doi:10.4149/neo_2020_190722N653

LncRNA HOXA11-AS promotes migration and invasion through modulating miR-148a/WNT1/β-catenin pathway in gastric cancer

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Received July 22, 2019 / Accepted August 21, 2019

Increasing researches have focused on the biological functions of long noncoding RNAs (lncRNAs) in human cancers. HOXA11-AS, a widely known lncRNA, has been confirmed to be involved in the progression of several cancers, including gastric cancer (GC). Whereas, the detailed mechanism of this lncRNA in GC remains to be further illuminated. The abundances of HOXA11-AS, miR-148a and WNT1 in GC tissues and cell lines were examined by qRT-PCR. Clinicopathological and Kaplan-Meier survival analyses were determined to explore the relationship between HOXA11-AS expression and outcomes of patients. Transwell assay was performed for the evaluation of cell migration and invasion. Bioinformatics, dual-luciferase reporter and RNA immunoprecipitation assays were employed to analyze the correlation between HOXA11-AS and miR-148a or miR-148a and WNT1. The protein levels of WNT1 and β-catenin were assessed by western blot assay. Results showed that HOXA11-AS and WNT1 expression levels were upregulated, while miR-148a level was downregulated in GC tissues and cell lines relative to matched controls. Elevated expression of HOXA11-AS was associated with increased tumor size, lymph node metastasis, advanced TNM stage, as well as reduced survival of GC patients. HOXA11-AS induced migration and invasion of GC cells through serving as a molecular sponge for miR-148a. Furthermore, miR-148a inactivated WNT1/β-catenin signaling pathway via directly targeting WNT1. HOXA11-AS increased WNT1/β-catenin pathway activity, which was abolished by miR-148a overexpression in GC cells. In conclusion, overexpression of HOXA11-AS contributed to migration and invasion of GC cells via activation of WNT1/β-catenin signaling pathway through repressing miR-148a, providing a prospective therapeutic target for GC.

Key words: gastric cancer, HOXA11-AS, miR-148a, WNT1/β-catenin pathway, migration, invasion, gastric cancer

Gastric cancer (GC) is counted as a severe public health issue worldwide, ranking in the top of gastrointestinal cancer-related morbidity and mortality among women and men [1]. Relevant statistics show an estimated 26,240 new cases and 10,800 deaths in the United States in 2018 [2]. Cell migration and invasion are considered to be significant contributors to cancer-induced death [3]. Although effective strategies, like surgery and adjuvant chemotherapy, have achieved long-term survival, the clinical outcome of GC patients is still unsatisfied, especially for the individuals diagnosed at an advanced stage [4, 5]. Thus, it is urgent to clarify the aggressive mechanism of GC and develop a novel therapeutic target for GC treatment.

Recently, emerging technologies such as high-throughput sequencing and microarrays have confirmed the existence of a large number of noncoding RNAs [6, 7]. Long noncoding RNAs (lncRNAs) are a type of unique transcripts of more than 200 nucleotides in length with low or no protein-coding capacity. Growing evidence indicates that lncRNAs have the potential to mediate cell proliferation, cycle arrest, apoptosis, invasion and migration in various human cancers, including GC [8, 9]. Abnormally expressed lncRNAs are associated with the prognosis, clinicopathology and carcinogenesis of GC patients [10, 11]. Given the vital roles of lncRNAs in tumorigenesis via serving as oncogenes or tumor suppressors, targeted therapies based on lncRNAs may provide a broad application prospect for cancer treatment.

Homeobox A11 antisense lncRNA (HOXA11-AS), a highly conserved transcript, is located near the homeobox A11 (HOXA11) gene. HOXA11-AS was first discovered in mouse embryonic cDNA library using a probe from the sequences of sense HOXA11 cDNA [12]. Reliable pieces of

evidence have demonstrated that HOXA11-AS is obviously upregulated in several types of cancers and associated with tumor aggressiveness. For instance, by performing a meta-analysis for 608 patients from seven studies, Li et al. [13] revealed that an enhanced abundance of HOXA11-AS is related to the poor clinical outcomes and distant metastasis in a variety of cancers. Wang et al. [14] reported that HOXA11-AS could serve as an independent prognostic factor in glioma. Moreover, upregulated HOXA11-AS stimulates the pathological process by inducing cell proliferation and tumor growth *in vitro* and *in vivo*. In addition, HOXA11-AS expression is significantly raised in non-small cell lung cancer tissues and cell lines, and its knockdown suppresses cell invasion via recruiting EZH2 and DNMT1 to miR-200b promoter regions [15]. It is worth noting that HOXA11-AS exhibits a notable elevation in GC [16]. However, the underlying mechanism of HOXA11-AS in the progression of GC remains to be further clarified.

A well-accepted hypothesis suggests that lncRNAs can function as competing endogenous RNAs (ceRNAs) for miRNAs to mediate the expression of cancer-related target mRNAs [17]. The present study was performed to investigate the role of HOXA11-AS in GC. Results revealed that HOXA11-AS expression was significantly elevated in GC tissues and cell lines and associated with clinicopathologic parameters and poor survival of GC patients. Besides, upregulated HOXA11-AS promoted cell migration and invasion via miR-148a/WNT1/β-catenin pathway. Our study provided a novel insight for better understanding this ceRNA network in GC and elucidated a potential target for GC treatment.

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 matched adjacent noncancerous tissues were collected from 30 GC patients who underwent surgical resection between March 2010 and February 2012. All excised tissues were immediately frozen in liquid nitrogen and then stored at –80 °C for the following study. The clinicopathologic parameters of GC patients, containing age, gender, tumor size, lymph node metastasis and TNM stage, were summarized and are shown in Table 1.

The abovementioned patients diagnosed with GC were divided into high HOXA11-AS expression group (17 cases) and low HOXA11-AS expression group (13 cases). Afterward, all patients who underwent surgery were subjected to a follow-up for 5-year Kaplan-Meier survival analysis.

Cell culture and transfection. Human gastric epithelial cell line GES-1 was purchased from American Type Culture Collection (Manassas, VA, USA). 293T cells 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% CO_2.

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 (vector) as a negative control. Small interfering RNA (siRNA) against HOXA11-AS (si-HOXA11-AS), its matched control (si-NC), miR-148a mimic (miR-148a), mimic 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 1×10^5 cells/well, followed by the transfection of the above products with 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, USA) and miRNA was extracted with mirVanaTM miRNA Detection Kit (Thermo Fisher) referring to the manufacturer's procedures. The first-strand cDNA for HOXA11-AS and WNT1 was synthesized using a High Capacity Reverse Transcription System Kit (Takara, Dalian, China) and cDNA for miR-148a was synthesized using a TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher). The following cDNA primer for miR-148a was used: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCG-CACTGGATACGACACAAAG-3'.

Then, qRT-PCR reactions were performed using the Universal SYBR Green PCR Kit (Takara) on an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Results were assessed with $2^{-\Delta\Delta Ct}$ method using GAPDH or U6 small RNA as a housekeeping gene for normal-

Table 1. Association of HOXA11-AS expression with clinicopathological factors in gastric cancer.

Clinicopathological factors	Number	HOXA11-AS expression		p-value
		Down n (%)	High n (%)	
Age				
<50 years	16	7 (43.8%)	9 (56.2%)	>0.05
≥50 years	14	6 (42.9%)	8 (57.1%)	
Gender				
Female	12	6 (50%)	6 (50%)	>0.05
Male	18	7 (38.9%)	11 (61.1%)	
Tumor size				
>3 cm	19	5 (26.3%)	14 (73.7%)	<0.05
≤3 cm	11	8 (72.7%)	3 (27.3%)	
Lymph node metastasis				
Negative	20	12 (60%)	8 (40%)	<0.05
Positive	10	1 (10%)	9 (90%)	
TNM stage				
I–II	22	11 (50%)	11 (50%)	<0.05
III–IV	8	2 (25%)	6 (75%)	

ization of HOXA11-AS, WNT1 or miR-148a, respectively. qRT-PCR primers were displayed as below: HOXA11-AS (Forward, 5'-GAGTTTGAAGCCGTGGATGT-3'; Reverse, 5'-AGATGAGGGGAGAGGTGGAT-3'); WNT1 (Forward, 5'-TGCACGCACACGCGCGTACTGCAC-3'; Reverse, 5'-CAGGATGGCAAGAGGGTTCATG-3'); GAPDH (Forward, 5'-TATGATGATATCAAGAGGGTAGT-3'; Reverse, 5'-TGTATCCAAACTCATTGTCATAC-3'). miR-148a (Forward, 5'-GTGCAGGGTCCGAGGT-3'; Reverse, 5'-GCGTCAGTGCACTACAGAACTT-3'), U6 (Forward, 5'-CTCGCTTCGGCAGCACA-3'; Reverse, 5'-AACGCTTCAGGAATTTGCGT-3').

Transwell assay. The abilities of cell invasion and migration were evaluated using uncoated or Matrigel pre-coated Transwell chambers (BD Biosciences, San Jose, CA, USA) placed in a 24-well plate. Briefly, SGC-7901 and MGC-803 cells supplemented with the serum-free medium were added into the upper chambers, while the bottom chambers were filled with complete medium containing 10% FBS. At 16 h after the incubation, cells on the basal side of the membrane were fixed and stained with hematoxylin (Sigma, St. Louis, USA), followed by the count of migrated or invasive cells in three random fields using a microscope (Olympus, Tokyo, Japan).

Dual-luciferase reporter assay. Putative HOXA11-AS targeting miR-148a or miR-148a targeting WNT1 was predicted by starBase or TargetScan software. Partial sequences of HOXA11-AS or WNT1 3'-UTR containing putative binding sites of miR-148a were subcloned into the psiCHECK-2 vector (Promega, Madison, WI, USA) to generate wild-type luciferase reporter plasmids HOXA11-AS (HOXA11-AS-WT) or WNT1 (WNT1-WT). Then, KOD-plus-mutagenesis kit (Toyobo, Osaka, Japan) was employed to generate the mutant HOXA11-AS (HOXA11-AS-MUT) or WNT1 (WNT1-MUT) plasmids by mutating these nucleotides that potentially bind to miR-148a. Wild-type or mutant luciferase reporter plasmids along with miR-148a or miR-NC were transfected into 293T cells. At 48 h after transfection, the luciferase activity was determined using the Dual-Luciferase Assay Kit (GeneCopoeia, Rockville, MD, USA).

RNA immunoprecipitation (RIP). RIP assay was carried out using Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. In brief, SGC-7901 cell lysate was incubated with protein A/G magnetic beads and anti-Argonaute2 (Ago2, Millipore) for 24 h, with IgG antibody as a negative control. After washing with PBS, the beads were treated with proteinase K to remove the proteins and then HOXA11-AS expression in the immunoprecipitated complex was detected by qRT-PCR assay.

Western blot assay. SGC-7901 and MGC-803 cells were prepared with RIPA lysis (Thermo Fisher) buffer supplemented with proteinase inhibitor. Then, proteins in the supernatant were extracted by high-speed centrifugation and quantified using Pierce BCA Protein Assay Kit (Thermo Fisher). Equal amounts of proteins denatured at 98 °C for 5 min were separated by SDS-PAGE and electro-transferred to PVDF membranes (Millipore). Next, the membranes were blocked with 5% non-fat milk powder, and then incubated with primary antibodies against β-catenin and WNT1 overnight at 4 °C. After washing, the membranes were further incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA, USA) for another 1.5 h. Finally, the protein blots were visualized using ECL Kit (Biorbyt, Shanghai, China) on Bio-Rad ChemiDoc MP imaging system (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis. All experiments were independently performed for at least three repetitions. The data were exhibited as the mean ± standard deviation (S.D.). Statistical significance of differences between groups was assessed by Student's t-test or ANOVA using SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). A p-value less than 0.05 was identified as statistical significance.

Results

HOXA11-AS expression was upregulated in GC and associated with clinicopathologic features. In the present study, HOXA11-AS level in GC tissues was first detected by qRT-PCR. As a result, an enhanced abundance of HOXA11-AS was observed in 30 GC tumor tissues in comparison to that in normal samples (Figure 1A). Afterward, we further explored the correlation between HOXA11-AS and clinicopathologic features. Table 1 summarized that there was no statistical difference between HOXA11-AS expression and age or gender of patients ($p>0.05$). However, high expression of HOXA11-AS was associated with the increased tumor size, lymph node metastasis and the advanced TNM stage (stage III–IV) ($p<0.05$). Furthermore, the survival of GC patients was evaluated by Kapan-Meier survival analysis. As displayed in Figure 1B, the patients with high expression of HOXA11-AS exhibited a lower survival than those with low expression of HOXA11-AS ($p=0.0097$). Additionally, the expression of HOXA11-AS was also examined in GC cell lines by qRT-PCR. As shown in Figure 1C, HOXA11-AS level was significantly upregulated in SGC-7901 and MGC-803 cells compared with that in GES-1 cells. These results indicated that the enhanced abundance of HOXA11-AS in GC might be associated with the development and poor outcomes of GC.

HOXA11-AS promoted migration and invasion of GC cells. Seeing that altered expression of HOXA11-AS was observed in GC cells, we attempted to explore the effect of HOXA11-AS overexpression or knockdown on GC progression. As presented in Figures 2A and 2B, the abundance of HOXA11-AS was effectively upregulated by the transfection of HOXA11-AS overexpression plasmid in SGC-7901 cells but downregulated by the introduction of si-HOXA11-AS

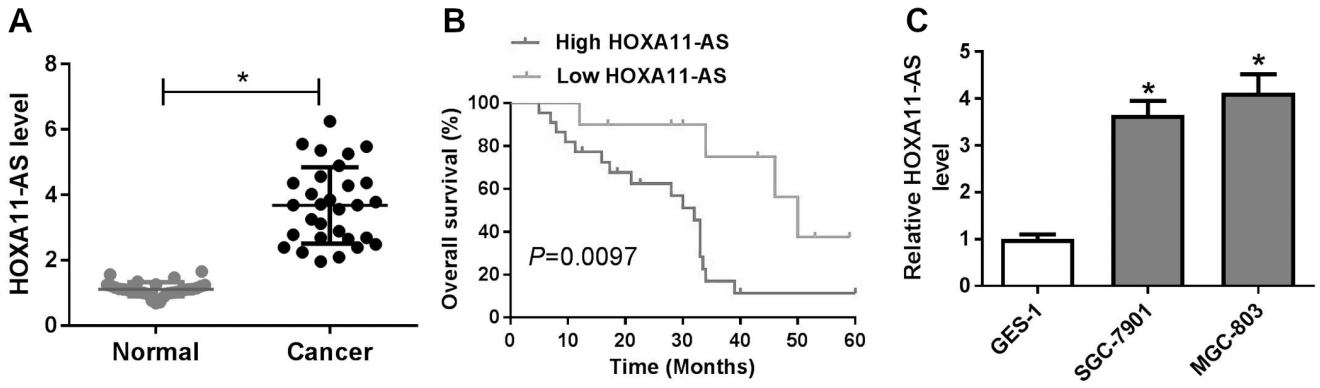

Figure 1. HOXA11-AS was upregulated in GC tissues and cell lines. A) The expression of HOXA11-AS was measured in GC tissues (n = 30) and matched normal tissues (n = 30) by qRT-PCR. B) Kaplan-Meier survival analysis was performed to evaluate the correlation between HOXA11-AS expression and overall survival of 30 GC patients. C) HOXA11-AS expression was detected in GES-1 and two GC cell lines (SGC-7901 and MGC-803) by qRT-PCR. *p<0.05.

in MGC-803 cells when compared to their corresponding controls, respectively. Moreover, the abilities of migration and invasion of GC cells were determined by Transwell assays. Results showed that overexpression of HOXA11-AS notably promoted the migration and invasion (Figures 2C and 2E) of SGC-7901 cells, while knockdown of HOXA11-AS remarkably inhibited migration and invasion in MGC-803 cells (Figures 2D and 2F).

HOXA11-AS served as a molecular sponge for miR-148a. The evidence has revealed that lncRNA can serve as a ceRNA targeting miRNA and mRNA expression to be involved in the progression of multiple cancers [18]. Thus, we expected to probe identifiable miRNA sequences on HOXA11-AS. Interestingly, bioinformatics analysis showed the existence of binding sites between miR-148a and HOXA11-AS using starBase software (Figure 3A), indicating that miR-148a might be a potential target of HOXA11-AS. Then, dual-luciferase reporter and RIP assays were carried out to verify the interplay between them. Results displayed that the luciferase activity of the HOXA11-AS-WT group was obviously reduced following the transfection of miR-148a compared to the miR-NC group, but it was not affected in HOXA11-AS-MUT group (Figure 3B). Moreover, RIP experiments revealed an increased abundance of HOXA11-AS in Ago2 immunoprecipitation complex compared to that in IgG control (Figure 3C). Taken together, these results suggested that HOXA11-AS could interact with miR-148a via complementary binding sites.

HOXA11-AS modulated cell migration and invasion through miR-148a. Next, qRT-PCR assay was performed to measure the expression of miR-148a in GC tissues and cell lines, as well as the regulation of HOXA11-AS on miR-148a expression. The results displayed a notably decreased expression of miR-148a in GC tumor tissues and cell lines relative to matched controls (Figures 4A and 4B). Moreover, miR-148a expression was downregulated following the transfection of HOXA11-AS in SGC-7901 cells, while upregulated after transfecting with si-HOXA11-AS in MGC-803 cells, implying that miR-148a expression was negatively modulated by HOXA11-AS (Figures 4C and 4D). To further investigate whether the effect of HOXA11-AS on cell migration and invasion was mediated by miR-148a in GC, SGC-7901 cells were transfected with vector, HOXA11-AS, HOXA11-AS+miR-NC or HOXA11-AS+miR-148a, and MGC-803 cells were transfected with si-NC, si-HOXA11-AS, si-HOXA11-AS+anti-NC or si-HOXA11-AS+anti-148a. By performing the Transwell assays, we observed that the abilities of cell migration and invasion were significantly increased by HOXA11-AS overexpression in SGC-7901 cells, which was weakened by the restoration of miR-148a (Figures 4E and 4G). Inversely, HOXA11-AS knockdown repressed the migration and invasion in MGC-803 cells, which was reversed by the deficiency of miR-148a (Figures 4F and 4H). Taken together, HOXA11-AS promoted cell migration and invasion by negatively regulating miR-148a in GC cells.

WNT1 was an authentic target of miR-148a. In view of the above efforts, we further expected to predict candidate targets of miR-148a using TargetScan online website. Among these candidate targets of miR-148a, WNT1 was selected because of its indicated oncogenic role in GC [19] (Figure 5A). To further confirm the prediction, WNT1-WT and WNT1-MUT reporter vectors containing wild-type or mutant miR-148a binding sites were constructed for the detection of luciferase activity. We found that the luciferase activity in the WNT1-WT group was dramatically reduced in miR-148a-overexpressed 293T cells compared with that in the miR-NC group, but there was no significant change in WNT1-MUT group (Figure 5B). Next, we found that the WNT1 mRNA level was remarkably upregulated in GC tumor tissues and cell lines relative to corresponding controls (Figures 5C and 5D). Besides, the effect of miR-148a on WNT1 expression was investigated in GC cells by overexpression or knockdown of miR-148a as shown in Figure 5E. Meanwhile, data of western blot assay described that the

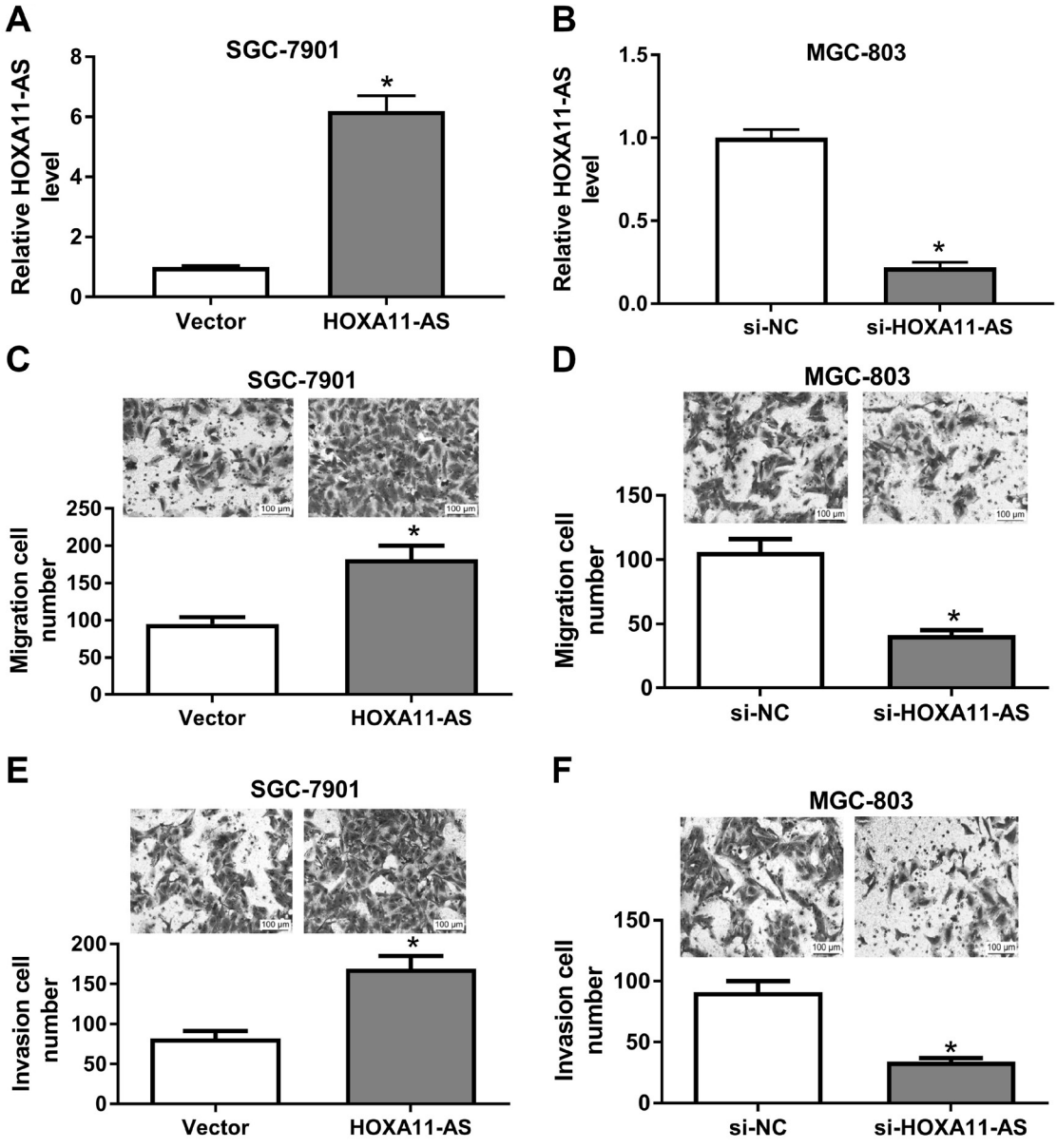

Figure 2. HOXA11-AS increased migration and invasion of GC cells. SGC-7901 cells were transfected with vector or HOXA11-AS, and MGC-803 cells were transfected with si-NC or si-HOXA11-AS. At 48 h after transfection, HOXA11-AS expression was determined by qRT-PCR (A and B). Cell migration (C and D) and invasion (E and F) were assessed by Transwell analyses. *p<0.05.

protein level of WNT1 was negatively mediated by miR-148a in SGC-7901 and MGC-803 cells (Figure 5F). Moreover, the protein expression of β-catenin, a key player of the WNT1/β-catenin signaling pathway, was also suppressed by miR-148a (Figure 5F). Thus, these data suggested that miR-148a inactivated the WNT1/β-catenin pathway via targeting WNT1 in GC cells.

HOXA11-AS activated WNT1/β-catenin pathway through repressing miR-148a. In the present study, rescue experiments were performed to explore whether HOXA11-AS had the potential to modulate the activity of WNT1/β-catenin pathway through sponging miR-148a. SGC-7901 cells were transfected with vector, HOXA11-AS, HOXA11-AS+miR-NC, or HOXA11-AS+miR-148a. MGC-803 cells were transfected with si-NC, si-HOXA11-AS, si-HOXA11-AS+anti-NC, or si-HOXA11-AS+anti-148a. At 48 h after the transfection, WNT1 and β-catenin protein levels were determined by western blot assay. Results showed that overexpression of HOXA11-AS stimulated the expression of WNT1 and β-catenin, while this effect was abrogated by the exogenous restoration of miR-148a (Figure 6A). Furthermore, knockdown of HOXA11-AS led to a great reduction of WNT1 and

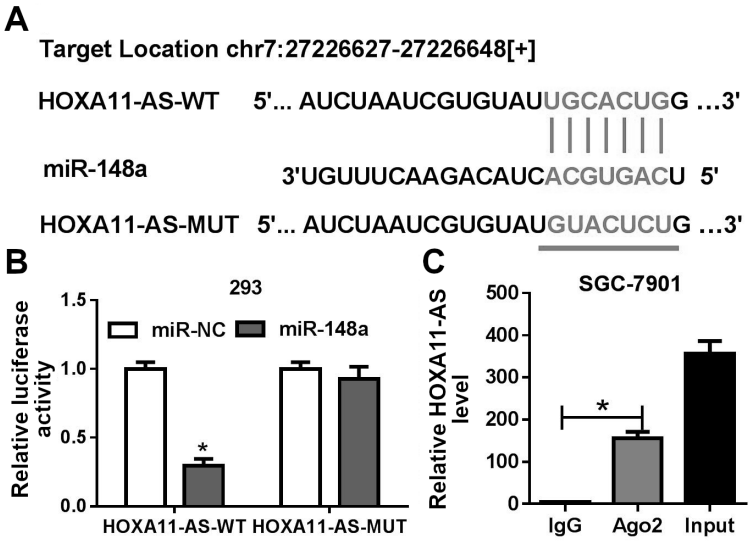

Figure 3. HOXA11-AS acted as a molecular sponge for miR-148a. A) The putative HOXA11-AS targeting miR-148a sequences were predicted using starBase software. B) The activity of HOXA11-AS-WT or HOXA11-AS-MUT construct in 293T cells transfected with miR-NC or miR-148a was determined by dual-luciferase reporter assay. C) RIP analysis was carried out to assess the association between HOXA11-AS and miR-148a in SGC-7901 cells. *p<0.05.

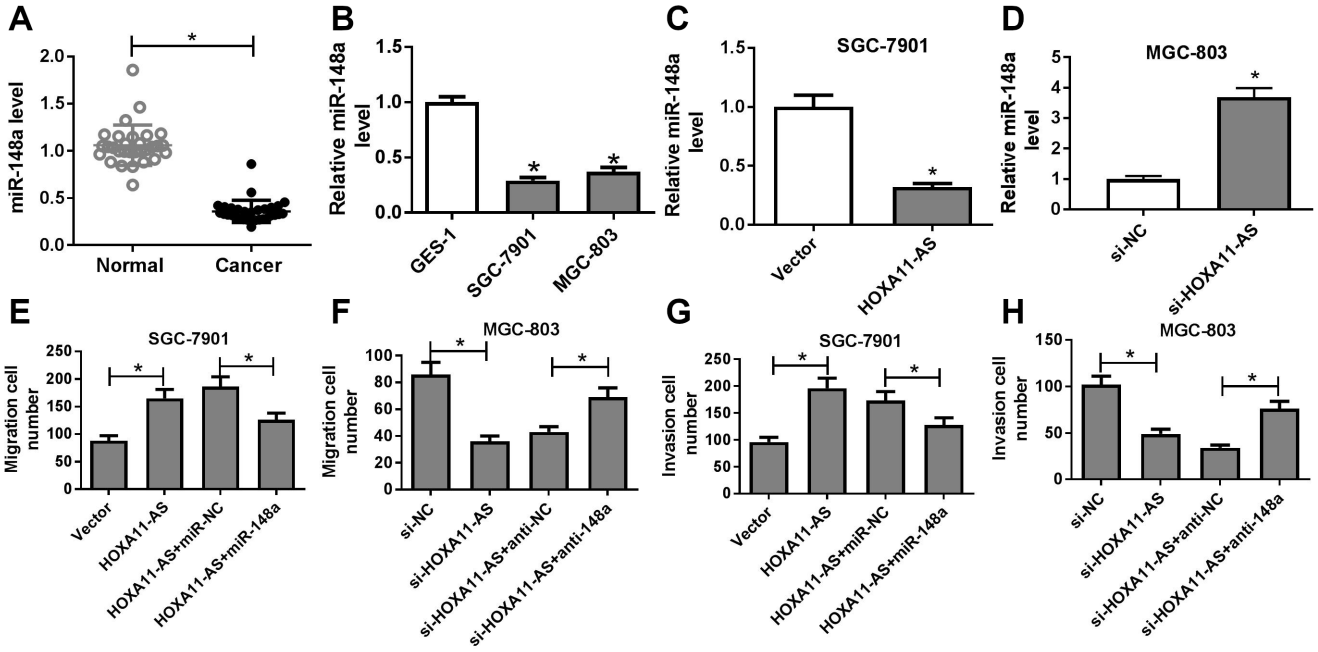

Figure 4. HOXA11-AS promoted cell migration and invasion through sponging miR-148a. (A and B) The expression level of miR-148a was detected in GC tissues (A) and cell lines (B) by qRT-PCR. The expression of miR-148a was assessed following the transfection of HOXA11-AS (C) or si-HOXA11-AS (D) in SGC-7901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

β-catenin protein levels, which was abated by knockdown of miR-148a (Figure 6B). These results indicated that HOXA11-AS activated WNT1/β-catenin pathway by decreasing miR-148a in GC cells.

Discussion

GC is a severe threat to human health and life, leading to a large number of new cases and deaths. LncRNAs, initially

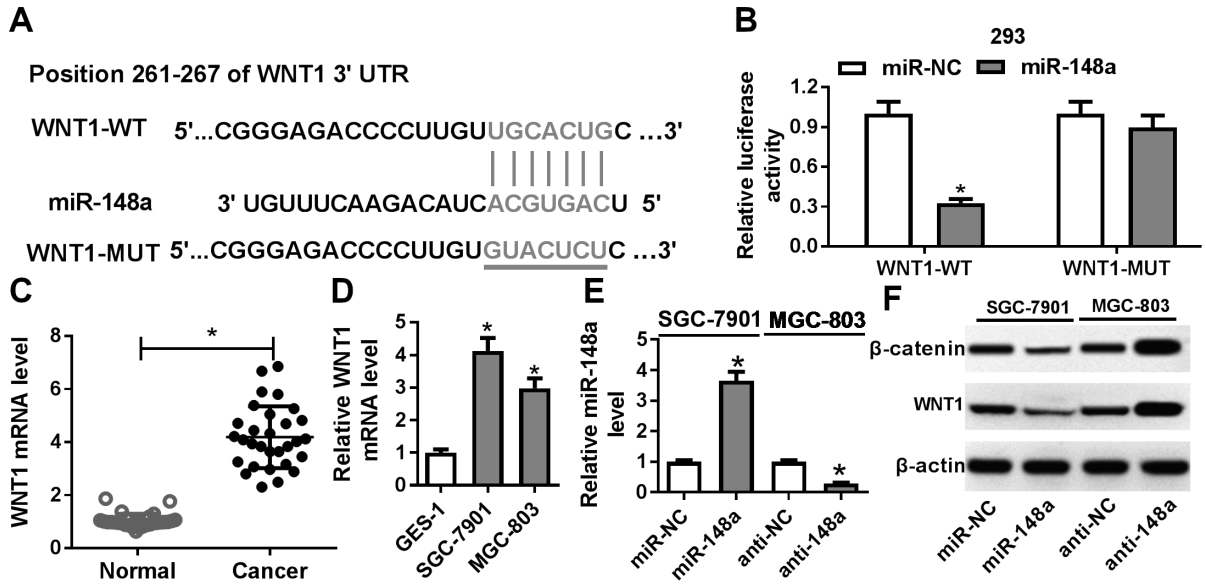

Figure 5. miR-148a directly targeted the 3'-UTR fragments of WNT1. A) The assumed binding sites of miR-148a within WNT1 3'-UTR, as well as the mutant sites containing mutant miR-148a binding sites. B) After 293T cells were co-transfected with miR-NC or miR-148a and luciferase constructs carrying the wild-type or mutated 3'-UTR of WNT1, the luciferase activity was analyzed by a dual-luciferase reporter assay. C and D) WNT1 mRNA level was determined in GC tissues and cell lines by qRT-PCR. E) The level of miR-148a was detected in SGC-7901 cells transfected with miR-148a or miR-NC and MGC-803 cells transfected with anti-148a or anti-NC by qRT-PCR. F) The protein levels of WNT1 and β-catenin in SGC-7901 or MGC-803 cells transfected with miR-148a mimic or inhibitor were determined by western blot assay. *p<0.05.

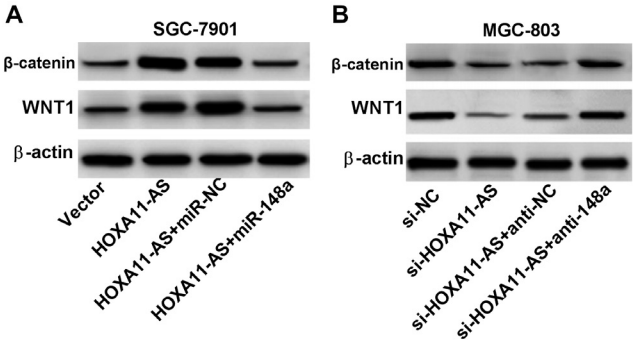

Figure 6. HOXA11-AS activated the WNT1/β-catenin pathway through miR-148a. SGC-7901 cells were transfected with vector, HOXA11-AS, or combined with miR-NC or miR-148a. MGC-803 cells were transfected with si-NC, si-HOXA11-AS, or combined with anti-NC or anti-148a. A and B) At 48 h after transfection, WNT1 and β-catenin protein levels were detected by western blot assay. *p<0.05.

thought to be a transcriptional noise, are emerging as pivotal players of a variety of processes in human cancers, including GC. For instance, lncRNA PANDAR was markedly upregulated in 100 GC tissues and correlated with advanced TNM stage, depth of invasion and lymphatic metastasis in GC [10]. Conversely, five lncRNAs (TINCR, CCAT2, AOC4P, BANCR, and LINC00857)-based index in plasma was significantly downregulated at 14 d after surgery, and high value of this index was associated with the malignancy in GC patients [20].

Increasing literature reported the central roles of HOXA11-AS in various solid cancers. For example, as reported by Yim et al. [21], upregulated HOXA11-AS accelerated cell proliferation and invasion and was associated with the poor outcomes of patients in ovarian cancer. Zhang et al. [22] disclosed an elevated level of HOXA11-AS in NSCLC tissues and cells and HOXA11-AS reduction repressed cell proliferation, migration, invasion and angiogenesis, but induced cell cycle arrest and apoptosis. Moreover, Qu et al. [23] suggested that high expression of HOXA11-AS was associated with the poor survival of patients with laryngeal squamous cell cancer and induced cell migration and invasion. In the present study, we focus on the biological role of lncRNA HOXA11-AS in GC. Results showed that the HOXA11-AS level was elevated in GC tissues and cell lines, and correlated with poor outcomes of patients. Furthermore, gain- and loss-of-function assays indicated that HOXA11-AS induced the malignant phenotypes of GC cells through stimulating cell migration and invasion. Our data are in agreement with the evidence showing that HOXA11-AS-reduction weakened migration, invasion ability of GC cells, as well as inhibited metastasis *in vivo* [24]. These results suggested that HOXA11-AS exerted oncogenic activity in GC, which highlighted the need for further exploration of the regulatory mechanism of HOXA11-AS implicating in GC.

Emerging evidence supported the involvement of lncRNA-associated ceRNA regulatory network in the carcinogenesis of GC [25, 26]. It has been reported that elevated expression of HOTAIR was related to the poor outcomes

of GC patients and induced cell malignant phenotypes by the release of HER2 via competitively binding miR-331-3p [27]. Therefore, we provided the hypothesis that HOXA11-AS implicated in the development of GC via acting as a ceRNA. Bioinformatic, dual-luciferase reporter, and RIP analyses confirmed that HOXA11-AS could act as a sponge for miR-148a. Functional restoration experiments revealed that the introduction of miR-148a weakened HOXA11-AS-induced cell migration and invasion, hinting that HOXA11-AS conferred the malignant phenotypes to GC via directly sponging miR-148a.

Mounting literature suggested that low expression of miR-148a was closely associated with the progression of GC. For instance, a significant loss of miR-148a was detected in most of 20 GC tissues using the miRNA microarray. Abnormally expressed miR-148a was closely correlated with the poor clinical outcomes and involved in the regulation of cell invasion via targeting MMP7 [28]. Elevated expression of miR-148a attenuated cell growth and invasiveness through binding to the 3'-UTR of TGFβ2 and SMAD2 in GC cells [29]. WNT1, a member of the WNT gene family, is widely implicated in cell fate and embryonic development. To date, reliable evidences has suggested the central role of WNT1 in oncogenesis via regulating β-catenin-dependent signaling pathway [30, 31]. It has been reported that miR-148a blocked the migrated and invasive capacities of breast cancer cells by targeting WNT1 and inactivating the WNT1/β-catenin signaling pathway [32].

To further explore the mechanism of HOXA11-AS in the carcinogenesis of GC, the target mRNA of HOXA11-AS-targeted miR-148a was predicted, and results confirmed WNT1 as a functional target of miR-148a. In GC cells, the expression levels of WNT1 and downstream protein β-catenin were remarkably reduced by miR-148a, while actively induced by HOXA11-AS. Furthermore, miR-148a overexpression abolished the promotion of HOXA11-AS on WNT1/β-catenin signaling, supporting that HOXA11-AS activated WNT1/β-catenin signaling pathway through modulation of miR-148a/WNT1 axis.

In conclusion, our data indicated the correlation between HOXA11-AS expression and clinicopathology of patients with GC. Moreover, HOXA11-AS contributed to cell migration and invasion via modulating miR-148a/WNT1/β-catenin pathway. These findings uncovered a novel regulatory mechanism for GC development, which might provide a potential target for GC treatment.

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