Tao Guo1,2, Xiao Yuan3, De-Feng Liu3, Shi-Hao Peng3 and A-Man Xu1*  
1Department of Gastrointestinal Surgery, The First Affiliated Hospital of Anhui Medical University, Shushan, Hefei, Anhui, 230031, China  
2Department of Gastrointestinal Surgery, The Fourth Affiliated Hospital of Anhui Medical University, Baohu, Hefei, Anhui, 230041, China  
3General Surgery Department, The Fourth Affiliated Hospital of Anhui Medical University, No.218, Shushan, Hefei, Anhui, 230041, China  

Received: 03 April, 2019  
Accepted: 22 July, 2019  
Published: 23 July, 2019  

*Corresponding author: A Man Xu, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Anhui Medical University, No.218, Shushan, Hefei, Anhui, 230041, China  
Tel: +86-0551-6287-5159; +86-137-0569-5470; Email: licorice666@sina.com

Keywords: Gastric cancer; HOXA11-AS; miR-148a; WNT1/β-catenin pathway; Migration; Invasion

https://www.peertechz.com

Research Article

Long non-coding RNA HOXA11-AS facilitates cell migration and invasion by regulating microRNA-148a/WNT1 axis in gastric cancer

Abstract

Recently, long non-coding RNAs (lncRNAs) have emerged as crucial players in the initiation and progression of multiple cancers including gastric cancer (GC). LncRNA HOXA11 antisense RNA (HOXA11-AS) has been found to be an oncogenic factor in GC. However, the molecular mechanisms responsible for these oncogenic properties have not been thoroughly elucidated. In the present study, the abundances of HOXA11-AS, miR-148a and WNT1 in GC tissues and cell lines were examined by qRT-PCR. Kaplan-Meier survival analysis was conducted to explore the relationship between HOXA11-AS expression and GC patient prognosis. Transwell assay was performed to evaluate cell migratory and invasive capacities. Bioinformatics, dual-luciferase reporter, RNA immunoprecipitation (RIP), and RNA-pull down assays were employed to analyze the interaction between miR-148a and HOXA11-AS or WNT1. Tumor xenograft experiments were performed to further examine the influence of HOXA11-AS knockdown on GC tumorigenesis in vivo. The protein levels of WNT1 and β-catenin were assessed by western blot assay. We found that HOXA11-AS and WNT1 expression was upregulated, while miR-148a expression was downregulated in GC tissues and cell lines. HOXA11-AS expression was associated with tumor size, lymph node metastasis, TNM stage, and overall survival in GC. HOXA11-AS promoted migration and invasion of GC cells through downregulating miR-148a. And, miR-148a inactivated WNT1/β-catenin signaling pathway via directly targeting WNT1. Moreover, HOXA11-AS induced the activation of WNT1/β-catenin pathway, which was abrogated by miR-148a overexpression in GC cells. Additionally, HOXA11-AS knockdown suppressed the growth of GC xenograft tumors by upregulating miR-148 and inactivating WNT1/β-catenin signaling pathway in vivo. These results suggest that HOXA11-AS overexpression promoted the tumorigenesis and progression of GC through activating WNT1/β-catenin pathway via repressing miR-148a, providing a prospective therapeutic target for GC.

Introduction

Gastric cancer (GC) is a serious public-health issue worldwide, ranking the first in gastrointestinal cancer-related morbidity and mortality [1]. An estimated 262,400 new cases and 108,000 deaths from GC occurred in 2018 in the United States alone [2]. Despite the great advances in the management of GC, the prognosis remains dismal, especially for individuals diagnosed with advanced disease [3,4]. Cell migration and invasion are considered to be the major events that can drive cancer progression [5]. And, an in-depth understanding on the molecular basis involved in aggressive behaviors of GC contributes to the identification and development of novel biomarkers or therapeutic targets.

LncRNAs are a group of transcripts larger than 200 nucleotides that can regulate various cellular processes, such as cell proliferation, apoptosis, invasion, and migration [6,7]. Moreover, LncRNAs are closely associated with the occurrence and development of various cancers including GC [8,9]. LncRNA homeobox A11 antisense (HOXA11–AS), a highly conserved transcript, is located near the homeobox A11 (HOXA11) gene. And, Li et al. pointed out that abnormal expression of HOXA11–AS was associated with tumor aggressiveness and poor clinical outcomes in multiple cancers [10]. Wang et al. revealed that HOXA11–AS expression was associated with glioma grade and poor prognosis, and HOXA11–AS overexpression promoted glioma tumorigenesis by regulating cell cycle progression [11]. Moreover, it is worth noting that HOXA11–AS level exhibits a significant elevation in GC [12]. However, the roles and detailed mechanisms of HOXA11–AS involved in GC tumorigenesis and progression remain unclear.

Citation: Guo T, Yuan X, Liu DF, Peng SH, Xu AM (2019) Long non-coding RNA HOXA11-AS facilitates cell migration and invasion by regulating microRNA-148a/WNT1 axis in gastric cancer. Arch Clin Gastroenterol 5(2): 019-026. DOI: http://doi.org/10.17352/2455-2283.000063

Keywords: Gastric cancer; HOXA11-AS; miR-148a; WNT1/β-catenin pathway; Migration; Invasion

https://www.peertechz.com

Check for updates
A hypothesis suggests that IncRNAs can function as competing endogenous RNAs (ceRNAs) of miRNAs to regulate the expression of cancer-related target mRNAs [13]. In the present study, we performed a series of experiments to investigate the roles of HOXA11-AS in GC as well as its potential molecular mechanisms. Results showed that HOXA11-AS expression was significantly upregulated in GC tissues and cell lines, and associated with the poor clinical outcome in GC. Besides, enforced expression of HOXA11-AS facilitated cell migration and invasion by miR-544a/WNT1/β-catenin pathway. Our study provided a novel ceRNA network in GC and a potential biomarker or therapeutic target for GC management.

Materials and Methods

Patients and tissue specimens: Our study was performed with the approval of the Research Ethics Committee of The First Affiliated Hospital of Anhui Medical University and all participants signed written informed consent prior to this research. Fresh tumor tissues and matching adjacent noncancerous tissues were collected from 30 GC patients underwent surgical resection between March 2010 and February 2012. All excised tissues were immediately frozen in liquid nitrogen and stored at -80°C. The clinicopathological parameters of GC patients, containing age, gender, tumor size, lymph node metastasis, and TNM stage, were summarized and shown as table 1.

All patients were followed-up and their survival patterns were monitored. Those who died from other diseases rather than GC were left out from the study. Patients diagnosed with GC were divided into high HOXA11-AS expression group (17 cases) and low HOXA11-AS expression group (13 cases). The overall survival patterns of GC patients were analyzed according to the difference of HOXA11-AS expression in tumor tissue samples.

Cell culture and transfection: Human gastric epithelial cell line GES-1 was obtained from the American type culture collection (ATCC, Manassas, VA, USA). Human embryonic kidney cell line 293T and gastric cancer cell lines SGC-7901 and MGC-803 were purchased from the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). All cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Gibco), and 1% penicillin/streptomycin (Solarbio, Beijing, China) at 37°C in a humidified air of 5% CO2.

The full-length fragments of HOXA11-AS were inserted into pcDNA3.1 vector to generate HOXA11-AS-overexpression plasmid (HOXA11-AS), with pcDNA3.1 vector as a negative control (vector). Small interfering RNA (siRNA) against HOXA11-AS (si–HOXA11-AS) and its control (si–NC), miR-148a mimic (miR-148a) and its negative control (miR–NC), miR-148a inhibitor (anti–148a) and its corresponding control (anti–NC) were purchased from GenePharma (Shanghai, China). SGC-7901 and MGC-803 cells were seeded into 6-well plates with a density of 1 × 105 cells/well, followed by the transfection of above products using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

RNA extraction and quantitative real-time PCR (qRT-PCR): Total RNA was isolated from GC specimens or cultured cells using Trizol reagent (Thermo Fisher, Wilmington, DE, USA) and miRNA was extracted using mirVanaTM miRNA Isolation Kit (Thermo Fisher) referring to the manufacturer’s procedures. First-strand cDNA was synthesized using High Capacity Reverse Transcription System Kit (Takara, Dalian, China). The reverse transcription primer sequences for miR-148a were 5'-GTC GTA TCC AGT GCA GGG TCC GCC GCA GTG CAT ACG ACA CAA G-3'. Then, qPCR reaction was conducted using Universal SYBR Green PCR Kit (Takara) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Results were calculated by 2–ΔΔCt method. GAPDH served as the housekeeping gene to normalize the expression of HOXA11-AS and WNT1. U6 small nuclear RNA (snRNA) served as the internal control to normalize miR-148a expression. qPCR primer sequences were displayed as below: HOXA11-AS (Forward, 5'-GAG TTT GAA GCC GTG GAT GT-3'); Reverse, 5'-AGA TGA GGG GAG ACG TGG AT-3'; WNT1 (Forward, 5'-TGG AGC CAC CAC CGG CTA GTG CAC-3'; Reverse, 5'-CAG GAT GGC AAG AGG GTT CAT G-3'); miR-148a (Forward, 5'-TGT ATC CAA ACT CAT TGT CAT TGT CAT AC-3'); mutant HOXA11-AS (HOXA11-AS-MUT) or WNT1 (WNT1-MUT) reporter by mutating the nucleotides complementary to miR-148a or miR-148a targeting WNT1 were predicted by miRcode (Thermo Fisher) referring to the manufacturer’s procedures. miRNA was extracted using mirVanaTM miRNA Isolation Kit (Thermo Fisher, Wilmington, DE, USA) and total RNA was isolated from GC specimens or cultured cells using Trizol reagent (Thermo Fisher, Wilmington, DE, USA) and reverse transcription primer sequences for miR-148a were 5'-GTC GTA TCC AGT GCA GGG TCC GCC GCA GTG CAT ACG ACA CAA G-3'. Then, qPCR reaction was conducted using Universal SYBR Green PCR Kit (Takara) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Results were calculated by 2–ΔΔCt method. GAPDH served as the housekeeping gene to normalize the expression of HOXA11-AS and WNT1. U6 small nuclear RNA (snRNA) served as the internal control to normalize miR-148a expression. qPCR primer sequences were displayed as below: HOXA11-AS (Forward, 5'-GAG TTT GAA GCC GTG GAT GT-3'); Reverse, 5'-AGA TGA GGG GAG ACG TGG AT-3'; WNT1 (Forward, 5'-TGG AGC CAC CAC CGG CTA GTG CAC-3'; Reverse, 5'-CAG GAT GGC AAG AGG GTT CAT G-3'); miR-148a (Forward, 5'-TGT ATC CAA ACT CAT TGT CAT TGT CAT AC-3'); mutant HOXA11-AS (HOXA11-AS-MUT) or WNT1 (WNT1-MUT) reporter by mutating the nucleotides complementary to miR-148a. Luciferase reporter was transfected into 293T cells along with miR-148a or miR–NC. At 48 h after transfection, luciferase activities were determined using a Dual–Luciferase Assay Kit (GeneCopoeia, Rockville, MD, USA).

RNA immunoprecipitation (RIP): RIP assay was carried out using the Magna RIP™ RNA–Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) according to manufacturer’s instructions. In brief, SGC-
7901 cell lysates were incubated with protein A/G magnetic beads conjugated with anti-Argonaute2 (anti-Ago2, Abcam, Cambridge, MA, USA) or anti-IgG (Abcam). After 24 h of incubation, the beads were treated with proteinase K to remove the proteins and HOXA11-AS level in immunoprecipitation complex was detected by qRT–PCR assay.

RNA pull-down: RNA–pull down assay was performed to detect the interaction between HOXA11-AS and miR-148a. In brief, SGC–7901 cells were transfected with biotin–labeled miR–148a–WT (Bio–miR–148a–WT) or miR–148a–MUT (Bio–miR–148a–MUT) for 48 h. Then, cell lysates were incubated overnight at 4°C with Streptavidin–Dyna beads (In Vitrogen). Next, RNA was isolated from beads and HOXA11-AS level was determined by RT–qPCR assay.

Western blot assay: SGC–7901 and MGC–803 cells were prepared with RIPA lysis buffer (Thermo Fisher) supplemented with protease inhibitor (Thermo Fisher), followed by high-speed centrifugation to remove cell debris and insoluble substance. Then, protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher). Equal amounts of proteins were separated by SDS–PAGE and electrophoretically transferred to PVDF membranes (Millipore). Next, the membranes were blocked overnight at 4°C with 5% non-fat milk, incubated for 2 h with primary antibodies against β–catenin and WNT1 at 37°C. After washing, the membranes were probed with horseradish peroxidase (HRP)–conjugated secondary antibodies (Cell Signaling Technology, Inc., Danvers, MA, USA) for another 1.5 h. Finally, protein bands were visualized using an ECL kit (Biorbyt, Shanghai, China) on Bio–Rad ChemiDoc MP imaging system (Bio–Rad Laboratories, Hercules, CA, USA).

Tumor xenograft experiment: Male BALB/c nude mice (6–week–old) were purchased from Charles river (Beijing, China) and fed under a standard condition for one–week. SGC–7901 cells (2×10^6) infected with sh–NC or sh–HOXA11–AS lentiviruses (Hanbio Biotechnology Co., Ltd., Shanghai, China) were subcutaneously inoculated into the back of BALB/c nude mice. Tumor volume was measured every week and calculated using the following formula: volume = (length × width^2)/2. At four weeks after injection, mice were sacrificed, and tumors were resected and weighed. All in vivo experiments were carried out with the national Guidelines of Animal Care and Use and the approval of Animal Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Statistical analysis: All data were analyzed through SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). Data from more than 3 independent experiments were exhibited as the mean ± standard deviation (SD). Statistical difference between two groups was assessed by Student’s t-test. Difference analysis of multiple data was performed by one–way ANOVA, followed by Student–Newman–Keuls–q (SNK–q) test. P values less than 0.05 was identified as statistically significant.

Results

HOXA11–AS expression was upregulated and associated with poor prognosis in GC: The level of wild type HOXA11–AS in GC tissues and adjacent normal tissues was firstly detected by qRT–PCR. Results showed that wild type HOXA11–AS level was strikingly upregulated in 30 GC tissues than that in adjacent normal tissues (Figure 1A). Afterwards, we further explored the correlation of HOXA11–AS expression and multiple clinicopathological parameters. Results presented that HOXA11–AS expression in GC tissues was not associated with age and gender of GC patients (Table 1, P>0.05), but was related with tumor size, lymph node metastasis and TNM stage (Table 1, P<0.05). Also, Kaplan–Meier survival analysis revealed that patients with high HOXA11–AS expression had a poor overall survival compared with low HOXA11–AS expression group (Figure 1B). Also, a notable upregulation of HOXA11–AS level was observed in SGC–7901 and MGC–803 cells compared with GES–1 cells (Figure 1C). These results suggested that HOXA11–AS expression was upregulated and associated with poor prognosis in GC.

HOXA11–AS contributed to cell migration and invasion in GC: To further explore the effects of HOXA11–AS on GC progression, HOXA11–AS overexpression plasmid (HOXA11–AS) and siRNA (si–HOXA11–AS) were constructed and synthesized. Following
transfection efficiency analysis disclosed that the transfection of HOXA11-AS overexpression plasmid effectively elevated HOXA11-AS expression in SGC-7901 cells, while si-HOXA11-AS introduction markedly reduced HOXA11-AS expression in MGC-803 cells (Figure 2A,B). Transwell assay showed that HOXA11-AS overexpression notably promoted SGC-7901 cell migration and invasion (Figure 2C,E), and HOXA11-AS knockdown markedly suppressed MGC-803 cell migration and invasion (Figure 2D,F).

**HOXA11-AS could interact with miR-148a**

Bioinformatics prediction analysis by miRcode website presents that there exists some complementary sequences between HOXA11-AS and miR-148a (Figure 3A). Following dual-luciferase reporter assay demonstrated that miR-148a overexpression resulted in the obvious reduction of luciferase activity of HOXA11-AS-WT reporter in 293T cells, but had no much impact on luciferase activity of HOXA11-AS-MUT reporter (Figure 3B), suggesting that HOXA11-AS could bind with miR-148a through putative binding sites. RIP assay further disclosed that HOXA11-AS could be copiously enriched by Ago2 antibody rather than IgG antibody in SGC-7901 cells (Figure 3C), indicating that HOXA11-AS had the potential to interact with miR-148a in space. However, there are other miRNAs that may be targeted by HOXA11-AS, not only miR-148a. RNA pull-down assay followed by RIP is a powerful tool to confirm the specific interaction between miRNA and lncRNA [14]. The results of RNA pull-down assay showed that the transfection of Bio-miR-148a-WT could induce the considerable enrichment of HOXA11-AS level in SGC-7901 cells compared with Bio-NC group, while the introduction of Bio-miR-148a-MUT had no much influence on HOXA11-AS level relative to Bio-NC group (Figure 3D). In a word, these data unveiled that HOXA11-AS could bind with miR-148a by predicted binding sites.

**HOXA11-AS modulated cell migration and invasion through miR-148a**: Subsequent qRT-PCR revealed that miR-148a expression was significantly downregulated in GC tissues (n=30) and cells compared with their counterparts (Figure 4A,B). And, miR-148a expression was remarkably reduced in HOXA11-AS-overexpressed SGC-7901 cells, but was dramatically increased in HOXA11-AS-depleted MGC-803 cells (Figure 4C,D), suggesting that HOXA11-AS could negatively regulate miR-148a expression in GC cells. Next, rescue experiments were performed to investigate whether the effects of HOXA11-AS on GC cell migration and invasion were mediated by miR-148a. Results showed that miR-148a overexpression suppressed the elevation of cell migratory and invasive capacities induced by HOXA11-AS in SGC-7901 cells (Figure 4E,G). And, miR-148a knockdown reversed the detrimental effects of HOXA11-AS loss on cell migration and invasion in MGC-803 cells (Figure 4F,H). Taken together, HOXA11-AS contributed to cell migration and invasion through downregulating miR-148a in GC.

**WNT1 was an authentic target for miR-148a**: Next, TargetScan online website was used to seek for potential targets of miR-148a. Among these candidate targets, WNT1 was selected because of its oncogenic role in GC as previously reported [15] (Figure 5A). To further confirm the interaction between miR-148a and WNT1, WNT1-WT and WNT1-MUT reporters containing wild-type or mutant miR-148a binding sites were constructed. Subsequent luciferase reporter assay showed that the luciferase activity of WNT1-WT reporter was dramatically reduced in miR-148a-overexpressed 293Tcells compared with miR-NC group (Figure 5B). However, miR-148a overexpression
or not had no much impact on luciferase activity of WNT1-MUT reporter (Figure 5B). That was to say, miR-148a could interact with WNT1 3’UTR by predicted sites. Next, we found that WNT1 expression was notably upregulated in GC tissues and cell lines relative to corresponding negative controls (Figure 5C,D). Also, RT-qPCR assay demonstrated that miR-148a level was markedly elevated in SGC-7901 cells transfected with miR-148a mimic, but was remarkably downregulated in MGC-803 cells transfected with anti-miR-148a (Figure 5E). And, protein levels of WNT1 and β-catenin were strikingly reduced in miR-148a-overexpressed SGC-7901 cells, but was noticeably increased in miR-148a-silenced MGC-803 cells (Figure 5F), suggesting that miR-148a inactivated WNT1/β-catenin pathway via targeting WNT1.

**Figure 4: HOXA11-AS promoted cell migration and invasion through downregulating miR-148a.** (A and B) The expression level of miR-148a in GC tissues and cell lines was detected by qRT-PCR. (C and D) SGC-7901 cells were transfected with Vector or HOXA11-AS, and MGC-803 cells were transfected with si-NC or si-HOXA11-AS, followed by the detection of miR-148a expression by qRT-PCR. (E and F) SGC-7901 cells were transfected with vector, HOXA11-AS, HOXA11-AS+miR-NC, HOXA11-AS+miR-148a. MGC-803 cells were transfected with si-NC, si-HOXA11-AS, si-HOXA11-AS+anti-NC, si-HOXA11-AS+anti-148a. At 48 h after transfection, the migratory ability of SGC-7901 and MGC-803 cells was evaluated by Transwell migration assay. (G and H) The invasive ability of SGC-7901 and MGC-803 cells was assessed by Transwell invasion assay.*p<0.05 compared to matching controls.

**Figure 5: miR-148a directly targeted the 3’UTR fragment of WNT1.** (A) Predicted binding sites of miR-148a within WNT1 3’UTR by TargetScan website, as well as the mutant sites in WNT1-MUT reporter. (B) 293T cells were co-transfected with miR-NC or miR-148a mimic and luciferase reporter carrying wild-type or mutant 3’UTR of WNT1. Forty-eight hours later, luciferase activities were detected by Dual-Luciferase reporter assay. (C and D) WNT1 miRNA level in GC tissues and cell lines was determined by RT-qPCR. (E) miR-148a level was determined by RT-qPCR assay in SGC-7901 cells transfected with miR-NC or miR-148a mimic and MGC-803 cells transfected with anti-NC or anti-148a. (F) The protein levels of WNT1 and β-catenin were determined by western blot assay in SGC-7901 cells transfected with miR-NC or miR-148a mimic and MGC-803 cells transfected with anti-NC or anti-148a.*p<0.05 compared to corresponding controls.

**HOXA11-AS activated WNT1/β-catenin pathway through repressing miR-148a.** Next, Rescue experiments were performed to explore whether HOXA11-AS could regulate WNT1/β-catenin pathway through sponging miR-148a. Results showed that upregulation of HOXA11-AS stimulated WNT1 and β-catenin expression, while these effects of HOXA11-AS were weakened by miR-148a overexpression in SGC-7901 cells (Figure 6A). And, miR-148a loss alleviated the inhibitory effects of HOXA11-AS knockdown on WNT1 and β-catenin expression (Figure 6B). These results indicated that HOXA11-AS activated WNT1/β-catenin pathway through downregulating miR-148a.

**HOXA11-AS knockdown inhibited GC xenograft tumor growth via the regulation of miR-148a/WNT1/β-catenin pathway in vivo.**

Subsequent in vivo experiments presented that HOXA11-AS knockdown resulted in the remarkable reduction of GC xenograft tumor volume (Figure 7A) and weight (Figure 7B), suggesting that HOXA11-AS loss hindered GC tumorigenesis in vivo. Next, qRT-PCR assay validated that HOXA11-AS expression was notably reduced in GC xenograft tumors infected with sh-HOXA11-AS lentiviruses (Figure 7C). And, increased miR-148a expression (Figure 7D) and reduced WNT1and β-catenin expression (Figure 7E) was observed in HOXA11-AS-depleted GC xenograft tumors. MMP-2 and MMP-9, two major members of matrix metalloproteinase (MMP) family, can lead to tumor metastasis by degrading collagen in the extracellular matrix (ECM) (16, 17). Our study further demonstrated that HOXA11-AS deficiency triggered the remarkable downregulation of MMP-2 and MMP-9 levels in GC xenograft tumors (Figure 7E). In a word, these data indicated that knockdown of HOXA11-AS suppressed GC xenograft tumor growth and metastasis via regulating miR-148a/WNT1/β-catenin pathway in vivo.

**Discussion**

GC is a serious threat for human health. Till now, surgery is still the main therapeutic strategy for GC, but high recurrence rate heavily impairs the clinical outcomes after surgical resection. lncRNAs, initially thought to be transcriptional noise, are emerging as pivotal players in a variety of cancers including GC. Over the past decades, IncRNA HOXA11-AS has been found to be implicated in the initiation and progression of multiple solid tumors. For instance, HOXA11-AS overexpression accelerated cell proliferation and invasion in ovarian cancer [18]. HOXA11-AS loss repressed cell proliferation, migration, invasion, and angiogenesis, while induced cell cycle arrest and apoptosis in non–small cell lung cancer (NSCLC) [19]. And, Qu et al. suggested that HOXA11-AS expression was associated with advanced pathogenic status and poor prognosis of laryngeal squamous cell cancer (LSCC) patients, and HOXA11-AS depletion suppressed LSCC cell proliferation, migration and invasion [20]. Consistent with previous studies, we observed that HOXA11-AS level was obviously elevated in GC tissues and cell lines, associated with tumor size, lymph node metastasis, advanced tumor stages and poor survival in GC. Previous studies used two different cell lines to investigate the effects of elevation or decrease of IncRNA HOTAIR and TINCR on cancer progression [21,22]. In this text, SGC-7901 cells were selected to conduct the gain–of–function investigations and MGC–803 cells were...
In this study, we demonstrated that WNT1 was a target of miR-148a, and miR-148a inhibited WNT1 expression by direct interaction. β-catenin is previously identified as a key molecule of WNT/β-catenin signaling pathway [33]. Here, we also confirmed that β-catenin level was downregulated in miR-148a-overexpressed GC cells, but was upregulated in miR-148a-depleted GC cells. And, we did not find the evidence to support the suppose that β-catenin can be directly targeted by miR-148a. Thus, we believed that the inhibitory effect of miR-148a on β-catenin expression was realized possibly by targeting WNT1. Following study further showed that miR-148a overexpression abolished the promotive effects of HOXA11-AS on WNT1 and β-catenin expression, suggesting that HOXA11-AS activated WNT1/β-catenin signaling pathway through modulation of miR-148a/WNT1 axis.

A large number of genes, such as transcription factor 3 (TCF3), zinc finger E-box binding homeobox transcription factor 1 (ZEB1), inhibitor of differentiation 2 (ID2), and SNAI2, have been found to be associated with the malignant metastatic phenotypes of tumor cells [34-37]. HOXA11-AS/miR-148a/WNT1/β-catenin reported in our study is one of multitudinous HOXA11-AS-involved regulatory mechanisms that are correlated with cell invasion and migration in GC.

In the following study, we intend to further explore whether HOXA11-AS can regulate GC initiation and progression by other signaling pathways.

In conclusion, our data indicated the correlation of HOXA11-AS expression and clinicopathological parameters of patients with GC. And, HOXA11-AS accelerated cell migration and invasion via modulation of miR-148a/WNT1/β-catenin pathway. Our findings uncovered a novel regulatory mechanism.
for GC development and provided a potential therapeutic target for GC.

**Authors’ contributions**

TG and XY designed the study. DFL collected the data. TG, XY and SHP carried out the statistical analyses. TG, XY and AMX prepared the figures. All authors wrote the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Our study was performed with the approval of the Research Ethics Committee of The First Affiliated Hospital of Anhui Medical University and all participants signed written informed consent prior to this research.

**References**

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Intl J Cancer 136: E359-E386. Link: https://bit.ly/2AjawM9

2. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68: 11. Link: https://bit.ly/25v7B8

3. Durães C, Almeida GM, Seruca R, Oliveira C, Carneiro F (2014) Biomarkers for gastric cancer: prognostic, predictive or targets of therapy? Virchows Arch 464: 367-378. Link: https://bit.ly/2MbxrBV

4. Liang H, Kim YH (2013) Identifying molecular drivers of gastric cancer through next-generation sequencing. Cancer Lett 340: 241-246. Link: https://bit.ly/2YOEdza

5. Han T, Kang D, Ji D, Wang X, Zhan W, et al. (2013) How does cancer cell metabolism affect tumor migration and invasion? Cell Adh Migr 7: 395-403. Link: https://bit.ly/2Ypx4Ym

6. Huaute M (2015) The emerging role of lncRNAs in cancer. Nat Med 21: 1253. Link: https://bit.ly/2y1Bx4Q

7. Sun M, Nie FQ, Wang ZX, De W (2016) Involvement of IncRNA dysregulation in gastric cancer. Histol Histopathol 31: 33-39. Link: https://bit.ly/2XV7Th4

8. Ma P, Xu T, Huang M, Shu Y (2016) Increased expression of LncRNA PANDAR predicts a poor prognosis in gastric cancer. Biomed Pharmacother 78: 172-176. Link: https://bit.ly/2YoyXu

9. Zhang L, Zhou Y, Huang T, Cheng AS, Yu J, et al. (2017) The interplay of LncRNA-H19 and its binding partners in physiological process and gastric carcinogenesis. Int J Mol Sci 18: 450. Link: https://bit.ly/2XVRj3

10. Li N, Yang M, Shi K, Li W (2017) Long non-coding RNA HOXA11-AS in human cancer: A meta-analysis. Clin Chim Acta 474: 165-170. Link: https://bit.ly/2G9CDDo

11. Wang Q, Zhang J, Liu Y, Wei Z, Zhou J, et al. (2016) A novel cell cycle-associated IncRNA, HOXA11-AS, is transcribed from the 5′-end of the HOXA transcript and is a biomarker of progression in glioma. Cancer Lett 373: 251-259. Link: https://bit.ly/2MmZgc9

12. Sun M, Nie F, Wang Y, Zhang Z, Hou J, et al. (2016) LncRNA HOXA11-AS promotes proliferation and invasion of gastric cancer by scaffolding the chromatin modification factors PRC2, LSD1, and DNMT1. Cancer Res 76: 6299-6310. Link: https://bit.ly/2MTFeOH

13. Tay Y, Rinn J, Pandolfi PP (2014) The multilayered complexity of ceRNA crosstalk and competition. Nature 505: 344-352. Link: https://go.nature.com/2JWNoX2

14. Ruan X, Li P, Cao H (2018) Identification of Transcriptional Regulators That Bind to Long Noncoding RNAs by RNA Pull-Down and RNA Immunoprecipitation. Methods Mol Biol 1783: 185. Link: https://bit.ly/2MSfsReX

15. Qian C, Liu F, Ye B, Zhang X, Liang Y et al. (2015) Notch4 promotes gastric cancer growth through activation of WNT1/β-catenin signaling. Mol Cell Biochem 401: 165-174. Link: https://bit.ly/2YfW4fB

16. Zheng H, Takahashi H, Murai Y, Cui Z, Nomoto K, et al. (2006) Expressions of MMP-2, MMP-9 and VEGF are closely linked to growth, invasion, metastasis and angiogenesis of gastric carcinoma. Anticancer Res 26: 3579-3583. Link: https://bit.ly/2LyB65

17. Xie Y, Lan F, Yang X, Huang Q, Lin Y, et al. (2014) IL-18-induced activation of p38 promotes metastasis in gastric adenocarcinoma via upregulation of AP-1/c-fos, MMP2 and MMP9. Mol Cancer 13: 18. Link: https://bit.ly/2MBupM7

18. Yim GW, Kim HJ, Kim LK, Sang WK, Kim S, et al. (2017) Long non-coding RNA HOXA11 antisense promotes cell proliferation and invasion and predicts patient prognosis in serous ovarian cancer. Cancer Res Treat 49: 656-668. Link: https://bit.ly/2K3nWF6

19. Zhang Y, Chen W, Gan T, Zhang X, Xie Z, et al. (2017) Clinical Significance and Effect of IncRNA HOXA11-AS in NSCLC. A study based on bioinformatics, in vitro and in vivo verification. Sci Rep 7: 5567. Link: https://bit.ly/2KMvAAC

20. Qu L, Jin M, Yang L, Sun C, Wang P, et al. (2018) Expression of long non-coding RNA HOXA11-AS is correlated with progression of laryngeal squamous cell carcinoma. Am J Transl Res 10: 573. Link: https://bit.ly/2G0f11c

21. Liu M, Jia J, Wang X, Liu Y, Wang C et al. (2018) Long non-coding RNA HOTAIR promotes cervical cancer progression through regulating BCL2 via targeting miR-143-3p. Cancer Biol Ther 19: 391-399. Link: https://bit.ly/2JW1iBK

22. Liu X, Ma J, Xu F, Li L (2018) TINCR suppresses proliferation and invasion through regulating miR-544a/FBXW7 axis in lung cancer. Biomed Pharmacother 99: 9-17. Link: https://bit.ly/2y1CSBJ

23. Liu Z, Chen Z, Fan R, Jiang B, Chen X, et al. (2017) Over-expressed long noncoding RNA HOXA11-AS promotes cell cycle progression and metastasis in gastric cancer. Mol Cancer 16: 82. Link: https://bit.ly/30PQdMf

24. Xia T, Liao Q, Jiang X, Shao Y, Xiao B, et al. (2014) Long noncoding RNA associated-competing endogenous RNAs in gastric cancer. Sci Rep 4: 6088. Link: https://bit.ly/2JIBYyp

25. Hu Y, Tian H, Xu J, Fang JY (2016) Roles of competing endogenous RNAs in gastric cancer. Briefings in Brief Funct Genomics 15: 266-273. Link: https://bit.ly/2SwTgKvY

26. Sakamoto N, Naito Y, Oue N, Sentani K, Uraoka N, et al. (2014) Roles of miR-148a in gastric cancer invasion by regulating MMP7 expression. MiR-148a expression in gastric cancer tissue detected by in situ hybridization. See also Sakamoto, N., et al. Cancer Sci 105: 236-243. Link: https://bit.ly/30PQoLY

27. Zhang W, Yan Li (2016) miR-148a downregulates the expression of transforming growth factor-β2 and SMAD2 in gastric cancer. Int J Oncol 48: 1877-1885. Link: https://bit.ly/2YqjFy8

28. Clevers H (2006) WNT/beta-catenin signaling in development and disease. Cell 127: 469-480. Link: https://bit.ly/2ZBLwuw

29. Birchmeier W, Rosário M, Birchmeier W. (2006) Balancing cell adhesion and WNT signaling, the key role of beta-catenin. Curr Opin Genet Dev 16: 51-59. Link: https://bit.ly/2XZ529i

30. Jiang Q, He M, Ma MT, Wu HZ, Yu ZJ, et al. (2015) MicroRNA-148a inhibits breast cancer migration and invasion by directly targeting WNT1. Oncol Rep 35: 1452-1456. Link: https://bit.ly/2Y5TTm6

31. Zhang W, Sun Z, Su L, Wang F, Jiang Y, et al. (2018) miRNA-185 serves as a prognostic factor and suppresses migration and invasion through WNT1 in colon cancer. Eur J Pharmacol 825: 75-84. Link: https://bit.ly/2y1LLS5
32. Fan D, Ren B, Yang X, Liu J, Zhang Z (2016) Upregulation of miR-501-5p activates the WNT/β-catenin signaling pathway and enhances stem cell-like phenotype in gastric cancer. J Exp Clin Cancer Res 35:177. Link: https://bit.ly/30T3BUt

33. Xu XH, Sun PL, Li JZ, Jheon S, Lee CT, et al. (2011) Aberrant WNT1/β-Catenin Expression is an Independent Poor Prognostic Marker of Non-small Cell Lung Cancer After Surgery. J Thorac Oncol 6: T16-724. Link: https://bit.ly/30N60Qz

34. Li RT, Li YH, Hu X, Lian HW, Wang L, et al. (2016) Transcription factor 3 controls cell proliferation and migration in glioblastoma multiforme cell lines. Biochem Cell Biol 94:1. Link: https://bit.ly/2GpFxR9

35. Liu Y, Zhang N, Wang Y, Xu M, Liu N, et al. (2012) Zinc finger E-box binding homeobox 1 promotes invasion and bone metastasis of small cell lung cancer in vitro and in vivo. Cancer Sci 103:1420-1428. Link: https://bit.ly/2y1PpMB

36. Coma S, Amin DN, Shimizu A, Lasorella A, Lavarone A, et al. (2010) Id2 Promotes Tumor Cell Migration and Invasion through Transcriptional Repression of Semaphorin 3F. Cancer Res 70: 3823-3832. Link: https://bit.ly/2JL7CV7

37. Fenouille N, Tichet M, Dufres M, Pottier A, Mogha A, et al. (2012) The Epithelial-Mesenchymal Transition (EMT) Regulatory Factor SLUG (SNAI2) Is a Downstream Target of SPARC and AKT in Promoting Melanoma Cell Invasion. PLoS One 7: e40378. Link: https://bit.ly/300vRp