A novel long non-coding RNA NFIA-AS1 is down-regulated in gastric cancer and inhibits proliferation of gastric cancer cells

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Abstract

Gastric cancer is one of the most common malignant gastrointestinal tumors whose morbidity and mortality account for the second and third place respectively in malignant tumors in China. As an important participant in tumor biology, the abnormal expression of long non-coding RNA (lncRNAs) in cancer cells is closely related to the occurrence and development of tumors and plays the role of oncogenes or tumor suppressor genes. In this study, we identified a novel lncRNA NFIA antisense RNA 1 (NFIA-AS1) and explored its role and clinical significance in gastric cancer. Real-time quantitative PCR was performed to detect the expression of NFIA-AS1 in tumor tissues and corresponding normal tissues from 42 pairs of gastric cancer samples. The lower expression of NFIA-AS1 was significantly associated with larger tumor size, lower histological grade, and advanced TNM stage. Kaplan-meier analysis showed that NFIA-AS1 expression could be used as an independent predictor of overall survival. We also demonstrated that overexpression of NFIA-AS1 significantly inhibited the proliferation of gastric cancer cells through affecting p16 levels. In conclusion, our results suggest that the lncRNA NFIA-AS1 may play the role of tumor suppressor gene, and serve as a biomarker for prognosis or progression of gastric cancer.

Keywords: gastric cancer, long non-coding RNA, NFIA antisense RNA 1 (NFIA-AS1), cell proliferation

Introduction

Gastric cancer is the second leading cause of cancer-related death in the world as well as one of the most common malignant gastrointestinal tumors in China1–3. The rapid development and metastasis of gastric cancer lead to its poor prognosis. Although surgical treatment and targeted molecular therapies have made significant progresses in the clinical treatment of gastric cancer, the overall 5-year survival rate of patients is still abysmal. To improve early diagnosis and targeted therapy of gastric cancer...
Effectively, there is an urgent need to discover the underlying molecular mechanisms and to identify new prognostic biomarkers and therapeutic targets[1].

Besides approximately 2% protein-coding genes, the vast majority of the human genome consists of non-coding RNAs (ncRNAs), which are not capable of being translated into proteins[4]. These ncRNAs affect the regular expression of many genes, including oncogenes and tumor suppressor genes, and may play an essential regulatory role in complex organisms, making them a new category of targets for cancer drug development[4]. ncRNAs are divided into two major classes based on the transcript size: small ncRNAs (<200 nt) and long non-coding RNAs (lncRNAs)[5–6]. lncRNA is a class of transcripts longer than 200 nucleotides that do not encode any proteins. Recently, many studies have demonstrated that lncRNAs are involved in a variety of diseases and have multiple functions in a wide range of biological processes, particularly in human cancer[7–9]. Multiple pieces of evidence have suggested that lncRNAs had carcinogenic and tumor suppressive effects through regulating gene expression at the level of transcription, post-transcription in tumorigenesis, which makes lncRNA as an ideal candidate for a better understanding of cancer developments, including gastric cancer[10–12]. Pseudogene-derived lncRNA POU5F1B is highly expressed in gastric cancer and is associated with clinical prognosis in patients with gastric cancer[9]. Zhang et al showed that H19 could promote the proliferation, invasion, and metastasis of gastric cancer cells and inhibit cell apoptosis[14].

Recently, many reports have shown that lncRNA is highly expressed in gastric cancer tissues, while a limited number of studies have investigated the low-expression of lncRNA[18]. In our current study, we sought to determine the clinical significance and function of dysregulated lncRNAs in the development of gastric cancer. Therefore, it is necessary to not only identify new lncRNAs, but also explore their biological roles in gastric cancer. In recent study, we present a novel lncRNA, NFIA antisense RNA 1 (NFIA-AS1), which is located at chromosome 1p31.3 and is transcribed into a 4,574 nt transcript. NFIA-AS1 is significantly more down-regulated in gastric cancer than that in corresponding adjacent tissues. We then found that low expression of NFIA-AS1 is associated with poor prognosis in patients with gastric cancer. Overexpression of NFIA-AS1 was found to inhibit the proliferation of gastric cancer cells both in vitro and in vivo. Besides, our results suggest that NFIA-AS1 as a tumor suppressor gene may partially affect p16 expression in gastric cancer. Collectively, the results from this study indicate that lncRNA NFIA-AS1 may represent a new prognostic indicator of gastric cancer and may be a potential therapeutic target for gastric cancer diagnosis and gene therapy.

Materials and methods

Tissue collection

Forty-two pairs of gastric cancer and the corresponding adjacent tissues were taken from patients who were diagnosed with gastric cancer according to histopathological evaluation and underwent surgery in Jiangsu Provincial Hospital and Nanjing First Hospital from April 2008 to April 2012. No local treatment, radiotherapy or chemotherapy was performed to treat these patients before surgery. All tissues were immediately placed in liquid nitrogen and stored until total RNA was extracted. The research on this subject was approved by the Ethics Committee of Nanjing Medical University (Nanjing, China), and informed consent was obtained from all the patients. All specimens from patients with gastric cancer had a histopathological diagnosis and detailed clinical, pathological data. The clinical characteristics of the participants are summed up in Table 1.

Cell lines and cell culture

Four human gastric cancer cell lines (SGC7901, BGC823, HGC27 and AGS), and a gastric epithelial cell line (GES-1) were purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). SGC7901 were cultured in DMEM and AGS, BGC823 and HGC27 were cultured in RPMI 1640 (GIBCO-BRL, NY, USA) medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin in an incubator at 37 °C with 5% CO2.

Transfection of gastric cancer cells

The full-length transcript sequence of human NFIA-AS1 gene (NR_104180) was synthesized according to the RefSeq database (http://www.ncbi.nlm.nih.gov/refseq/) by chemical synthesis and then cloned into a pcDNA vector (Invitrogen, NY, USA). The pcDNA-NFIA-AS1 or empty vector was transfected into SGC7901 and AGS cells using X-treme GENE HP DNA transfection reagent (Roche, Basel, Switzerland), according to the manufacturer's instructions. The empty pcDNA vector was used as the control.

RNA extraction and qRT-PCR analysis

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Invitrogen, Grand Island,
NFIA-AS1 inhibits gastric cancer cell proliferation

MTT analysis and cell colony formation assays

MTT analysis was performed using the MTT kit (BioFROXX, Einhausen, Germany) to observe the proliferative activity of gastric cancer cells. The transfected cells were seeded in each well of a 96-well plate and evaluated at every 24 hours according to the manufacturer’s instructions. For the colony formation assay, cells were placed in 6-well plates and maintained in medium containing 10% FBS for about 12 days, and the medium was replaced every 4 days. Colonies were fixed with methanol and stained with 0.1% crystal violet (Sigma, St. Louis, USA). The result of the colony formation assay was determined by counting the number of stained colonies.

EdU analysis

Proliferating cells were evaluated using the 5-ethynyl-2-deoxyuridine (EdU) labeling/detection kit (Ribobio, Guangzhou, China) according to the manufacturer’s introductions. The gastric cancer cells were cultured in 24-well plates at 5×10^3 cells/well. Twenty-four hours after transfection with plasmid DNA, 50 μmol/L EdU labeling medium was added to the cell culture and incubated at 37 °C for 2 hours under 5% CO_2. Next, the cultured cells were fixed with 4% paraformaldehyde (pH7.4) for 30 minutes and treated with 0.5% Triton X-100 at room temperature for 30 minutes. After being washed with phosphate-buffered saline (PBS), the samples were stained with anti-EdU working solution at room temperature for 30 minutes. Subsequently, the cells were incubated with 100 μL of Hoechst 33342 (5 μg/mL) at room temperature for 30 minutes and then observed under a fluorescence microscope. The percentage of EdU-positive cells was calculated from three random fields in three wells for each treatment group.

Transwell assay

For the transwell assay, 5×10^4 or 8×10^4 gastric cells were placed in the medium containing 10% FBS into the upper chamber of an insert (8 μm pore size, Millipore, Billerica, USA), while the lower chamber was added with medium containing 20% FBS. For cell invasion analysis, the matrigel matrix was used (Corning, NY, USA). After incubation for 24 hours, the cells remaining on the upper membrane were removed with cotton wool, whereas the cells that had migrated or invaded through the membrane were stained with methanol and 0.1% crystal violet, and then imaged and counted using an IX71 inverted microscope (Olympus, Tokyo, Japan).

### Table 1 The clinic-pathological factors of gastric cancer patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>Expression of NFIA-AS1 Low [n (%)]</th>
<th>High [n (%)]</th>
<th>P-value*</th>
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<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male</td>
<td>23</td>
<td>12 (52.2)</td>
<td>11 (47.8)</td>
<td>0.757</td>
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<tr>
<td>Female</td>
<td>19</td>
<td>9 (47.4)</td>
<td>10 (52.6)</td>
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<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤60 years</td>
<td>17</td>
<td>10 (58.8)</td>
<td>7 (41.2)</td>
<td>0.346</td>
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<tr>
<td>&gt;60 years</td>
<td>25</td>
<td>11 (44.0)</td>
<td>14 (56.0)</td>
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<td>Histological grade</td>
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<td>20 (57.1)</td>
<td>15 (42.9)</td>
<td>0.079</td>
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<td>Low</td>
<td>31</td>
<td>18 (58.1)</td>
<td>13 (41.9)</td>
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<tr>
<td>Middle or high</td>
<td>11</td>
<td>3 (27.3)</td>
<td>8 (72.7)</td>
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<tr>
<td>Tumor stage</td>
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<tr>
<td>I</td>
<td>7</td>
<td>1 (14.3)</td>
<td>6 (85.7)</td>
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<tr>
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<td>35</td>
<td>20 (57.1)</td>
<td>15 (42.9)</td>
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<td>Tumor size</td>
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<tr>
<td>≤5 cm</td>
<td>15</td>
<td>4 (26.7)</td>
<td>11 (73.3)</td>
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<tr>
<td>&gt;5 cm</td>
<td>27</td>
<td>17 (63.0)</td>
<td>10 (37.0)</td>
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<tr>
<td>N0</td>
<td>13</td>
<td>5 (38.5)</td>
<td>8 (61.5)</td>
<td></td>
</tr>
<tr>
<td>N1 or above</td>
<td>29</td>
<td>16 (55.2)</td>
<td>13 (44.8)</td>
<td></td>
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</table>

*For analysis of correlation between lncRNA NFIA-AS1 levels and clinical features, Pearson’s Chi-square tests were used.

USA) at 48 hours after transfection. For quantitative real-time PCR (qRT-PCR) analyses, RNA was reversely transcribed to cDNA by using a Reverse Transcription Kit (Takara, Dalian, China). QRT-PCR was performed with SYBR Green (Takara, Dalian, China). The results were calculated and normalized to GAPDH using the 2^−ΔΔCt method.
Flow cytometry analysis

For the cell cycle analysis, the cells were stained with PI using the Cycle TESTTM PLUS DNA Reagent Kit (BD Biosciences, San Jose, USA) following the manufacturer's protocol and were then analyzed by FACScan. The percentages of cells in the G0–G1, S, and G2–M phases were counted and compared. All of the samples were analyzed in triplicate.

Tumor formation assay in a nude mouse model

Four-week-old male athymic BALB/c nude mice were maintained under specific pathogen-free conditions and operated according to regimen approved by Animal Core Facility of Nanjing Medical University. SGC7901 cells were stably transfected with pcDNA-NFIA-AS1, or empty vector was harvested at a concentration of 2×10⁷ cells/mL, and 0.1 mL of the suspension cells were injected subcutaneously into either side of the nude mice. The tumor size (length × width² × 0.5) was measured every 2 days in mice from the control (4 mice) or pcDNA-NFIA-AS1 (4 mice) groups. On the sixteenth day after injection, tumors were removed from all mice and fixed in 4% of paraformaldehyde after measuring tumor weight. Primary tumors were excised and used for immunostaining analysis of Ki-67 protein expression.

Western blotting assay

Cell protein lysates were separated by 10% SDS-PAGE, transferred to 0.2 µm PVDF membranes (Millipore), and incubated with specific antibodies. Autoradiograms were quantified by densitometry (Quantity One software, Bio-Rad, USA). GAPDH antibody was used as a control. Anti-p16 was purchased from Abcam (ab108349, USA).

Statistical analysis

Statistical analysis was performed using the SPSS version 17 software package. Statistical significance was properly tested by the Student's t-test or Chi-square test. Survival analysis was performed using the Kaplan-Meier method, and the log-rank test was used to compare the differences between patient groups. A P value <0.05 was considered to be statistically significant.

Results

Expression of NFIA-AS1 was down-regulated in human gastric cancer tissues and associated with poor prognosis of gastric cancer

First, we detected the levels of lncRNA NFIA-AS1 in gastric tumor tissues compared with normal tissues adjacent to cancer by qRT-PCR. The results showed that, among all the 42 pairs of gastric cancer patients, the expression levels of IncRNA NFIA-AS1 in tumor tissues were lower than those in the corresponding normal tissues, with a median ratio of 0.64 compared with the normal group (Fig. 1A). Next, we examined the correlation between NFIA-AS1 expression levels and the clinicopathological factors of gastric cancer patients. The results showed that the expression of NFIA-AS1 was correlated with tumor size, histological grade, and TNM stage. The lower expression of NFIA-AS1 was positively related to greater tumor size, lower histological grade, and advanced TNM stage, respectively (Fig. 1B–D). The clinical characteristics of all patients were shown in Table 1. Besides, we evaluated the association between NFIA-AS1 expression levels and the prognosis of patients with gastric cancer. For this, the 42 gastric cancer patients were divided into two groups: relatively high-NFIA-AS1 group (n=21, NFIA-AS1 expression ratio ≥ median ratio) and relatively low-NFIA-AS1 group (n=21, NFIA-AS1 expression ratio ≤ median ratio) according to the median ratio of relative NFIA-AS1 expression (0.64) in tumor tissues compared with the normal group (Fig. 1E). Kaplan-Meier survival analysis and log-rank test showed that lower expression levels of NFIA-AS1 were associated with shorter overall survival (P<0.001, Fig. 1F).

Overexpression of NFIA-AS1 inhibited the proliferation of gastric cancer cells in vitro

To assess the biological function of NFIA-AS1, we first examined the expression levels of NFIA-AS1 using qRT-PCR in a variety of cell lines, including SGC7901, BGC823, AGS, HGC27, and normal gastric epithelium cell line GES-1. The results showed that the expression levels of NFIA-AS1 were significantly downregulated in two gastric cancer cell lines (SGC7901 and AGS) compared with that in GES-1 (Fig. 2A). Then we selected AGS and SGC7901 cell lines for the next experimental study. Many lncRNAs were reported in previous studies in gastric cancer. For example, HOXC-AS3 could promote the proliferation, migration, and invasion of gastric cancer cells[19]. To observe the function of NFIA-AS1 in gastric cancer cells, pcDNA-NFIA-AS1 was transfected into both AGS, and SGC7901 cells, and the expression levels of NFIA-AS1 were assessed using qRT-PCR analysis compared to the empty vector control (Fig. 2B). After transfection, MTT and colony formation assays were performed. Compared
with cells transfected with the empty vector, the transfection of pcDNA-NFIA-AS1 significantly reduced the viability of SGC7901 and AGS cells (Fig. 2C and 2D). Similarly, EdU (red)/DAPI (blue) immunostaining also demonstrated that overexpression of NFIA-AS1 significantly reduced the rate of proliferating cells in both AGS and SGC7901 cells (Fig. 2E). These findings suggest that NFIA-AS1 may act as a tumor suppressor involved in the inhibition of gastric cancer cell proliferation.

NFIA-AS1 affected gastric cancer cell cycle and inhibited cell migration in vitro

To further investigate whether NFIA-AS1 affects the proliferation of gastric cancer cells by altering the normal division process of cells, the cell cycle changes of gastric cancer cells were detected by flow cytometry after overexpression of NFIA-AS1 in both SGC7901 and AGS cells. The results showed that the cell cycle progression of pcDNA-NFIA-AS1 transfected SGC7901 cells was significantly stagnant in G1–G0 phase compared with cells transfected with empty vector. Similar effects were also observed in AGS cell line (Fig. 3A), which indicated that NFIA-AS1 may regulate the proliferation of gastric cancer cells by controlling the cell cycle progression of gastric cancer. In addition to the proliferative capacity of gastric cancer cells, tumor cell migration and invasion are crucial aspect of cancer progression. Thus, we evaluated gastric cancer cell migration and invasion by performing a transwell assay. As shown in Fig. 3B, overexpression of NFIA-AS1 inhibited the migration of SGC7901 and AGS cells, and significantly reduced the number of migrating cells. At the same time, we performed cell invasion experiments in SGC7901 cells. We could observe that the cell invasion ability of SGC7901 cells was weakened with overexpressed NFIA-AS1 levels (Fig. 3C). These results imply that NFIA-AS1 has a tumor-suppressing property and inhibits the migration of gastric cancer cells.

NFIA-AS1 regulated cell cycle and cell proliferation in gastric cancer through affecting p16 levels

To further investigate the role of NFIA-AS1 in GO/G1 growth arrest, we examined the expression of inactivation of CDK inhibitors (CKIs). As shown in Fig. 4A, the results showed significant up-regulation of p16 and p15 in both SGC7901 and AGS cells with overexpression NFIA-AS1 compared with those with an empty vector. However, the expression levels of p21, p27, and p57 were not increased (Fig. 4A). The
Fig. 2  Effects of NFIA-AS1 on gastric cancer cell growth in vitro. A: The relative expression level of NFIA-AS1 in one normal gastric cell line and four gastric cancer cells. B: qRT-PCR analyses of NFIA-AS1 expression level following treatment SGC7901 and AGS cells with pcDNA-NFIA-AS1 or the empty vector. C: At 24 hours after transfection, MTT assay was performed to determine the cell vitality of SGC7901 and AGS cells. D: Representative results of colony formation of SGC7901 and AGS cells transfected with the empty vector or pcDNA-NFIA-AS1. E: Proliferating SGC7901 and AGS cells were labeled with EdU. The positive point revealed Edu staining (red). Cell nuclei were stained with DAPI (blue). Representative images and data based on three independent experiments. Error bars indicate mean±standard errors of the mean. *P<0.05, **P<0.01.
result of Western blotting also showed that the expression of p16 was up-regulated at protein levels in gastric cancer cells transfected with pcDNA-NFIA-AS1 compared with those with an empty vector while the changes of p15 protein levels is not obvious (not shown). As we suspected that NFIA-AS1 may only affect the mRNA level of p15, but has little effect on its protein level, we then focused on the impact of NFIA-AS1 on p16. Then we carried out a correlation study for NFIA-AS1 and p16 in clinical samples, the results showed that there was a weak correlation between the expression of NFIA-AS1 and p16 in the 42 gastric cancer samples we tested (Fig. 4D). This data suggested that NFIA-AS1 may work as a tumor suppressor by affecting p16 expression through post-transcriptional regulation in gastric cancer cells.

NFIA-AS1 overexpression inhibited tumor growth of gastric cancer cells in vivo

To explore whether the expression level of NFIA-AS1 affects tumor growth in vivo, we stably transfected pcDNA-NFIA-AS1 or the empty vector, and SGC7901 cells were inoculated into 4-week-old male nude mice. All mice developed xenograft tumors at the injection site. On the 16th day after injection, as shown in Fig. 5A and B, tumor growth in NFIA-AS1 overexpression group was significantly slower than that in the empty vector group. The average tumor weight in the pcDNA-NFIA-AS1 group was lower than that in the control group (Fig. 5C). Moreover, the H&E staining showed the typical characteristics of tumor cells, and by using immunohistochemical staining, the proliferation index Ki-67 was

Fig. 3 NFIA-AS1 influenced cell cycle and inhibited gastric cancer cell migration and invasion in vitro. A: At 48 hours after transfection, cell cycle of SGC7901 and AGS was analyzed by flow cytometry. The bar chart represents the percentage of cells in G1–G0, S, or G2–M phase, as indicated. B and C: At 24 hours after transfection, Transwell assay was performed to investigate the changes in migratory and invasive abilities of GC cells. Error bars indicate mean±standard errors of the mean. *P<0.05, **P<0.01.
significantly decreased in the NFIA-AS1-transfected tumors (Fig. 5D). Subsequently, we performed qRT-PCR analysis of p16 mRNA expression in xenograft models (n=4), and as shown in Fig. 5E, the expression of p16 was significantly up-regulated in tumor models. These results demonstrated that NFIA-AS1 could suppress tumor growth of gastric cancer cells in vivo.

Discussion

In the past decades, the role of lncRNAs in human diseases, including cancer, have attracted increasing attention[20–22]. Many studies have shown that a lncRNA can play a crucial role in various cancer types as a potential oncogene or tumor suppressor. In our previous study, we found that E2F1-induced overexpression of LINC00668 affects the proliferation of gastric cancer cells by epigenetically silencing of CKIs[23]. We also explored whether up-regulation of HOXC-AS3 predicts poor prognosis of gastric cancer and promotes the expression of the oncogene HDAC5 by binding YBX1[19]. However, most studies have shown that lncRNAs may promote tumor proliferation, only a few numbers of lncRNAs are involved in the inhibition of tumor development because a small part of lncRNAs shows low expression in tumors. For example, Yin et al found that lncRNA GAS5 could inhibit colorectal cancer cell proliferation and was associated with poor prognosis in gastric cancer patients[18]. In addition to lncRNAs, we found that many miRNAs are also involved in the regulation of proliferation of gastric cancer cells. For example, miRNA-429 can suppress the growth of gastric cancer cells in vitro[24].

In our present study, we found a novel lncRNA NFIA-AS1 and the average level of NFIA-AS1 in gastric cancer tissues was significantly lower than those in corresponding non-tumor tissues. It has been pointed out that lncRNA-MEG3 expression is down-regulated in gastric cancer tissues and cell lines, and it can inhibit cell proliferation and metastasis by regulating miRNA-21 in gastric cancer[25]. Moreover, in our study, the low expression level of NFIA-AS1 in gastric cancer patients was positively correlated with migration depth and TNM stage and also associated with poor prognosis. NFIA-AS1 could be an independent prognostic indicator. These results suggested that NFIA-AS1 might play an important role in gastric cancer progression and may act as a tumor suppressor in cancer development. Many studies have reported that aberrant expression of
lncRNAs is involved in a variety of tumor progression and can be used as a prognostic indicator. For example, Song et al found that lncRNA UCA1 promotes tumor cell metastasis and predicts poor prognosis in patients.

In our study, NFIA-AS1 is a long non-coding RNA which is located at chromosome 1p31.3 and is transcribed into a 4,574 nt transcript. Here, we mainly focus on the role of NFIA-AS1 in inhibiting cell proliferation and metastasis. Our current findings suggested that ectopic expression of NFIA-AS1 inhibits proliferation of gastric cancer cells through MTT analyses, cell colony formation assays and EdU analysis and blocks the normal cycle of cells. Moreover, NFIA-AS1 also plays a role in regulating the migration and invasion of gastric cancer cells. The results of the animal experiment show that overexpression of NFIA-AS1 suppressed gastric cancer cell tumorigenesis in vivo. These findings indicate that NFIA-AS1 could function as a tumor suppressor via regulating cell growth and cell cycle and may be useful in the development of novel prognostic or progression markers for gastric cancer.

In the current study, p16 (INK4A) is considered to be a tumor suppressor gene encoding specific inhibitors of cyclin-dependent kinases (CDK) 4 and 6 and found to change in a wide range of human cancers. p16 inhibits cell proliferation by blocking cell cycle progression and promoting cell apoptosis and differentiation. The functional role of p16 has been demonstrated in many cancers, including gastric cancer. The relationship between lncRNA and p16 has also been studied. For example, expression of p16 is often inhibited in a variety of cancers to promote cell proliferation and may be useful in the development of novel prognostic or progression markers for gastric cancer.

In this study, we examined the effects of overexpression NFIA-AS1 on the level of several CKI family proteins involved in the S checkpoint of the cell cycle in SGC7901 and AGS cells. The results confirm that the

Fig. 5 The impact of NFIA-AS1 on tumorigenesis in vivo. A: Empty vector and pcDNA-NFIA-AS1 cells were stably transfected into SGC7901 cells, which were injected in the nude mice (n=4), respectively. B: Tumor sizes were calculated after injection every 2 days. Bars indicate SD. C: Tumor weights are represented as means of tumor weights±SD. D: The tumor sections were under H&E staining and IHC staining using antibodies against Ki-67. E: Relative p16 expression was detected in xenograft models by qRT-PCR. Error bars indicate means±standard errors of the mean. **P<0.01.
expression levels of p15 and p16 are upregulated in NFIA-AS1 overexpression gastric cells. We subsequently examine the protein level of p16 in overexpressing NFIA-AS1 cells, and the results were consistent with the results of qRT-PCR that the expression of p16 is elevated. Then the correlation analysis of NFIA-AS1 and p16 in clinical samples showed that there was a weak correlation between the expression of NFIA-AS1 and p16. Here, we provide the evidence for the first time that NFIA-AS1 regulates the proliferation of gastric cancer cells by affecting the expression of p16 and exerting a tumor suppressor function in human gastric cancer cells, and these findings indicate that lncRNA NFIA-AS1 may function as a tumor suppressor and play an inhibitory role in the development of gastric cancer. However, further research is needed to elucidate the specific regulatory mechanisms of NFIA-AS1-mediated upregulation of p16 in gastric cancer cells.

We demonstrated that NFIA-AS1 is down-regulated in human gastric cancer tumor tissues and can be considered as an independent prognostic factor in patients with gastric cancer. Overexpressed NFIA-AS1 could inhibit cell proliferation and migration by affecting the expression level of the tumor suppressor p16. Our study may provide a new strategy for molecular therapy of gastric cancer and promote the development of diagnostic and therapeutic approaches to this tumor suppressing lncRNA.

Acknowledgments

This work was supported by grants from the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX17_1301 to E.Z.) and the National Natural Science Foundation of China (81730066 to D.M.).

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**CLINICAL TRIAL REGISTRATION**

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