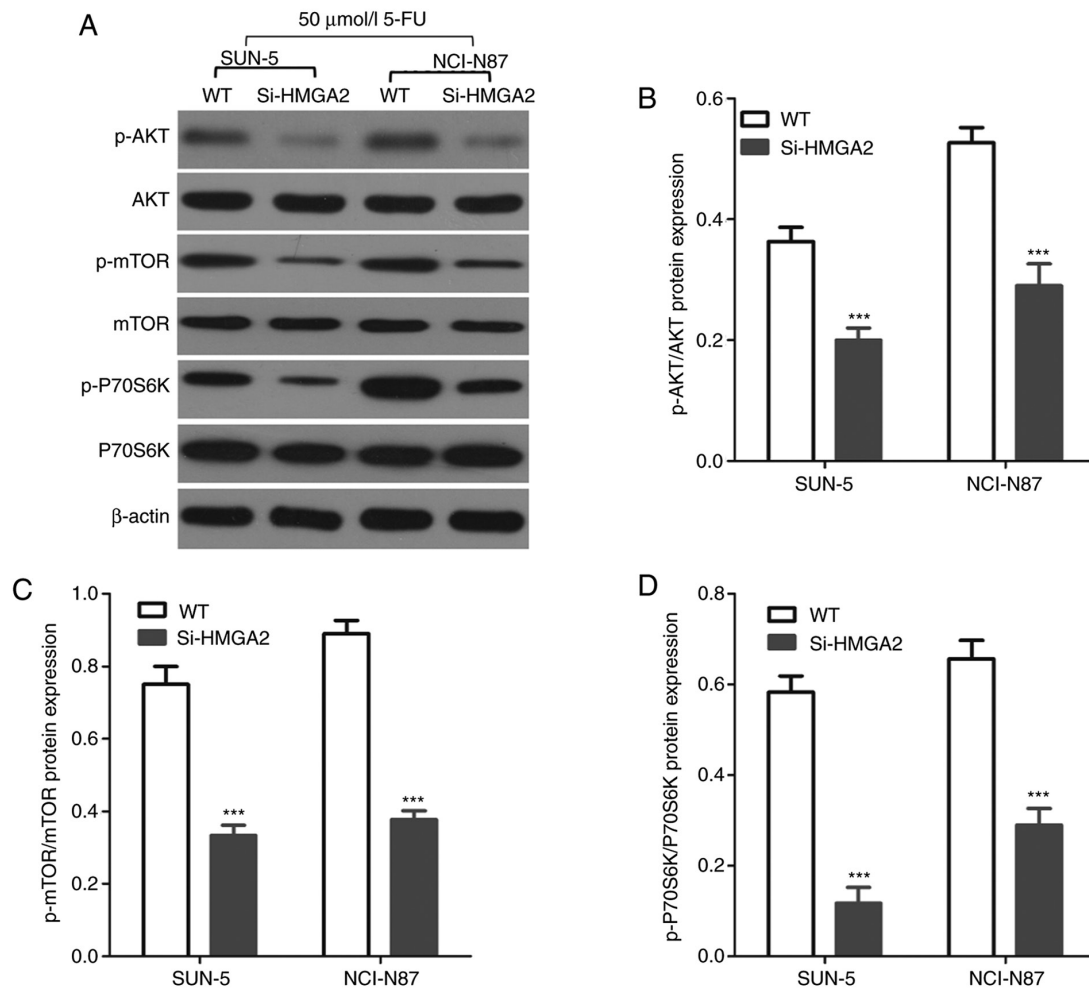


Figure 4. HMGA2 knockdown inhibits the AKT/mTOR/P70S6K pathway. (A) Western blotting was used to measure the expression of AKT, mTOR and P70S6K phosphorylation in gastric cancer cells following HMGA2 knockdown. Quantification of (B) AKT, (C) mTOR and (D) P70S6K phosphorylation in gastric cancer cells following HMGA2 knockdown. Experiments were performed in triplicate. ***P<0.001 vs. Si-NC group. HMGA2, high mobility group A2; P70S6K, P70S6 kinase; WT, wild type; 5-FU, 5-fluorouracil; si, small interfering.

expression knocked down compared with those of control cells (Fig. 4). Taken together, these data suggest that HIT000218960 enhanced resistance to 5-fluorouracil by promoting HMGA2 expression and by activating the AKT/mTOR/P70S6K pathway in gastric cancer cells (Fig. 5).

Discussion

Although 75% of human genomic DNA is transcribed into RNA, only 2% of the genome actually encodes proteins whereas 98% of transcripts are non-coding RNAs (19,20). Single-stranded RNA molecules with a length of 20-24 nucleotides are considered to be non-coding RNA molecules, whilst those with a length of >200 nucleotides are categorized as lncRNAs (19,20). lncRNAs were originally considered to be 'junk' RNA, but have been discovered to serve important roles in dose compensation effects, epigenetic regulation, cell cycle regulation and cell differentiation regulation (21,22). The abnormal expression of numerous lncRNAs have been demonstrated in malignant tumor tissues, including urothelial carcinoembryonic antigen 1 in lung cancer and colorectal neoplasia differentially expressed in hepatocellular carcinoma (23,24), where they can regulate tumor progression

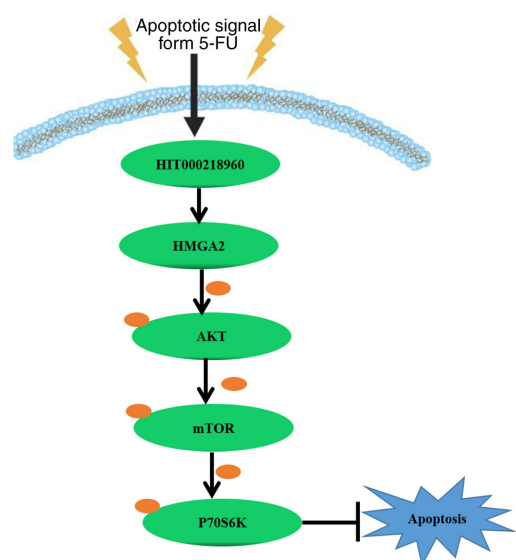


Figure 5. Mechanistic model of the present study. HIT000218960 enhanced resistance to 5-fluorouracil by promoting HMGA2 expression and activating the AKT/mTOR/P70S6K pathway in gastric cancer cells. Orange ovals represent phosphorylation modification. 5-FU, -fluorouracil; HMGA2, high mobility group A2; AKT, protein kinase B; mTOR, mammalian receptor of rapamycin; P70S6K, P70S6 kinase.

and are associated with the efficacy and prognosis of chemotherapy (25). Additionally, lncRNAs are involved in regulating cancer cell proliferation, invasion, migration and sensitivity to chemotherapeutic agents *in vitro* (26,27).

The lncRNA HIT000218960 was originally reported to be highly expressed in papillary thyroid cancer (12). Additionally, Sun *et al* (11) previously demonstrated that HIT000218960 was highly expressed in gastric cancer tissues compared with that in normal tissues and found that HIT000218960 promoted the proliferation and migration of the gastric cancer cells. The results of the present study also revealed that HIT000218960 was highly expressed in gastric cancer tissues and cell lines, consistent with the study by Sun *et al* (11). Furthermore, in the present study, patients with gastric cancer who exhibit high HIT000218960 levels did not respond as effectively to chemotherapy. To validate this observation, gastric cancer cell lines overexpressing HIT000218960 and those with HIT000218960 expression knocked down were generated. Higher levels of HIT000218960 expression were found to be associated with increased resistance to 5-FU-induced apoptosis in gastric cancer cells.

lncRNAs are non-coding RNAs that exert biological functions by regulating the expression of other genes (25). The results of the present study demonstrated that the relative expression levels of HIT000218960 were positively correlated with that of HMGA2 proteins in gastric cancer tissues, where HIT000218960 positively regulated the expression of HMGA2 in gastric cancer cells. HMGA2 has been previously documented to promote cancer cell proliferation and invasion, such as in breast cancer, colon cancer and lung cancer (28). Additionally, HMGA2 protein expression levels are positively associated with the degree of deterioration of various malignant tumors, such as gastric cancer, breast cancer and lung cancer and so on (28). As an oncogene, HMGA2 has been discovered to protect cancer cells from the toxicity of chemotherapy drugs (29,30). A previous study reported that HMGA2 exhibits dRP/AP lyase site cleavage activity and protects cancer cells from DNA-damage-induced cytotoxicity during chemotherapy (29). Furthermore, another previous study demonstrated that three-dimensional collagen I promotes gemcitabine resistance through HMGA2-dependent histone acetyltransferase expression in pancreatic cancer cells (30). The present study revealed that HMGA2 knockdown increased 5-FU-induced apoptosis in gastric cancer cells. Previous studies have shown that elevated HMGA2 expression in gastric cancer tissues is significantly associated with patient prognosis (31,32). In addition, HMGA2 has also been shown to promote gastric cancer cell proliferation, invasion, migration and resistance to chemotherapy (33,34). The findings of the present study indicated that the enhanced resistance of gastric cancer cells to 5-FU is mediated via the promotion of HMGA2 expression. In addition, HIT000218960 could positively regulate HMGA2 expression in gastric cancer.

The AKT/mTOR/P70S6K pathway is an important signaling pathway that regulates cell survival and apoptosis (35). It is also closely associated with the mitochondria-mediated (36) and the Fas death receptor-mediated cell death pathways (37,38). In the AKT/mTOR/P70S6K pathway, phosphatidylinositol-dependent kinase 1 phosphorylates AKT, which in turn

activates mTOR and p70S6K, ultimately resulting in the inhibition of apoptosis (39,40). A previous study demonstrated that HMGA2 overexpression suppresses cyclin dependent kinase inhibitor 2A levels in human umbilical cord blood-derived stromal cells (hUCBSCs) to regulate aging and proliferation of hUCBSCs through the Akt/mTOR/p70S6K pathway (18). Furthermore, another study revealed that microRNA-195 inhibits the proliferation and apoptosis of esophageal carcinoma cells through the mTOR/p70S6K signaling pathway by inhibiting HMGA2 (41). Therefore, the key components of the Akt/mTOR/p70S6K pathway were analyzed in the present study, where the results demonstrated that AKT, mTOR and P70S6K phosphorylation were significantly higher in gastric cancer cells compared with those with HMGA2 expression knocked down. These data indicated that HIT000218960 activated the AKT/mTOR/p70S6K pathway by promoting HMGA2 levels.

The present study is associated with a number of limitations. Although the association between HIT000218960 expression and the efficacy of chemotherapy in patients with gastric cancer was elucidated, the limited number of patients limited the reliability of clinical conclusions. Further analysis with larger numbers of patients with gastric cancer will lead to a more concrete clinical conclusion with regards to the ability of HIT000218960 expression to predict the prognosis of patients with gastric cancer following chemotherapy. The role of HIT000218960 has been previously studied in gastric cancer (42). In this previous study, 103 gastric cancer tissues and 62 normal gastric mucosa tissues were collected before the expression of HIT000218960 in these tissues were analyzed. In addition, their association with the prognosis of patients with gastric cancer was assessed. HIT000218960 was also found to be highly expressed in patients with gastric cancer, which was associated with poor prognosis. However, it should be pointed out that since these 103 patients with gastric cancer were not only treated with chemotherapy, their clinical data could not be unified with the present study. Nevertheless, this suggests that HIT000218960, which is highly expressed in gastric cancer tissues, may be associated with poorer prognosis by enhancing chemotherapy resistance.

In conclusion, higher levels of HIT000218960 predicted poor prognosis for patients with gastric cancer after treatment with chemotherapy. Mechanistically, this may be caused by increased HMGA2 expression and activation of the AKT/mTOR/p70S6K pathway, in turn inhibiting 5-FU-induced apoptosis of gastric cancer cells (Fig. 5).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

YC conceived and designed the current study. All authors read and approved the final manuscript. LB and KD performed the experiments, collected data, drafted the current study and critically revised the manuscript for important intellectual content. DT, XS and SW analyzed and interpreted the experimental data. LB and KD confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was performed with the approval of The Ethics Committee of The 970th Hospital of the PLA joint Logistics Support Force, (Yantai, China). All patients were informed and signed a consent form.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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