Long non-coding RNA lnc-GNAT1-1 inhibits gastric cancer cell proliferation and invasion through the Wnt/β-catenin pathway in *Helicobacter pylori* infection

LIPING LIU[1,2], TIANKUI SHUAI[3], BIN LI[2], LEI ZHU[2] and XUN LI[1]

[1]Department of Critical Care Medicine, The First Hospital of Lanzhou University; [2]Department of Critical Care Medicine, The Donggang Branch of First Hospital of Lanzhou University; [3]Department of General Surgery, The First Hospital of Lanzhou University, Lanzhou, Gansu 730000, P.R. China

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Abstract. The aim of the present study was to investigate the effect of long non-coding (lnc)-G protein subunit α transducin 1 (GNAT1)-1 in gastric cancer cells infected with *Helicobacter* (*H.*) *pylori*. Reverse transcription-quantitative polymerase chain reaction was used to assess lnc-GNAT1-1 expression in normal gastric and gastric cancer cells infected with *H. pylori*. The overexpression of lnc-GNAT1-1 in SGC-7901 and MNK45 cells was induced by transfection. Migration and invasion assays were performed in transfected gastric cancer cells to evaluate the effect of lnc-GNAT1-1. Histological and western blot analyses were used to determine the alterations in the Wnt/β-catenin signaling pathway protein expression. Cells transfected with lnc-GNAT1-1 were used to initiate gastric cancer tumor xenografts to compare tumor growth in mice inoculated with untransfected cells. The results revealed that *H. pylori* infection significantly downregulated lnc-GNAT-1 expression. Lnc-GNAT1-1 overexpression inhibited gastric cancer cell migration and invasion. Wnt/β-catenin pathway protein expression was decreased by lnc-GNAT1-1 overexpression. In addition, lnc-GNAT1-1 overexpression reduced tumor growth. Thus, lnc-GNAT1-1 is downregulated in gastric cancer infected with *H. pylori* and lnc-GNAT1-1 overexpression inhibited gastric cancer growth through the Wnt/β-catenin signaling pathway.

Introduction

Gastric cancer is one of the most common cancers with a high prevalence worldwide (1). In total, >80% of diagnoses occur when gastric cancer has already progressed to later stages of the disease, which significantly impedes the effectiveness of therapy in this highly aggressive disease. Thus, the prognosis of patients with gastric cancer remains poor (2). Early diagnosis of gastric cancer and effective treatment to prevent gastric cancer progression is critical in improving the clinical management of gastric cancer. Hence, the underlying molecular mechanisms of gastric cancer require further investigation in order to develop successful therapeutic strategies to improve patient survival.

Helicobacter pylori (*H. pylori*) infection is an important oncogenic driver of gastric cancer. It was reported in 2008 that an estimated 660,000 cases of cancer were attributable to *H. pylori* infection, which is ~5.2% of all cancer cases (3). Upon *H. pylori* infection, a cascade of molecular alterations that may ultimately lead to tumorigenesis is induced in gastric cells (4). Long non-coding RNAs (lncRNAs), which constitute a large portion of the human genome, are increasingly identified as novel cancer regulators (5). They are typically >200 nucleotides in length and modulate gene expression post-transcriptionally (6). In gastric cancer, a number of lncRNAs have been confirmed to be involved in cancer promotion or suppression (5,7-9). The lncRNAs HOX Transcript Antisense RNA and H9 have been identified as potent gastric cancer inducers by increasing tumor cell invasiveness and metastasis (10). In addition, downregulation of the lncRNA Maternally Expressed 3 is associated with poor survival in patients with gastric cancer (11). Strategies to regulate these lncRNAs may therefore be devised to impede gastric cancer progression. Lnc-G protein subunit α transducin 1 (GNAT1)-1 is a novel cancer suppressor (12). In colorectal cancer, downregulation of lnc-GNAT1-1 is characteristic of high risk patients with poor prognosis. Lnc-GNAT1-1 suppresses colorectal cancer by modulating the Raf kinase inhibitor protein (RKIP)-nuclear factor (NF)-κB-protein snail homolog 1 (Snail) circuit (12). However, to the best of our knowledge, the regulatory role of lnc-GNAT1-1 in gastric cancer has not yet been reported. Understanding the role and mechanism of lnc-GNAT1-1 in gastric cancer may contribute to the discovery of novel diagnostic and therapeutic tools to improve the clinical management of gastric cancer.

Correspondence to: Dr Xun Li, Department of Critical Care Medicine, The First Hospital of Lanzhou University, 1 Donggang West Road, Chengguan, Lanzhou, Gansu 730000, P.R. China
E-mail: li_xunsab@163.com

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The present study focused on the role of lnc-GNAT1-1 in gastric cancer induced by *H. pylori* infection and aimed to suggest potential strategies for gastric cancer treatment based on lnc-GNAT1-1 regulation. The results of the present study may aid in uncovering the molecular mechanism of *H. pylori* in gastric cancer tumorigenesis and provide a gene therapy tool for the treatment of this highly aggressive cancer.

Materials and methods

Cell culture. Normal gastric epithelial cells were obtained from Wuhan Feiyi Group (Wuhan, China). Human gastric cancer cell lines SGC-7901 and MKN45 were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). All cell lines were maintained in RPMI-1640 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37˚C in a humidified atmosphere of 95% air and 5% CO_2.

Infection of cells with H. pylori and cell transfection. Cell transfection was performed as previously described (12). Human gastric cancer cell lines SGC-7901 and MKN45, obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China), were grown in Minimum Essential Medium with Earle's salts (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) with 10% FBS in a 5% CO_2 atmosphere at 37˚C, and collected during the logarithmic growth phase, and cells were seeded into 6-well plates at a density of $5x10^5$ cells per well. Standard *H. pylori* (ATCC), which contained the entire cytotoxin-associated gene pathogenicity island (*cag*PAI), including *cag*A, grown in Brucella broth (Shanghai Biomart Technology Co., Ltd., Shanghai, China) were incubated with SGC-7901 and MKN45 cells to infect cells at a ratio of bacteria to SGC-7901 and MKN45 cells of 100:1 and with a multiplicity of infection of 10 ($5x10^6$ plaque forming units). Isogenic mutants lacking *cag*PAI or *cag*A (ATCC) were also inoculated into the gastric epithelial cells as control measures. Following 24 h, total RNA was extracted from the gastric epithelial cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed to cDNA using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) under the following temperature protocol: 55˚C for 30 min and 85˚C for 20 min. Transfection was performed using a 2nd generational system using 293 cells. PCR was performed to amplify an EcoRI-EcoRI fragment containing full-length lnc-GNAT1-1 cDNA using Phusion® High-Fidelity DNA Polymerase (New England Biolabs, Inc., Ipswich, MA, USA) using following thermocycling conditions: 98˚C for 20 sec; followed by 30 cycles of 98˚C for 5 sec, 55˚C for 10 sec and 72˚C for 30 sec; and then a final extension step at 72˚C for 5 min. Primers were directly provided by GenePharma Co., Ltd. (Shanghai, China; sequences not available). This fragment was inserted into EcoRI linearized pIRSE2-EGFP vectors (Clontech Laboratories, Inc., Mountainview, CA, USA) to establish lnc-GNAT1-1 expressing vectors. Lipofectamine® 2000 reagent (cat. no. 11668-019; Invitrogen, Thermo Fisher Scientific, Inc.) was then used to transfect 10 nM vectors into $5x10^7$ SGC-7901 and MKN45 cells. Empty pIRSE2-EGFP vectors were used as negative control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.). First-strand cDNA was generated using a Reverse Transcription system kit (Takara Biotechnology Co., Ltd., Dalian, China). RNA was heated to 70˚C for 5 min, chilled on ice for 5 min and then incubated at 25˚C for 5 min. Reagents within the Reverse Transcription kit were used to polyadenylate all lcnRNAs prior to cDNA conversion, as some of lncRNAs did not have poly A tails. qPCR was performed using a SYBR-Green PCR kit (ABgene UK Ltd.; Thermo Fisher Scientific, Inc.) in a StepOne System (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. PCR thermocycling conditions used were as follows: 50˚C for 60 sec; followed by 40 cycles of 95˚C for 15 sec and 56˚C for 35 sec. The primer sequences used were as follows: lnc-GNAT1-1 forward 5'-ATGTGTCGC CAGGTTGCTGTA-3', and reverse, 5'-CCGCTGAGGACT AGAGTAGC-3'; and GAPDH forward 5'-GCAAGAGCACAA GAGGAAGA-3', GAPDH reverse, 5'-ACTGTGAGGAGG GGAGATTC-3'. GAPDH was used as an endogenous control. RT-qPCR results were quantified with ABI Prism 7900HT (Applied Biosystems; Thermo Fisher Scientific, Inc.) and were analyzed using the $2^{-\Delta\Delta Cq}$ method (13).

Invasion assay. Invasion assays were performed using Transwell invasion chambers coated with Matrigel (50 μl/filter; BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. A total of $2x10^4$ cells were transfected with pLV-Control (Ctrl) or pLV-lnc-GNAT1-1 and cultured for 48 h prior to transfer to the upper Matrigel-coated invasion chamber in a 1% fetal calf serum (FCS; Gibco; Thermo Fisher Scientific, Inc.) and Dulbecco's modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F12; Gibco; Thermo Fisher Scientific, Inc.). DMEM/F12 containing 10% FCS (Sigma-Aldrich; Merck KGaA) was added to the lower chamber. Following incubation for 24 h at 37˚C with 5% CO_2, invasive cells in the lower chamber were stained with 1% crystal violet (Sigma-Aldrich; Merck KGaA) for 30 min at 25˚C. Cells were subsequently counted under a light microscope (magnification, x100). Assays were repeated six times.

Migration assay. Migration assays were performed in a 24-well Transwell chamber system. Following transfection for 24 h, cells were seeded in the upper chamber at $2x10^4$ cells/ml in 0.1 ml serum-free DMEM/F12 media. Media supplemented with 10% FBS was placed in the bottom well at a volume of 0.8 ml. Cells were incubated for 24 h at 37˚C with 5% CO_2. Following this, migrated cells in the lower chamber were stained with 1% crystal violet for 30 min at 25˚C. Cells were counted under a light microscope (magnification, x100). All experiments were repeated six times over multiple days.

In vivo assay of tumor growth. A total of 8 of BALB/c nude mice (age, 6-8 weeks; weight, 20-22 g; 4 male and 4 female) were purchased from Charles River Laboratories, Inc. (Beijing, China) and housed at a temperature of 25˚C with a humidity of 50%, a 12/12 h light/dark cycle and free access to food and water. The protocol of the present study was approved by the Ethics Review Committee of The First

Hospital of Lanzhou University (Lanzhou, China). SGC-7901 or MKN45 cells ($1x10^7$) that had been stably transfected with lnc-GNAT1-1-expressing vectors were suspended in 100 μl PBS and subcutaneously inoculated into the bilateral armpit, a thin subcutaneous fat and muscle layer between the fore limb and trunk of 4 BALB/c nude mice. An equivalent amount of SGC-7901 or MKN45 cells were injected into a further 4 BALB/c nude mice to generate a control group, and the mice did not demonstrate any clinical signs of tumor burden. The tumors were measured every 7 days following implantation, and the volume of each tumor was calculated by length x $width^2$ x 0.5. At 4 weeks following inoculation, mice were sacrificed and tumor tissues were collected and weighed. Humane end-points were strictly observed. Mice exhibiting signs of moderate to severe discomfort were euthanized. This was accomplished by anesthetizing the animals with 2,2,2-tribromoethanol (0.25 ml/kg) (14) followed by cervical dislocation, in accordance with the American Veterinary Medical Association guidelines on euthanasia (15). Analgesics were not used as they had the potential to affect the experimental outcomes of the study.

Western blot analysis. Total protein from cell lysates of both tumor samples and tissue samples from healthy mice were extracted using 1% SDS buffer (cat. no. P0013K; Beyotime Institute of Biotechnology, Haimen, China), and protein concentration was then determined using a BCA protein assay. Protein samples (40 μg/lane) were separated by 10% SDS-PAGE gel and transferred onto nitrocellulose membranes. Following blocking for 1 h at 4˚C in 5% non-fat milk and then washing with Tris-buffered saline with 10% Tween 20, membranes were incubation with anti-β-catenin (1:1,000; cat. no. 9582; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-Cyclin D (1:1,000; cat. no. sc-8396; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-c-Myc (1:900; cat. no. sc-789; Santa Cruz Biotechnology, Inc.) and anti-β-actin (1:2,000; cat. no. ab8227; Abcam, Cambridge, UK) primary antibodies overnight at 4˚C. Following this, blots were incubated with anti-rabbit IgG-horseradish peroxidase secondary antibodies (1:3,000; cat. no. TA130024; OriGene Technologies, Inc., Rockville, MD, USA) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescent reagent (PerkinElmer, Inc., Waltham, MA, USA) and quantified with ImageJ 1.37 software (version 1.37; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. Data are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). One-way analysis of variance followed by Tukey's post-hoc test was used to determine significant differences between >2 groups. Comparisons between two groups were performed using Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

H. pylori infection downregulates lnc-GNAT1-1 expression in normal gastric and gastric cancer cell lines. The effect of *H. pylori* infection on the expression of lnc-GNAT1-1 in normal and gastric cancer cells was investigated. The RNA expression of lnc-GNAT1-1 was detected by RT-qPCR analysis. As shown in Fig. 1A, normal gastric GES cells had significantly lower lnc-GNAT1-1 levels following *H. pylori* infection at a MOI of 1:1 and 10:1 (1:1, P=0.02 and 10:1, P=0.007; Fig. 1A). A higher MOI induced a greater reduction in lnc-GNAT1-1 expression, indicating a dose-dependent manner in this effect. The reduction in lnc-GNAT1-1 expression was also observed in SGC-7901 cells (1:1, P=0.01 and 10:1, P=0.002; Fig. 1B) and MKN45 cells (1:1, P=0.02 and 10:1, P=0.004; Fig. 1C). The RNA expression of lnc-GNAT1-1 was significantly decreased in SGC-7901 and MKN45 cells (P=0.009 and P=0.012, respectively; Fig. 1D) compared with normal gastric GES cell at the same MOI (10:1). Therefore, the results revealed that normal gastric cells and gastric cancer cells were subject to lnc-GNAT1-1 reduction upon *H. pylori* infection; however, the lnc-GNAT1-1 reduction was more marked in gastric cancer cells.

Ectopic lnc-GNAT1 overexpression inhibits gastric cancer cell invasion and migration. To further confirm the role of lnc-GNAT1-1 in gastric cells under *H. pylori* infection, lnc-GNAT1-1 was overexpressed through transfection of a lnc-GNAT1-1 expression vector. Lentiviral transfection was used to transfect lnc-GNAT1-1 expression vector to induce lnc-GNAT1-1 overexpression. RT-qPCR was used to analyze lnc-GNAT1-1 expression in transfected SGC-7901 and MNK45 cells, compared with that of untransfected cells. As presented in Fig. 2, SGC-7901 and MNK45 cells transfected with lnc-GNAT-1 had significantly higher levels of lnc-GNAT1-1 (P=0.004).

In addition, the effects of lnc-GNAT1-1 overexpression on gastric cancer cell migration and invasion was explored. The results revealed that lnc-GNAT1-1 overexpression significantly reduced the migration and invasion ability of SGC-7901 (Fig. 3A-C) and MNK45 cells (Fig. 3D-F).

Lnc-GNAT1 downregulates Wnt/β-Catenin pathway protein expression in gastric cancer cells infected with H. pylori. To investigate the association between Wnt/β-catenin signaling and tumor growth, the expression of key proteins in the Wnt/β-catenin signaling pathway was evaluated. β-catenin, cyclin D and c-Myc expression was significantly decreased in tumors overexpressing lnc-GNAT1-1 (Fig. 4).

To elucidate the underlying mechanism of lnc-GNAT1-1 in inhibiting gastric cancer migration and invasion, the association between lnc-GNAT1-1 overexpression and the Wnt/β-catenin pathway was also investigated in gastric cancer cell lines. Consistently, lnc-GNAT1-1 overexpression significantly decreased Wnt/β-catenin pathway protein expression in SGC-7901 cells (Fig. 5A and B) and MNK45 cells (Fig. 5C and D). These results indicated that lnc-GNAT1-1 may have the potential to be developed as a cancer therapeutic that may impede cancer cell migration through Wnt/β-catenin pathway protein expression inhibition. Thus, the efficacy of lnc-GNAT1 overexpression in attenuating tumor growth *in vivo* was further investigated.

Overexpression of lnc-GNAT1-1 inhibits tumor tissue growth in nude mice. MNK45 cells transfected with or without

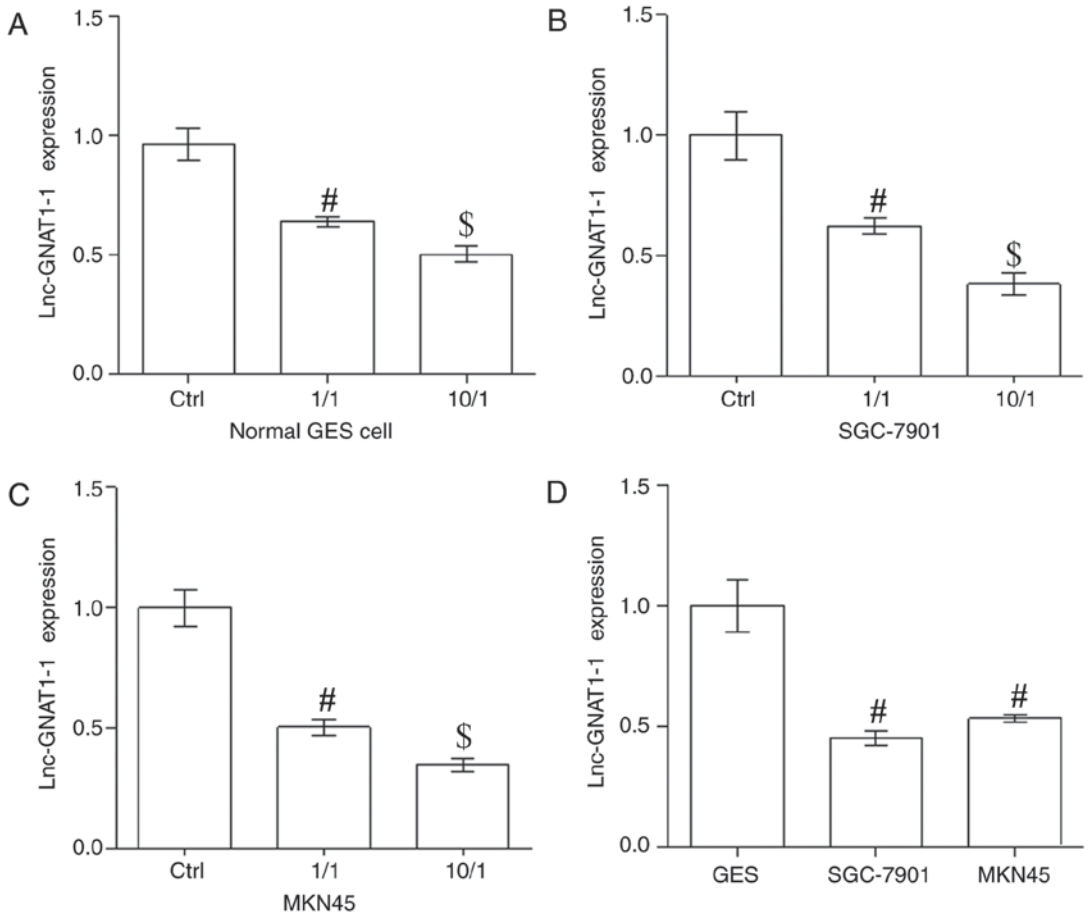

Figure 1. *Helicobacter pylori* infection downregulates lnc-GNAT1-1 expression in (A) the normal gastric cell line GES and gastric cancer cell lines (B) SGC-7901 and (C) MKN45. #P<0.05 vs. control group; $P<0.05 vs. 1/1 group. (D) The reduction in expression was more prominent in gastric cancer cell lines. #P<0.05 vs. GES group. Experiments were repeated three times. Data are presented as the mean ± standard deviation. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control.

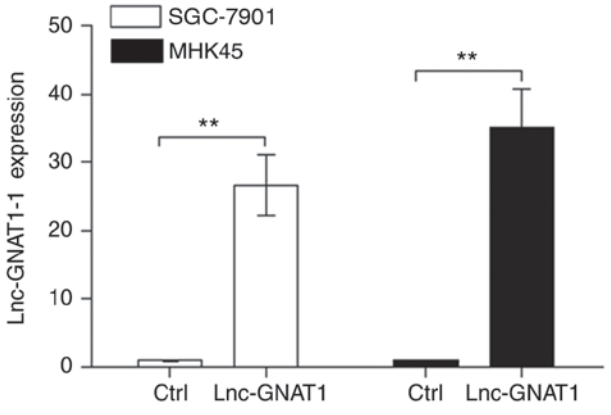

Figure 2. Lnc-GANT1-1 overexpression in gastric cancer cells is induced by lentiviral transfection. Experiments were repeated three times. Data are presented as the mean ± standard deviation. **P<0.01, as indicated. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control.

lnc-GNAT1-1 were used to construct gastric cancer tumor xenografts and tumor growth was subsequently monitored. As shown in Fig. 6, tumor volume and tumor weight were monitored 4 weeks following inoculation. A much slower rate of volume increase was observed in gastric tumors derived from MNK45 cells overexpressing lnc-GNAT1-1 (Fig. 6A). As presented in Fig. 6B and C, tumor size and tumor weight of lnc-GNAT1-1 overexpressing tumors was also decreased when compared with the control group.

Taken together, these results indicated that lnc-GNAT1-1 overexpression may impede gastric cancer progression.

Discussion

It is well established that *H. pylori* infection is an important risk factor in gastric cancer. *H. pylori* is categorized as a group I carcinogen by the International Agency for Research on Cancer (16). Therefore, substantial effort has been devoted to reduce *H. pylori* infection in order to decrease the incidence of gastric cancer; this has been successful in developing countries (17). Despite significant progress, chemotherapy and radiation therapy are combined with surgery to improve therapeutic outcomes, and advanced strategies and effective treatments are required for patients with gastric cancer. At present, the highly invasive gastrectomy remains the principle treatment for gastric cancer (18) and more efficient approaches in gastric cancer treatment must be developed.

Encouraged by progress in gene therapeutics for various types of cancer (19), the present study aimed to evaluate the role of an emerging cancer regulating lncRNA, lnc-GNAT1-1, in gastric cancer. The results revealed that

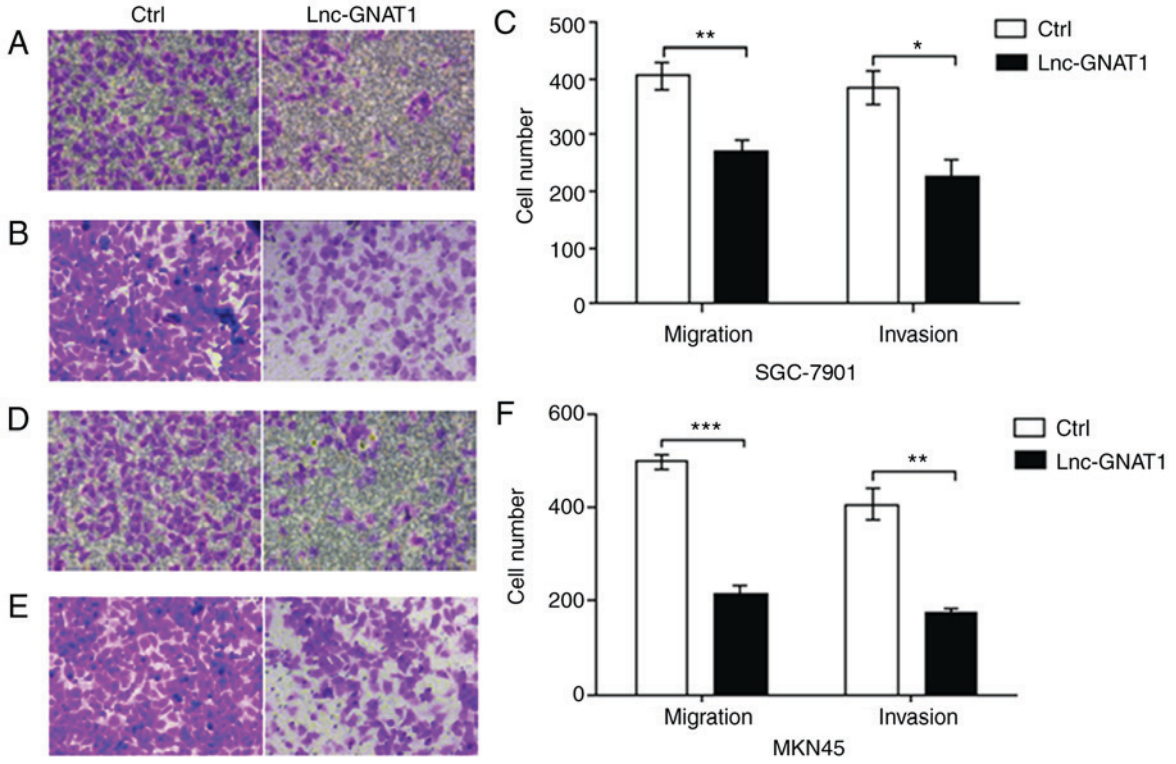

Figure 3. Lnc-GNAT1-1 overexpression inhibits gastric cancer (A) migration and (B) invasion in SGC-7901 cells. (C) Quantitative analysis of the effect of lnc-GNAT1-1 overexpression on the migratory and invasive abilities of SGC-7901 cells. Lnc-GNAT1-1 overexpression inhibits gastric cancer (D) migration and (E) invasion in MKN45 cells. (F) Quantitative analysis of the effect of lnc-GNAT1-1 overexpression on the migratory and invasive abilities of MKN45 cells. *P<0.05, **P<0.01 and ***P<0.001, as indicated. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control; Cells were observed under a light microscope (magnification, x100).

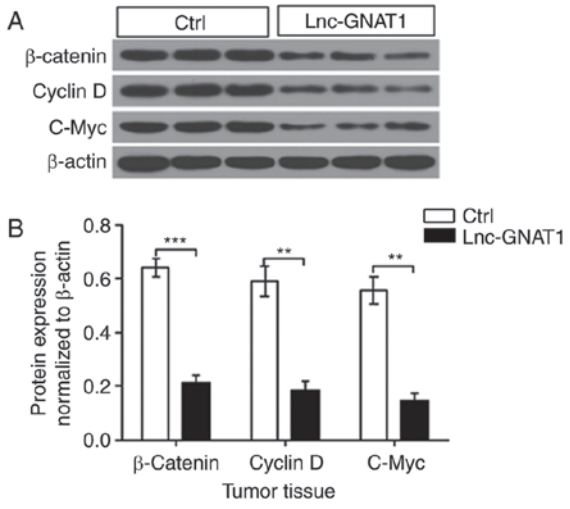

Figure 4. Overexpression of lnc-GNAT1-1 inhibits tumor proliferation by blocking the Wnt/β-catenin signaling pathway protein expression. (A) β-catenin, Cyclin D and c-Myc expression in xenograft tumors obtained from mice transfected with or without lnc-GNAT1-1 were determined by western blot analysis. (B) Quantitative analysis of β-catenin, Cyclin D and c-Myc protein expression normalized to β-actin. Experiments were repeated three times. Data are presented as the mean ± standard deviation. **P<0.01 and ***P<0.001, as indicated. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control.

lnc-GNAT1-1expression was significantly downregulated by *H. pylori* infection. A previous report has demonstrated that lnc-GNAT1-1 may act as a cancer suppressor by inhibiting cancer cell invasion and migration (12), suggesting that decreased levels of lnc-GNAT1-1 may bean indicator for high-risk gastric cancer.

In the present study, lnc-GNAT1-1 overexpression induced by lentiviral transfection resulted in decreased cancer cell invasion and migration *in vitro*. *In vivo*, gastric tumor growth was also attenuated by lnc-GNAT1-1 overexpression, which demonstrated the function of lnc-GNAT1-1 in gastric cancer. Compared with surgery, gene therapy strategies are less invasive. In addition, as normal tissues express high levelslnc-GNAT1-1 (12), non-specific transfection of lnc-GNAT1-1 expressing vector presumably would not induce toxic effects on normal tissues, making it a potential tumor-specific treatment strategy.

The Wnt/β-catenin signaling pathway promotes the growth of aggressive cancer cells (20). The present study identified the Wnt/β-catenin signaling pathway as an underlying mechanism of lnc-GNAT1-1 in gastric cancer cell regulation. Activation of the Wnt/β-catenin pathway is a putative mechanism in the promotion of cancer cell invasion, migration and dissemination (21). *H. pylori* infection has also been associated with increased Wnt/β-catenin signaling to generate gastric cancer cells with stem cell-like properties (22). However, to the best of our knowledge, the association between lnc-GNAT1-1and the Wnt/β-catenin signaling pathway has not been previously reported. Previous findings have suggested that lnc-GNAT1-1 is associated with RKIP-NF-κB-Snail pathway modulation (12). As Wnt/β-catenin and the RKIP-NF-κB-Snail signaling

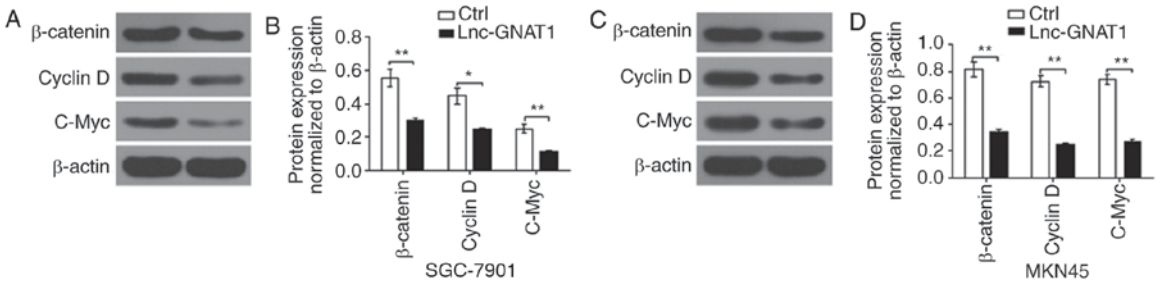

Figure 5. Wnt/β-catenin signaling pathway downregulation by lnc-GNAT1-1 overexpression in gastric cancer cell lines infected with *H. pylori*. (A) β-catenin, Cyclin D and c-Myc expression in SGC-7901 cells, and (B) expression quantification normalized to β-actin. (C) β-catenin, Cyclin D and c-Myc expression in SGC-7901 cells, and (D) expression quantification normalized to β-actin. Experiments were repeated three times. Data are presented as the mean ± standard deviation. *P<0.05 and **P<0.01, as indicated. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control.

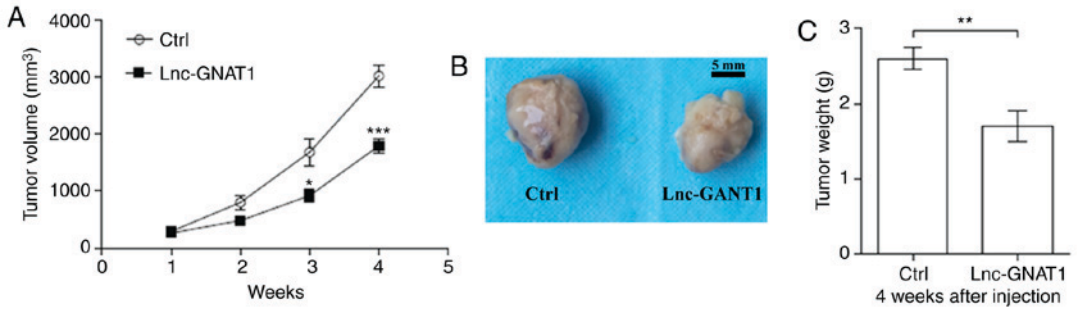

Figure 6. Overexpression of lnc-GNAT1-1 suppressed gastric tumor growth. (A) Tumor volume with or without lnc-GNAT1-1 overexpression. *P<0.05 and ***P<0.001 vs. Ctrl. (B) Representative image of dissected tumors harvested from mice bearing MNK45 cell xenografts with or without Lnc-GNAT1-1 transfection. (C) Comparison of tumor weights harvested from mice bearing MNK45 cell xenografts with or without lnc-GNAT1-1 transfection. **P<0.01, as indicated. Data are presented as the mean ± standard deviation. lnc-, long non-coding RNA; GNAT1-1, G protein subunit α transducin 1-1; Ctrl, control.

pathways may contribute to malignant cancer development, this finding may account for the therapeutic potential of lnc-GNAT1-1 in inhibiting gastric cancer progression (12). In the present study, it was demonstrated that *H. pylori* infection significantly downregulated the expression of lnc-GNAT-1. Furthermore, lnc-GNAT1-1 overexpression was revealed to decrease the protein expression associated with the Wnt/β-catenin pathway, suppress tumor growth and suppress gastric cancer cell migration and invasion abilities. However, the results of the present study suggested that lnc-GNAT1-1 did not fully suppress Wnt/β-catenin signaling, and thus there may be a requirement for other potent Wnt/β-catenin signaling suppressors combined with potential lnc-GNAT1-1 targeting therapies for the treatment of gastric cancer. It was not investigated if lnc-GNAT1-1 overexpression may be useful in the treatment of chemoresistant gastric cancer in the present study. However, it is probable that chemoresistance may be alleviated, as Wnt/β-catenin signaling has been reported to be involved in the development of chemoresistance (23).

In conclusion, the present study demonstrated that lnc-GNAT1-1 may have an important role in gastric cancer induced by *H. pylori* infection. Upon infection, lnc-GNAT1-1 expression was significantly downregulated, and lnc-GNAT1-1 overexpression inhibited gastric cancer cell migration and invasion. In addition, Wnt/β-catenin signaling pathway protein expression was reduced by lnc-GNAT1-1 overexpression. Furthermore, tumor growth was slower in mice inoculated with cells overexpressing lnc-GNAT1-1. Therefore, gene therapy targeting lnc-GNAT1-1 may be a potential strategy for gastric cancer suppression; however, further studies are required to validate lnc-GNAT1-1 as a useful biomarker for gastric cancer diagnosis.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LL, TS, BL, LZ and XL were responsible for the conception and design of the study. LL and XL performed the experiments. LL, TS and BL analyzed and interpreted the data. LL and BL drafted the article. LZ and XL were responsible for the revision of the manuscript.

Ethics approval and consent to participate

The protocol of the present study was approved by the Ethics Review Committee of the First Hospital of Lanzhou University (Lanzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66: 7, 2016.
2. Liu HS and Xiao HS: MicroRNAs as potential biomarkers for gastric cancer. World J Gastroentero 20: 12007-12017, 2014.
3. Plummer M, Franceschi S, Vignat J, Forman D and de Martel C: Global burden of gastric cancer attributable to *Helicobacter pylori*. Int J Cancer 136: 487-490, 2015.
4. Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, Hinoue T, Laird PW, Curtis C, Shen H, *et al*: Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513: 202-209, 2014.
5. Xia T, Liao Q, Jiang X, Shao Y, Xiao B, Xi Y and Guo J: Long noncoding rna associated-competing endogenous rnas in gastric cancer. Sci Rep 4: 6088, 2014.
6. Shi X, Sun M, Wu Y, Yao Y, Liu H, Wu G, Yuan D and Song Y: Post-transcriptional regulation of long noncoding rnas in cancer. Tumour Biol 36: 503-513, 2015.
7. Liu X, Sun M, Nie FQ, Ge YB, Zhang EB, Yin DD, Kong R, Xia R, Lu KH, Li JH, *et al*: Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. Mol Cancer 13: 92, 2014.
8. Song H, Sun W, Ye G, Ding X, Liu Z, Zhang S, Xia T, Xiao B, Xi Y and Guo J: Long non-coding RNA expression profile in human gastric cancer and its clinical significances. J Transl Med 11: 225, 2013.
9. Cao WJ, Wu HL, He BS, Zhang YS and Zhang ZY: Analysis of long non-coding RNA expression profiles in gastric cancer. World J Gastroentero 19: 3658-3664, 2013.
10. Li H, Yu B, Li J, Su L, Yan M, Zhu Z and Liu B: Overexpression of lncRNA H19 enhances carcinogenesis and metastasis of gastric cancer. Oncotarget 5: 2318-2329, 2014.
11. Sun M, Xia R, Jin F, Xu T, Liu Z, De W and Liu X: Downregulated long noncoding RNA MEG3 is associated with poor prognosis and promotes cell proliferation in gastric cancer. Tumor Biol 35: 1065-1073, 2014.
12. Ye C, Shen Z, Wang B, Li Y, Li T, Yang Y, Jiang K, Ye Y and Wang S: A novel long non-coding RNA lnc-GNAT1-1 is low expressed in colorectal cancer and acts as a tumor suppressor through regulating RKIP-NF-κB-Snail circuit. J Exp Clin Cancer Res 35: 187, 2016.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
14. Vieira RP, Ossig A, Perez J, Grassi VG, Petzhold CL, Peres AC, Costa JM and Lona LMF: Styrene atrp using the new initiator 2,2,2-tribromoethanol: Experimental and simulation approach. Polym Eng Sci 55: 2270-2276, 2015.
15. Mahady G, Pendland S, Yun G and Lu Z: Turmeric (*Curcuma longa*) and curcumin inhibit the growth of *Helicobacter pylori*, a group 1 carcinogen. Anticancer Res 22: 4179-4181, 2002.
16. Lee YC, Chen THH, Chiu HM, Shun CT, Chiang H, Liu TY, Wu MS and Lin JT: The benefit of mass eradication of *Helicobacter pylori* infection: A community-based study of gastric cancer prevention. Gut 62: 676-682, 2013.
17. Japanese Gastric Cancer Association: Japanese gastric cancer treatment guidelines 2010 (ver. 3). Gastric Cancer 14: 113-123, 2011.
18. Ibraheem D, Elaissari A and Fessi H: Gene therapy and DNA delivery systems. Int J Pharm 459: 70-83, 2014.
19. Nusse R and Clevers H: Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 169: 985-999, 2017.
20. Mao J, Fan S, Ma W, Fan P, Wang B, Zhang J, Wang H, Tang B, Zhang Q, Yu X, *et al*: Roles of Wnt/β-catenin signaling in the gastric cancer stem cells proliferation and salinomycin treatment. Cell Death Dis 5: e1039, 2014.
21. Yong X, Tang B, Xiao YF, Xie R, Qin Y, Luo G, Hu CJ, Dong H and Yang SM: *Helicobacter pylori* upregulates Nanog and Oct4 via Wnt/β-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. Cancer Lett 374: 292-303, 2016.
22. Sagara N and Katoh M: Mitomycin C resistance induced by TCF-3 overexpression in gastric cancer cell line MKN28 is associated with DT-diaphorase down-regulation. Cancer Res 60: 5959-5962, 2000.
23. Noda T, Nagano H, Takemasa I, Yoshioka S, Murakami M, Wada H, Kobayashi S, Marubashi S, Takeda Y, Dono K, *et al*: Activation of Wnt/beta-catenin signalling pathway induces chemoresistance to interferon-α/5-fluorouracil combination therapy for hepatocellular carcinoma. Br J Cancer 100: 1647-1658, 2009.