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ORIGINAL ARTICLE

Silence of Long Noncoding RNA NEAT1 Inhibits Malignant Biological Behaviors and Chemotherapy Resistance in Gastric Cancer

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Abstract Gastric cancer (GC) is the most common solid tumor in digestive system. Nuclear-enriched abundant transcript 1 (NEAT1) gene is a lncRNA, and reveal potential oncogene role in several malignant tumors. The aim of this study is to investigate the expression and clinical significance of Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) gene and its influence to malignant biologic behaviors and chemotherapy resistance to adriamycin in GC. This study found NEAT1 was up-regulated in GC tissues and cells, especially in in GC adriamycin-resistant cells. NEAT1 silence in SGC7901 cells could inhibit proliferation and invasion ability, and promote cell apoptosis significantly. NEAT1 silence in adriamycin-resistant SGC7901/ADR cells also depressed the half maximal inhibitory concentration (IC50) for adriamycin, chemotherapy resistance to adriamycin was inhibited significantly. NEAT1 knockdown promoted apoptosis in SGC7901/ADR cells induced by adriamycin. In summary, lncRNA NEAT1 is high-expressed in GC and functions as an oncogene to modulate apoptosis, invasion, proliferation and chemotherapy resistance of GC cells, which might be a novel potential therapeutic target for GC.

Keywords Long noncoding RNA · NEAT1 · Gastric cancer · Malignant behaviors · Chemotherapy resistance

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Introduction

Gastric cancer (GC) is the most common solid tumor originated from digestive system [1]. Because advanced GC has high frequent metastasis and relapse, its 5 years survival rate was 30% ~ 50%, and its prognosis is poor. Surgical resection is the preferred and key therapy strategy. Postoperative chemotherapy can prevent metastasis and recurrence of GC efficaciously, and is a well supplement for operation [2]. But, chemotherapy resistance has severely restricted the application of chemotherapy. Thus, it is necessary to find a novel therapeutic target and method to improve the poor prognosis of GC.

Over the past ten years, the long noncoding RNA (lncRNA) related research has been progressing rapidly. LncRNAs could act as a transcriptional regulator for numerous genes, and participate in almost all key biological characteristics, included proliferation, invasion and chemotherapy resistance [3–5]. The expression of some lncRNAs was dysregulated in some malignant tumors, and their anomalous functions was related to the tumorogenesis and progression [6, 7].

Nuclear-enriched abundant transcript 1 (NEAT1) gene had been found high-expression in esophageal squamous cell carcinoma, non-small cell lung cancer, and bladder cancer, revealed potential oncogene role [8–10]. Recent, Fu JW and Ma Y reported that NEAT1 was up-regulated in GC [11, 12]. Those discoveries suggested that NEAT1 might be an oncogene in GC. But, the impacts of NEAT1 on proliferation, apoptosis and chemotherapy resistance in GC cells are still unclear.

This study found NEAT1 was up-regulated in GC, and inhibited malignant behaviors included cell proliferation, apoptosis and invasion, and inhibited chemotherapy resistance in GC cells. These foundings were helpful to improve the effects of clinical treatment in GC.

Material and Methods

Clinical Specimens

Total 76 GC and control normal stomach tissues (NT) were gathered from Affiliated First Hospital of China Medical University from Feb 2016 to Aug 2016. The tissue samples were stored in liquid nitrogen. The clinicopathological data was affirmed by professional pathologist after operation. The Ethics Committees of Dalian Medical University approved this study, and permissions of surgical patients were achieved before operation.

Cell Culture

Human gastric cancer SGC7901 cells and normal gastric epithelial cell GES-1 were purchased from China Academy of Chinese Medical Sciences. Adriamycin-resistant SGC7901/ADR was preserved in our laboratory [13]. Those cells were cultured as previously described [13].

Real Time Quantitative PCR

Total RNA was extracted with Trizol reagent and synthesized cDNA with Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). One Step SYBR RT-PCR Kit (TaKaRa, Japan) was used to detect the NEAT1 expression. Its primers were 5′- TGGACTAGCTCAGGGACTTCAG -3′ (sense) and 5′- TCTCCTTGACCAAGACTTCCTTC -3′ (antisense). The primers of endogenous gene GAPDH were 5′-CGGAGTCAACGGATTTGGTCGTAT -3′ (sense) and 5′-R:AGCCTTCTCCATGGTGGTGAAGAC -3′ (antisense). Relative NEAT1 expression was quantified with the relative quantitative method [14].

Vector Construction and Transfection

The silence vector pS-NEAT1 was constructed by Fitgene Company (Guangzhou, China). The pSilencer vector (Invitrogen, Foster City, CA, USA) acted as negative control (pS-NC). pS-NEAT1 and pS-NC were transfected with Lipofectamine 3000 Reagent (Invitrogen, Foster City, CA, USA) according to manufacturer's protocol. G418 (Invitrogen, Foster City, CA, USA) was used to establish stable cell lines, and qRT-PCR was applied to detect the transfected efficiencies.

Proliferation and Chemotherapy Resistance Assay

Cell proliferation was assayed by MTT assay as previously described [14]. Cells were seeded into 96-well plates with 3000 cells per well and treated with adriamycin (0.005 μg/mL, 0.05 μg/mL, 0.5 μg/mL, 5 μg/mL, 50 μg/mL) 24 h later [13]. After 48 h, the cell viability was detected, and the dose-response curve was drew to count the half maximal inhibitory concentration (IC50) using a Probit regression model.

Cell Invasion Assay

Cell invasion assay was detected by Transwell chamber (Costar, Corning, NY, USA) with polycarbonic membrane (6.5mmin diameter, 8 μm pore size) and Matrigel (BD, NJ, USA) according to previous report [15].

Apoptosis Detection

Cell poptosis rate was detected using Annexin V-FITC apoptosis detection kit (Biosci, Hangzhou, China) with flow cytometry. The apoptosis data was achieved and analyzed according to our previous report [14].

Statistical Analysis

All data were showed as mean ± SD of five independent experiments and analyzed with SPSS 21.0 software (IBM, Somers, NY, USA). The difference comparison between them was used one-way ANOVA and pared Student's t-test. $P < 0.05$ means significant difference.

Results

NEAT1 was Up-Regulated in GC Specimens

Compared with control NT samples, the NEAT1 expression in GC tissues was up-regulated significantly ($p < 0.05$, Fig. 1). Furthermore, the expression of NEAT1 in SGC7901 cells was higher than that in GES-1 cells ($p < 0.05$).

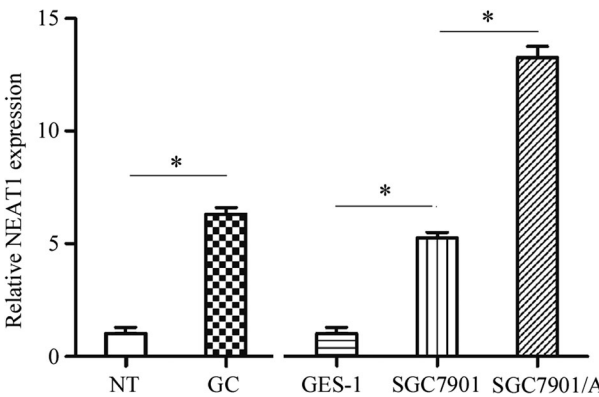

Fig. 1 Compared with control NT samples and GES1 cells, the NEAT1 expression in GC tissues and SGC7901 cells was up-regulated significantly. Moreover, the expression of NEAT1 in SGC7901/ADR cells was higher than that in SGC7901 cells. * $P < 0.05$

NEAT1 Silence Inhibits the Malignant Biological Behaviors of GC Cells

In order to validate the influence of NEAT1 on malignant biological behaviors of GC cells, the pS-NEAT1 vector was transfected into SGC7901 cells to silence the expression of NEAT1 ($P < 0.05$, Fig. 2a).

In comparison to the respective NC groups, the cell viability of SGC7901 cells with NEAT1 silence decreased significantly (Fig.2b), and the results of flow cytometry revealed NEAT1 silence promoted apoptosis markedly (Fig. 2c, $P<0.05$). As shown in Fig. 2d, NEAT1 silence in SGC7901 cells also restrained invasion ability ($p < 0.05$).

NEAT1 Knockdown Inhibited Chemotherapy Resistance to Adriamycin in GC Adriamycin-Resistant Cells

Fig. 3a showed the IC50 of SGC7901 and SGC7901/ADR cells to adriamycin were 1.52 ± 0.13 μg/mL and 5.41 ± 0.14 μg/mL respectively, the IC50 of SGC7901/ADR cells was much higher. SGC7901/ADR cells had stronger drug resistance to adriamycin ($p < 0.05$).

Furthermore, compared with SGC7901 cells, the NEAT1 expression was up-regulated in SGC7901/ADR cells ($p < 0.05$, Fig. 1). The NEAT1 expression in SGC7901/ADR cells were silenced by pS-NEAT1 transfection ($P < 0.05$, Fig. 3b).

Fig. 3a revealed IC50 of SGC7901/ADR cells to adriamycin were inhibited by NEAT1 konckdown from 5.41 ± 0.14 μg/mL to 2.38 ± 0.16 μg/mL ($p < 0.05$), which demonstrated that NEAT1 silence inhibits chemotherapy resistance of GC cells to adriamycin.

NEAT1 Silence Promoted Cell Apoptosis of Adriamycin-Resistant GC Cells Induced by Adriamycin

Fig. 3c showed the influence of NEAT1 silence on apoptosis rate induced by 0.5 μg/mL adriamycin in SGC7901/ADR cells. Compared with control group, NEAT1 silence promoted cell apoptosis from 6.36 ± 0.18% to 14.43 ± 0.26% in SGC7901/ADR cells ($p < 0.05$).

Discussion

In this study, low-expression of NEAT1 in GC tissues and cells was found, which was corresponded to recent report. Fu JW et al. reported that NEAT1 was up-regulated in GC and may act as a potential biomarker for therapeutic strategy and prognostic prediction [11]. Ma Y et al. also detected that NEAT1 were significantly elevated in gastric adenocarcinoma (GAC) tissues, and high NEAT1 expression was correlated with advanced GACs and GACs with lymph node metastasis [12]. Overall, these findings suggested NEAT1 might be an

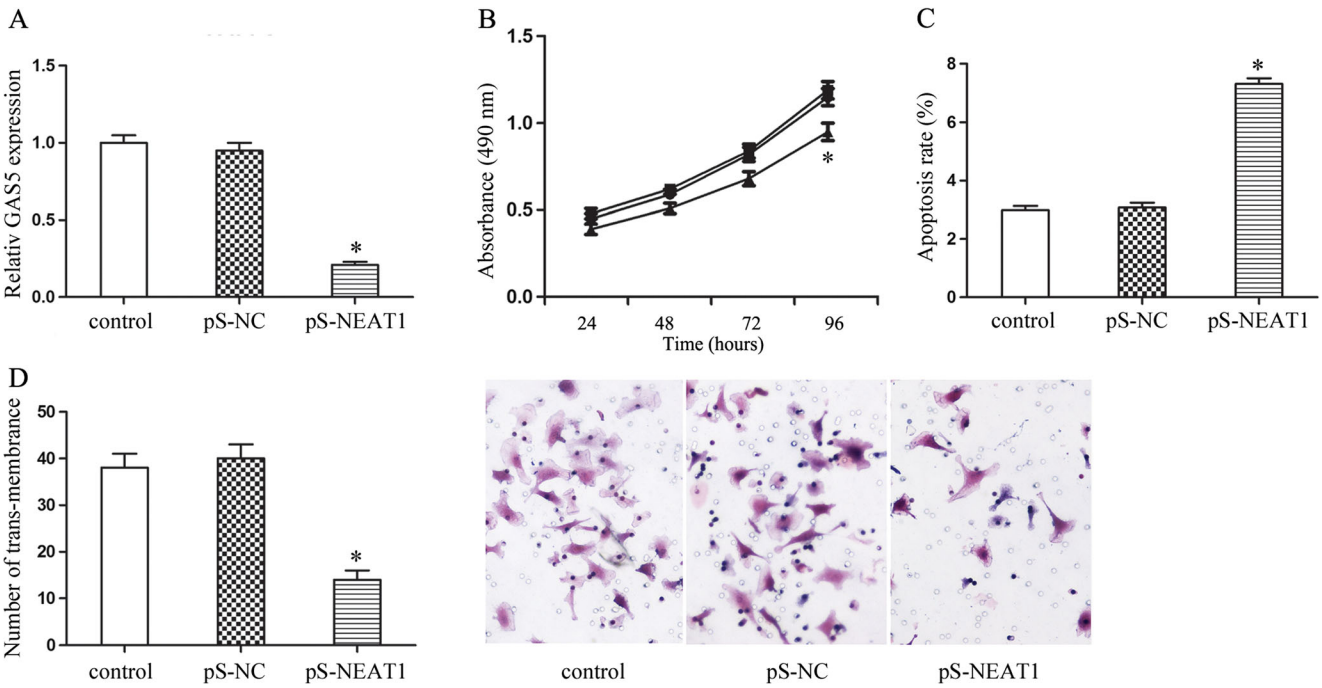

Fig. 2 **a** Tranfection with pS-NEAT1 into SGC7901 cells could silence the NEAT1 expression. **b** NEAT1 knockdown inhibited proliferation of SGC7901 cells. **c** NEAT1 silence promoted cell apoptosis of SGC7901 cells. **d** NEAT1 silence inhibited the invasion ability of SGC7901 cells.* $P < 0.05$

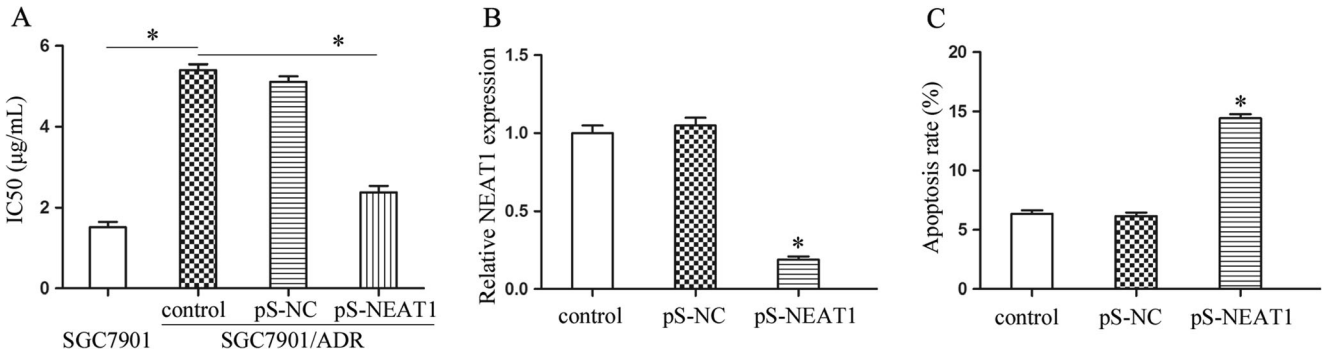

Fig. 3 **a** Compared with SGC7901 cells, the IC50 of SGC7901/ADR cells to adriamycin was much higher, and NEAT1 knockdown depressed IC50 of SGC7901/ADR cells to adriamycin. **b** Tranfection with pS-NEAT1 into SGC7901/ADR cells could silence the NEAT1 expression. **c** NEAT1 knockdown promoted apoptosis of SGC7901/ADR cells induced by adriamycin. * $P < 0.05$

oncogene in GC, and play a promotive role in progress and metastasis of GC. Nevertheless, the impacts of NEAT1 on proliferation, apoptosis and chemotherapy resistance in GC cells are still unclear.

To discuss the functions of NEAT1 in GC cells, the expression of NEAT1 was silenced by pS-NEAT1 transfection. Then, the results showed the NEAT1 silence could inhibit the proliferation and invasion ability of SGC7901 cells, and promote cell apoptosis of SGC7901 cells, which proved NEAT1 acted as an oncogene in GC cells.

NEAT1 is an essential architectural component of paraspeckle nuclear bodies, and may act as a transcriptional regulator for numerous genes, including some genes involved in cancer progression. Previous reports had suggested its oncogene role in various malignant tumors, including GC [8–12]. Zhen L's study demonstrated that NEAT1 knockdown reduced proliferation, invasion, and migration in glioma cells [16]. Moreover, knockdown of NEAT1 depressed cell proliferation, and induced apoptosis and cell cycle arrest at G1 phase in laryngeal squamous cell cancer cells [17]. Those findings indicated NEAT1 modulate malignant biologic behaviors and provided solid evidence for the NEAT1 functional role in GC.

In this study, the expression of NEAT1 was much higher in adriamycin-resistant SGC7901/ADR cells, which suggested that NEAT1 over-expression might be involved in chemotherapy resistance of GC cells to adriamycin. Adriaens C et al. discovered NEAT1 knockdown induced synthetic lethality with genotoxic chemotherapeutics, including PARP inhibitors, and nongenotoxic activation of p53 [18]. Chemotherapy is an effective approach to prevent metastasis and recrudescence of GC. But, the prognosis of GC patients is still poor, because of chemotherapy resistance. Up to now, none is known about the impact of NEAT1 to chemotherapy resistance in GC. In summary, NEAT1 silence inhibited significantly chemotherapy resistance to adriamycin in drug-resistant GC cells, which might be a novel potential therapeutic target for GC.

Recent literatures reported NEAT1 could specifically bind with some microRNA, such as let-7e and mir-98-5p, and then suppress the expression of their target genes to regulate biologic behaviors in some malignant cancer [19, 20]. NEAT1 might play its oncogene role in GC through regulating its target genes expression, but the target genes and regulative mechanism in GC cells is still unknown, which need more intensive investigations.

In conclusion, lncRNA NEAT1 is high-expressed in GC, and functions as an oncogene to modulate malignant behaviors and chemotherapy resistance to adriamycin in GC. These achievements might provide a novel therapeutic target, and improve the effects of clinical treatment in GC.

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Authors' Contributions JLZ and BJH participated in the study design and drafted the manuscript. BCZ and XXC carried out the in vitro studies and performed the statistical analysis. ZNW and HMX conceived of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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