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Long non-coding RNA AB073614 promotes metastasis of gastric cancer cells by upregulating IGF-2

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Abstract. – OBJECTIVE: Recently, long non-coding RNAs (lncRNAs) have been widely studied for their vital roles in human diseases. In this study, we investigated the effect of lncRNA AB073614 on the metastasis of gastric cancer (GC), and explored the possible underlying mechanism.

PATIENTS AND METHODS: AB073614 expression in GC tissue samples was detected by Real-time quantitative polymerase chain reaction (RT-qPCR). The roles of AB073614 in GC metastasis were identified through wound healing assay and transwell assay, respectively. Moreover, RT-qPCR and Western blot assay were used to explore the potential mechanism.

RESULTS: AB073614 expression level in GC samples was significantly higher than that of adjacent ones. Besides, the migration and invasion of GC cells were obviously repressed after AB073614 was knocked down. After AB073614 was knocked down *in vitro*, the mRNA and protein expressions of insulin-like growth factor 2 (IGF-2) was remarkably down-regulated. Furthermore, a negative relation was found between the expression of IGF-2 and AB073614 in GC tissues.

CONCLUSIONS: AB073614 could promote cell migration and invasion *via* up-regulating IGF-2. Our findings might provide a potential therapeutic target for GC patients.

Key Words:
Long noncoding RNA AB073614, Gastric cancer, IGF-2.

Introduction

Gastric cancer (GC) is the fourth most prevalent malignant disease and is also the third leading cause of cancer-related death in the world[1,2]. Due to the advances in diagnosis and therapy, the incidence and mortality of GC have been decreasing in recent years. However, GC remains a threat to public health. More than 24,590 patients are diagnosed with GC every year in America, with about 10,720 deaths[3]. Most of GC patients are diagnosed at an advanced stage because of atypical or absent symptoms at early stage. This greatly limits successful therapeutic interventions[4]. Therefore, it is urgent to identify the underlying mechanism of GC and to find out novel markers for its therapeutic strategy. Long non-coding RNAs (lncRNAs), a cluster of non-coding transcripts, have been proved to play an important role in various heterogeneous molecular activities. They function functioning as a ceRNA sponging miR-215, lncRNA UICLM enhances colorectal cancer liver metastasis and modulates the expression level of ZEB2[5]. LncRNA SNHG15, mediated by E2F1, participates in MNX1-AS1/miR-218-5p-SEC61A1 feedback loop and promotes the progression of colon adenocarcinoma[6]. Meanwhile, lncRNA CDKN-2B-AS1 promotes the growth and migration of hepatocellular carcinoma cells through miR-153-5p/ARID3A signaling pathway[7]. Overexpression of lncRNA FALEC facilitates the proliferation of melanoma cells by silencing p21. LncRNA FALEC upregulation is also associated with poor prognosis of patients with melanoma[8]. In addition, lncRNA BACE1-AS inhibits the proliferation and invasion of ovarian cancer stem cells, eventually functioning as a novel target for ovarian cancer[9]. However, the specific role of lncRNA AB073614 in the metastasis of GC has not been fully elucidated. In our study, the expression of AB073614 was significantly up-regulated in GC tissues. Functional assays showed that AB073614 promoted the migration and invasion of GC cells *in vitro*. In addition, we also explored the underlying mechanism of AB073614 function in GC development.

Patients and Methods

Cell Lines and Clinical Samples

Human GC tissues were collected from 48 GC patients who received surgery at Affiliated Wujiang Hospital of Nantong University between February 2015 and December 2017. No patients

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received radiotherapy or chemotherapy before operation. All collected fresh tissues were kept at -80°C for subsequent use. This study was approved by the Ethics Committee of Affiliated Wujiang Hospital of Nantong University. Signed written informed consents were obtained from all participants before the study.

Cell Culture

3 human GC cell lines (SGC-7901, BGC-823, HGC-27) and 1 normal human gastric epithelial cell line (GES) were cultured in Dulbecco's Modified Eagle'sMedium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and penicillin. All cells were maintained in an incubator with 5% CO_2 at 37°C.

Cell Transfection

The cDNA oligonucleotides targeting AK027294 (sh-AB073614) were provided by GenePharma and cloned into pGPH1/Neo vector (GenePharma, Shanghai, China). Subsequently, sh-AB073614 and negative control (NC) were transfected into GC cells according to re instructions. 48 h later, transfection effici transfected cells was verified by real-time q tative polymerase chain reaction (RT-qPCR).

RNA Extraction and RT-qPCR

Total RNA in tissues and cell s ex ted by TRIzol reagent (Invitrogen, C bad, C Then extracted RNA was re ibed t complementary deoxyribo e nu ds (cDNAs) in strict accordanc h revers ip- tion Kit (TaKaRa Biote logy Co., Ltd., an, China). Primers used PCR were shown as follows: AB07 , 5′-ATTTCT-GCTCCTGGGTCT AC-3′ an 5′-AGT-GGCTTGTCTC AGAGTC-3′; G raldehyde 3-phosphate d droge ase (GAPDH), forward 5′-CACCCA CTC CTTTG-3′ and reverse 5′-CCACC TG GCTG G-3′. Specific thermal cycle s at 95 5 s for 40 cycles at 95°C, and 35 s

Wo ling Assay

nsfec e first seeded into 6-well p nd Dulbecco's Modified Eagle n (DMEM) overnight. Next, they were s with a plastic tip, followed by culture in s ee DMEM. 48 h later, wound closed area was iewed and analyzed.

Transwell Assay

To detect the migration ty of ells, 4×10^4 cells in 200 µL se free DME ere transformed to the upp mber of a um pore size insert (Millipor a, MA A). Meanwhile, the low chamb with DMEM and fetal b e serum (n later, the top surface of mber as wipe y cotton swab before im ed w re-co ed methanol for 10 min. Th hen st d with crystal violet for 30 mi ct th vasion ability of GC cell $\times10^4$ c µL serum-free DMEM v transformed e upper chamber of an 8 size insert (Millipore, Billerica, MA,), s previously coated with 50 µg Ma igel (B nces, Franklin Lakes, N A). Meanwhi e lower chamber was f with DMEM and FBS. 48 h later, the top face of chambers was wiped by cotton swab ore immersed h pre-cooled methanol for 10 The cells e then stained with crystal vio- ually, cells were observed under a m and the number of migrating and invading was calculated.

Blot Analysis

proteins were extracted *via* radioimmunoprecipitation assay (RIPA) buffer. The concentration of protein sample was determined by the bicinchoninic acid (BCA) method (Beyotime, anghai, China). Target proteins were separated by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were incubated with primary antibodies of rabbit anti-GAPDH (Cell Signaling Technology, CST, Danvers, MA, USA) and rabbit anti-IGF-2 (Cell Signaling Technology, CST, Danvers, MA, USA) overnight. On the next day, the membranes were incubated with corresponding secondary antibodies. Chemiluminescent film was applied for assessing protein expression with Image J software (NIH, Bethesda, MD, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (Armonk, NY, USA) was adopted for all statistical analysis. Data were presented as mean ± standard deviation (SD). Each assay was independently repeated in triplicate. Student *t*-test was utilized to compare the difference between two groups. $p<0.05$ was considered statistically significant.

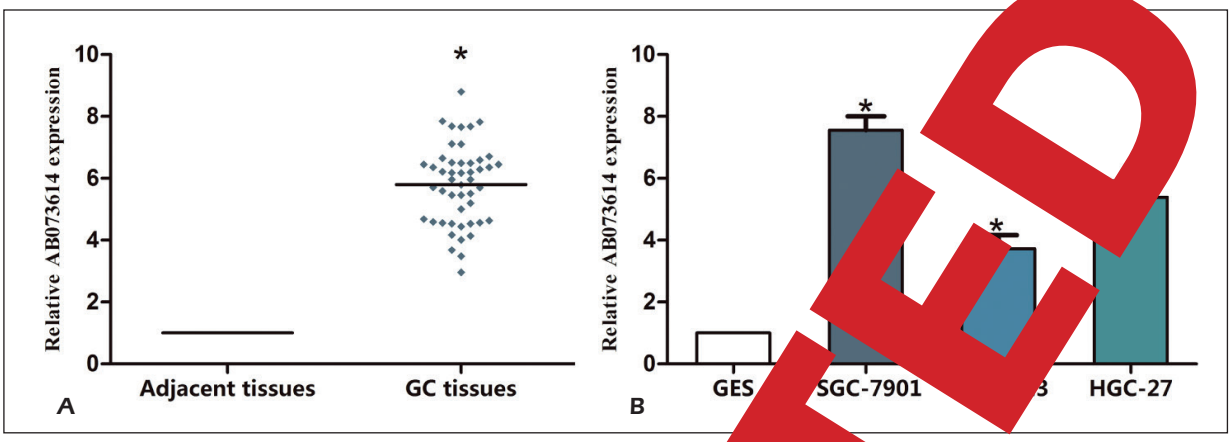

Figure 1. Expression of AB073614 was up-regulated in GC tissues and cells. **A**, AB073614 expression significantly increased in GC tissues compared with adjacent tissues. **B**, Expression levels of AB073614 were determined in GC cell lines (SGC-7901, BGC-823, HGC-27) and normal human gastric epithelial cell line (GES) by RT-qPCR. GAPDH was used as an internal control. *p<0.05.

Results

The Expression Level of AB073614 in GC Tissues and Cells

AB073614 expression in 48 patients' tissues and 3 GC cell lines was first detected by RT-qPCR. Results showed that AB073614 expression in GC tissues was significantly higher than that of adjacent tissues (Figure 1A). Similarly, AB073614 level in GC cells was remarkably higher than that of GES cells (Figure 1B).

Knockdown of AB073614 Inhibited GC Cell Migration and Invasion

In our study, SGC-7901 GC cells were selected for transfection of AB073614 shRNA. RT-qPCR was utilized to verify transfection efficiency (Figure 2A). Subsequent wound healing assay showed that the migration ability of GC cells was significantly repressed after knockdown of AB073614 (Figure 2B). Transwell assay revealed that after AB073614 was knocked down *in vitro*, the number of migrated cells decreased significantly (Figure 3A and 3B). Furthermore, transwell assay showed that after AB073614 knockdown, the number of invaded cells decreased remarkably as well (Figure 3C and 3D).

Interaction Between IGF-2 and AB073614 in GC

Further mechanism assays showed that compared with IGF-2 level in NC group, the expression level of IGF-2 in GC cells of sh-AB073614 group was significantly higher (Figure 4A). Western blot assay showed that after AB073614 was knocked down, the protein level of IGF-2 was obviously down-regulated (Figure 4B). Moreover, IGF-2 expression in GC tissues was remarkably up-regulated when compared with adjacent tissues (Figure 4C). Furthermore, IGF-2 expression level was positively correlated with AB073614 expression in GC tissues (Figure 4D).

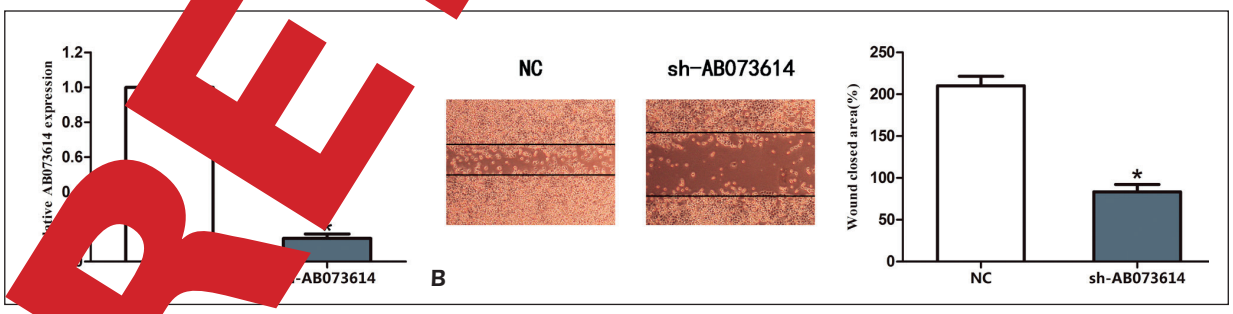

Figure 2. Silence of AB073614 inhibited GC cell migration. **A**, AB073614 expression in GC cells transfected with AB073614 shRNA (sh-AB073614) and negative control (NC) was detected by RT-qPCR. GAPDH was used as an internal control. **B**, Wound healing assay showed that silence of AB073614 significantly repressed the migration of GC cells (magnification: 10×). The results represented the average of three independent experiments. *p<0.05.

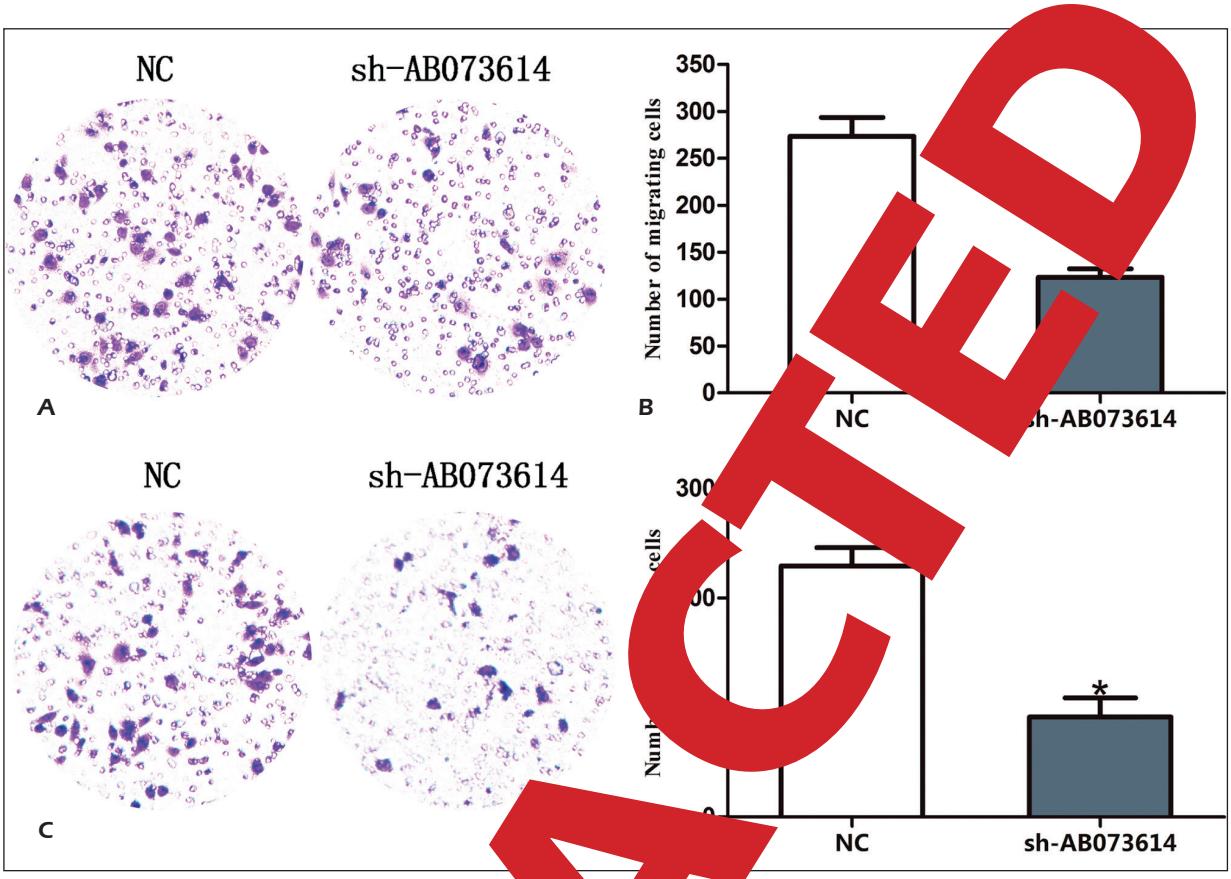

Figure 3. Silence of AB073614 inhibited GC cell migr vasion. **A**, The representative pictures of migrated cells in sh-AB073614 group and NC group (magnification: 40×). umber of migrated cells decreased significantly *via* silence of AB073614 *in vitro*. **C**, The representati tures of inv lls in sh-AB073614 group and NC group (magnification: 40×). **D**, Transwell assay showed that the invaded c reased significantly *via* silence of AB073614 *in vitro*. The results represented the average of thre experiment $p<0.05$, as compared with control cells.

Discussion

Evidence demonstr that lncRNA y a pivotal role in the pr of GC. For example, acting as a sp 3p, lncRNA SNHG20 promotes the prolifer invasion of GC cells *vi* regulating the ression of ZFX[10]. By cro k wit miR-21, lncRNA LINC-PINT functi as a or suppressor in GC. Meanwhile lso ociate h poor survival of GC patie NA 554202 enhances the proliferatio ion of GC cells by regula E-cadhe p21[12]. Overexpression CTD-251 5.4 is associated with ma ant p GC, which predicts poor p is ts[13]. In addition, lncRNA SN es as an important anti-oncogene in the prog f GC through trapping MTA2 in cytosol[14]. Li AB073614, as a novel lncRNA, promotes tumorigenesis of ovarian cancer and predicts poor prognosis for these patients[15]. AB073614 plays an important role in regulation of colorectal cancer proliferation and migration by modulating PI3K/AKT signaling pathway[16]. AB073614 promotes the proliferation and migration of glioma cells by regulating epithelial-mesenchymal transition. It also indicates poor prognosis of patients with glioma[17]. In this study, we found that AB073614 was significantly upregulated in GC samples. Besides, GC cell migration and invasion were remarkably suppressed through knockdown of AB073614, suggesting that AB073614 acted as an oncogene in GC cells. IGF-2 is a protein hormone, which plays a crucial role in anti-apoptosis and mitosis. Recently, IGF2 has been widely explored to be involved in the modulation of tumor growth. For instance, the mRNA expression of IGF2 is upregulated in fibroadenomas, especially in stromal cells[18]. Up-regulation of IGF2 is reported to be remarkably correlated with poor prognosis

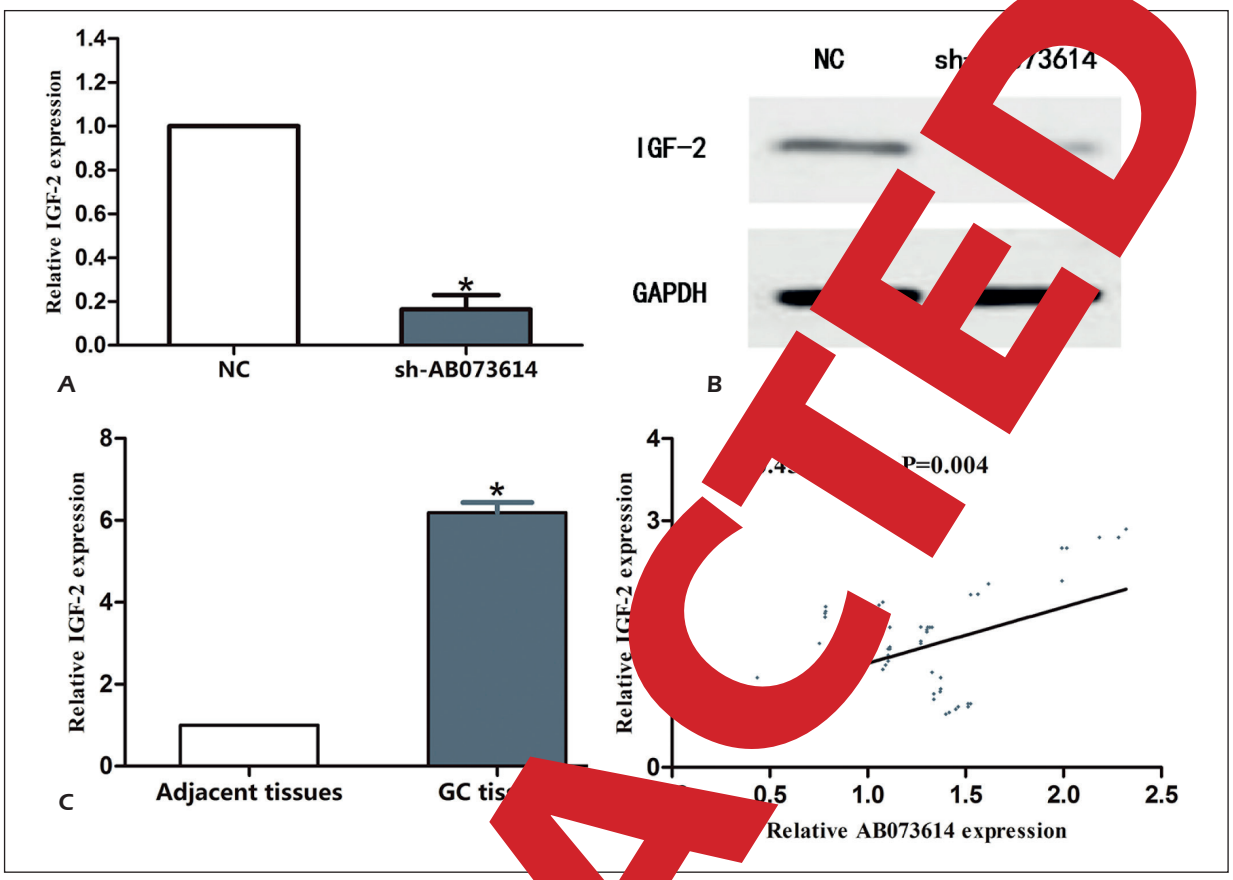

Figure 4. The association between AB073614 and IGF-2 in GC tissues. **A**, RT-qPCR results showed that IGF-2 expression in sh-AB073614 group was significantly lower than NC group. **B**, Western blot assay revealed that IGF-2 protein expression decreased remarkably in sh-AB073614 group compared with NC group. **C**, IGF-2 was significantly up-regulated in GC tissues compared with adjacent tissues. **D**, Correlation between the expression level of IGF-2 and AB073614 in GC tissues. The results represented the average of three independent experiments. *$p<0.05$.

of ovarian cancer patients. Three... pressing IGF2 expression and IGF... mediated ...OR pathway, curcumin functions as a tumor suppressor in the development of epithelial tumor[20]. Moreover, lncRNA ... enhance the aggressive phenotype of breast cancer cells, while, it positively regulates the expression of ...9/IGF2 *via* epigenetic modifications. Our study showed that IGF-2 expression was significantly downregulated *via* knockdown of AB073614 in GC cells. Besides, IGF-2 expression positively correlated with AB073614 expression in tissues.

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...ed that AB073614 could enhance GC cell migration and invasion through up-regulating IGF-2. Our findings suggested that AB073614 might be a candidate target for GC therapy.

Conflict of Interests

The authors declare that they have no conflict of interest.

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