Long noncoding RNA NEAT1 promotes tumorigenesis in H. pylori gastric cancer by sponging miR-30a to regulate COX-2/BCL9 pathway

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Abstract
Background: Helicobacter pylori (H. pylori) is a carcinogenic factor for gastric cancer. Our previous study demonstrated that H. pylori decreased the expression of microRNA (miRNA)-30a to promote the tumorigenesis of gastric cancer. However, the upstream regulatory molecules of miR-30a are not well elucidated. In this study, we found the long non-coding RNA (lncRNA) nuclear paraspeckle assembly transcript 1 (NEAT1) may sponge miR-30a to regulate COX-2/BCL9 pathway.

Methods: The expression of NEAT1 was detected in gastric cancer tissues and tumor-adjacent tissues by fluorescence in situ hybridization (FISH) analysis and RT-qPCR. LncRNA-miRNA interaction networks were constructed using the RNAhybrid and starBase v.2.0. and then validated using a dual-luciferase reporter assay. The effects of NEAT1 dysregulation on the proliferative, migratory, and invasive abilities of H. pylori filtrate-infected gastric cancer cells were observed by cell counting kit-8 (CCK-8), colony formation, wound healing test, and transwell assays. Western blot and RT-qPCR were performed to detect protein and RNA expression. Immunohistochemistry (IHC) was carried out to analyze the localization and expression of COX-2 and BCL9.

Results: FISH and RT-qPCR demonstrated that the expression of NEAT1 was upregulated in gastric cancer tissues, especially in H. pylori-infected gastric cancer tissues, and the expression of NEAT1 was negatively correlated with miR-30a (miR-30a-3p and miR-30a-5p). The upregulation of NEAT1 enhanced proliferation, migration, and invasion of H. pylori filtrate-infected gastric cancer cells, while the downregulation of NEAT1 decreased these abilities, and miR-30a could reverse the effect of NEAT1 on these abilities. The dual-luciferase reporter assay identified that NEAT1 directly
targeted miR-30a (miR-30a-3p and miR-30a-5p). Because miR-30a (miR-30a-3p and miR-30a-5p) negatively regulates the expression of downstream COX-2 and BCL9, NEAT1 was identified to upregulate indirectly the expression of COX-2 and BCL9. IHC showed that the expression of COX-2 and BCL9 was increased in *H. pylori* gastric cancer tissues.

**Conclusion:** The study demonstrated that IncRNA NEAT1 may act as a promoter of tumorigenesis in *H. pylori* gastric cancer, by sponging miR-30a (miR-30a-3p and miR-30a-5p) to regulate the COX-2/BCL9 pathway.

**KEYWORDS**

COX-2/BCL9 pathway, gastric cancer, *H. pylori*, miR-30a, NEAT1

## 1 | BACKGROUND

Gastric cancer is one of the most frequently diagnosed digestive cancers, accounting for over million new cases globally in 2018. Incidence rates of gastric cancer are markedly elevated in Asian countries, especially in China. Compelling evidence supports that *Helicobacter pylori* (*H. pylori*) is a first class carcinogen leading to gastric adenocarcinoma and is directly linked to the development of gastric cancer. Early diagnosis of gastric cancer for radical cure largely improved the overall survival of patients; however, since early-stage gastric cancer is asymptomatic, or characterized by non-specific symptoms, most gastric cancer patients are diagnosed at an advanced stage. Thus, there is an urgent need to identify early-stage markers of gastric cancer.

Non-protein-coding RNAs (ncRNAs) were found to participate in genome encoded-transcripts and almost all cellular processes. ncRNAs are classified as long noncoding RNAs (lncRNAs) and small ncRNAs, such as microRNAs (miRNAs). The interaction of lncRNAs, miRNAs, and mRNAs is critically implicated in various diseases, including cancer. Multiple ncRNAs act as oncogenes or tumor suppressor genes during carcinogenesis. Therefore, ncRNAs represent effective diagnostic biomarkers of cancer.

miR-30a has been identified to have tumor suppressor properties, and the double-stranded precursor generates two single-stranded miRNAs, including miR-30a-3p and miR-30a-5p, which regulate the proliferation and migration, respectively, of *H. pylori*-infected gastric cancer cells. Specifically, miR-30a-3p was found to target the 3’UTR of COX-2 mRNA and regulate nuclear translocation of β-catenin, and miR-30a-5p was shown to target the 3’UTR of BCL9 mRNA to affect TCF/LEF promoter activity and regulate downstream gene expression of β-catenin.

LncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) was reported to influence various diseases. NEAT1 is up-regulated in the Huntington’s disease brain tissues to alleviate neuronal injury, but repressed expression in the early stage of Alzheimer’s disease. NEAT1 also be induced by immune stimuli. All these reports suggest that NEAT1 is an effector during stress and disease development. Additionally, NEAT1 is aberrantly expressed in different types of cancer. High levels of NEAT1 may act as a biomarker of prostate cancer patients, and NEAT1 promotes cervical cancer cell invasion and the progression of sarcoma metastasis. NEAT1 is downregulated in acute promyelocytic leukemia. We applied bioinformatics analysis, and it was predicted that NEAT1 sponges miR-30a. Hence, our study aimed at evaluating the function of NEAT1 in *H. pylori* gastric cancer, which might be valuable for the diagnosis and treatment of gastric cancer.

## 2 | MATERIALS AND METHODS

### 2.1 | Clinical samples

Gastric cancer tissues and matching adjacent tissues were isolated from 38 patients (15 with *H. pylori* infection history, 23 without *H. pylori* infection history, between stage II and stage IV). None of the patients had other malignant tumors or had undergone chemotherapy or radiotherapy. All patients signed the informed consent. Sample collection and usage were performed in accordance with the relevant guidelines. The study was approved by ethics committee of Shuguang Hospital. All the specimens were immediately flash-frozen in liquid nitrogen.

### 2.2 | Preparation of *H. pylori* filtrate

*H. pylori* strain NCTC11637 (containing cacA and cagA gene) was obtained from Renji Hospital. *H. pylori* was cultured under microaerophilic conditions (12% CO₂ and 5% O₂) at 37°C, on Columbia agar plates (Oxoid) containing 5% sheep blood. In other experiments, *H. pylori* was used to prepare a bacterial lysate as previously described. *H. pylori* was suspended in phosphate-buffered saline (PBS), and the suspension mixture was kept in an ice bath, then pulse sonicated for 5 min at 50% capacity. The suspension was centrifuged at 10,000 rpm for 15 min to remove bacterial debris, and the collected supernatant was sterilized by passing through a 0.22 μm cellulose acetate filter. Protein concentration was determined by
the BCA protein quantitative method (Beyotime). The lysates were stored frozen at −80°C.

2.3 | Cell culture

Human MKN45 and SGC-7901 gastric cancer cell lines were commercially obtained from the American Type Culture Collection (ATCC) and cultured in RPMI 1640 medium containing 10% fetal bovine serum, 1% streptomycin and penicillin, at 37°C, 5% CO₂, and saturated humidity. MKN45 and SGC-7901 cells were treated with H. pylori filtrate at a concentration of 500 μg/ml.

2.4 | Cell viability, migration, and invasion assays

Cell viability was tested by CCK-8 and colony formation. Cells were seeded at 5 × 10⁵ per well in 96-well plates, and 10 μl CCK-8 reagent was added into every chamber according to the standard protocol (Beyotime). Subsequently, the absorbance at 450 nm was measured by a plate reader (Thermo Fisher Scientific). For colony formation assay, 100 cells were grown in 12-well plates for 2 weeks. The cells were then fixed with ethanol and stained with crystal violet (Beyotime). A wound healing test and transwell assay evaluated the invasive and migratory abilities of MKN45 and SGC-7901 cells. Cells in the bottom chamber were stained using crystal violet, visualized under an optical microscope, and counted.

2.5 | Construction of the expression vectors

All expression vectors involved in this study were constructed by Genomeditech. The NEAT1 gene (Gene ID283131, NR_028272.1) was amplified by RT-PCR and subcloned into the BamHI and NotI sites of the pcDNA3.1(+) expression vector and named pcDNA3.1(+)–NEAT1(+)–NEAT1(+) (Table S1). NEAT1 fragments (5′end 865nt-1397nt) containing the binding sites for miR-30a-3p (Table S2), NEAT1 fragments (5′end 1105nt-1126nt) containing the binding sites with point mutations for miR-30a-3p(Table S3), NEAT1 fragments (5′end 3167nt-3704nt) containing the binding sites for miR-30a-5p(Table S4), and NEAT1 fragments (5′end 3449nt-3455nt) containing the binding sites with point mutations for miR-30a-5p(Table S5) were amplified by RT–PCR and subcloned into the Nhel and Xhol sites of pmirGLO vector for the luciferase reporter assay.

2.6 | Cell transfection

Cell transfection was performed using the HilyMax kit (DOJINDO, Japan) according to the manufacturer’s instructions. NEAT1–targeting siRNA (siNEAT1), miR-30a-3p mimics or inhibitors, miR-30a-5p mimics or inhibitors, were commercially provided by Genomeditech (Shanghai, China). Empty pcDNA3.1(+) vector, mimics control, and non-targeting siRNAs were used as negative controls. The miRNA mimics or inhibitors, and the siNEAT1 were introduced into cells at a final concentration of 50 nM. The empty pcDNA3.1(+) vector and p-NEAT1 vector were introduced into cells at a final concentration of 1 μg/ml. The sequences of siNEAT1 are listed in Table S6; mimics or inhibitor sequences of miR-30a-3p and miR-30a-5p are listed in Table S7.

2.7 | Dual-luciferase reporter assay

Dual-luciferase assays were used to verify the predicted relationships between NEAT1 and miR-30a-3p or miR-30a-5p. The constructed luciferase reporters were co-transfected into MKN45 and SGC-7901 cells with Renilla reporters. For luciferase assays, cells in 24-well plates were co-transfected with miR-30a (miR-30a-3p or miR-30a-5p) mimics or inhibitor and 200 ng/well luciferase reporter constructs, and SV-Renilla luciferase plasmid was applied at 5 ng/well as the internal control. After 24 h transfection, the luciferase activity was detected using the Dual Luciferase Reporter Assay kit (Promega). Firefly luciferase activities were normalized to Renilla luciferase activity.

2.8 | Reverse transcription and quantitative real-time PCR

Total RNA was reverse transcribed using a PrimeScript RT Reagent Kit (Vazyme, Nanjing, China) and miRNAqPCRQuantitation Kit (Tiangen) to synthesize cDNA. Quantification of NEAT1 expression was normalized to GAPDH expression, and quantification of miR-30a-3p and miR-30a-5p expression was normalized to U6 expression using the 2−ΔΔCt method. RT-qPCR was performed using the following primers: IncRNA NEAT1 sense, 5′- GAGTGCGTGTTGAGTCTGGATTG-3′ and antisense, 5′- AACTTCTCCTCTGTTAGCTGGATG-3′; miR-30a-3p sense, 5′-ACACTCCAGTGCGTGTCACTGCGATG-3′ and antisense, 5′-CTCAACTTGCTGGAGTGCTGCAATTCTAGTTGAGGCCACTGACATCT-3′; miR-30a-5p sense, 5′-ACACTTCA GCTGCGTGAACGTCCTGCAG-3′ and antisense, 5′-CTCAACTTGCTGGAGTGCTGCAATTCTAGTTGAGGCCACTGACATCT-3′; miR-30a-5p sense, 5′-ACACTTCA GCTGCGTGAACGTCCTGCAG-3′ and antisense, 5′-CTCAACTTGCTGGAGTGCTGCAATTCTAGTTGAGGCCACTGACATCT-3′; and antisense, 5′-AACGCTTCAGAATTCTGGCCTGACATCT-3′.

2.9 | Western blot

Expression of COX-2 and BCL9 proteins in MKN45 and SGC-7901 cells was evaluated by Western blot. Cells were lysed in 1xRIPA buffer (Beyotime), and lysates were quantified, electrophoresed, and transferred onto PVDF membranes, and blocked in 10% skim milk for 1.5 h, then incubated with rabbit anti-COX-2(1:1000 dilution,
Abcam), rabbit anti-BCL9 (1:1000 dilution, Abcam), and mouse anti-GAPDH (1:1000 dilution, ProteinIntech) for 12 h at 4°C. After incubation with the secondary antibody (Beyotime), protein bands were visualized by ECL detection kit (Millipore).

2.10 Fluorescence in situ hybridization

The fluorescence in situ hybridization (FISH) assay was used to analyze the location and expression of NEAT1. Twenty-five formalin-fixed paraffin-embedded (FFPE) gastric cancer tissues (13 with H. pylori infection history, 12 without H. pylori infection history) were detected.

Tissues were cut into 3 µm thick paraffin sections, and slides were deparaffinized in xylene and ethanol solutions. Proteinase K reagent was added to fully cover the slides and then incubated at 37°C for 10 min, after which 50 µl of hybridization mix (including RNA probes) was applied to the slides, and each section was covered with a sterile coverslip. After hybridization for 1 h, the slides were washed 5x in saline sodium citrate buffer and blocked for 15 min with blocking solution in a humidified chamber. The blocking solution was removed, and the slides were incubated with the anti-FAM/×200 washed 5x with a sterile coverslip. After hybridization for 1 h, the slides were washed 5x in saline sodium citrate buffer and blocked for 15 min with blocking solution in a humidified chamber. The blocking solution was removed, and the slides were incubated with the anti-FAM/×200.

2.11 Immunohistochemistry

Immunohistochemistry (IHC) was carried out to analyze the location and expression of COX-2 and BCL9. Twenty-five formalin-fixed paraffin-embedded (FFPE) gastric cancer tissues (13 with H. pylori infection history and 12 without H. pylori infection history) were detected. Tissues were cut into 3 µm thick paraffin section. After deparaffinization, sections were blocked for endogenous peroxidase and transferred to antigen retrieval solution. Sections were then incubated with rabbit anti-COX-2 (1:100 dilution, Abcam) and rabbit anti-BCL9 (1:100 dilution, Abcam) at 37°C for 30 min. After incubation with secondary antibody (Beyotime), DAB was used to illuminate the positive staining signals and then counterstained with hematoxylin. The positive staining signals were analyzed by Image J.

3 RESULTS

3.1 Expression of NEAT1 and miR-30a in gastric cancer tissues

Our previous study proved that miR-30a affected the H. pylori-induced gastric cancer, and bioinformatics analysis by RNAbhybrid and starBase v.2.0 revealed that lncRNA NEAT1 could effectively bind to the two strands of miR-30a, including miR-30a-3p and miR-30a-5p. Hence, we detected the expressions of NEAT1, miR-30a-3p, and miR-30a-5p in gastric cancer tissues to determine whether these genes are correlated with H. pylori gastric cancer. FISH detected the expression of NEAT1 and demonstrated that NEAT1 was overexpressed in gastric cancer tissues, especially in H. pylori-infected gastric cancer tissues. Compared to non–H. pylori-infected tumor-adjacent tissues, NEAT1 was also overexpressed in H. pylori-infected tumor-adjacent tissues (Figure 1A, B). Real-time qPCR also demonstrated that NEAT1 was significantly overexpressed in H. pylori-infected gastric cancer tissues and slightly overexpressed in H. pylori-infected tumor-adjacent tissues, which confirmed the results of FISH (Figure 1B). In contrast, miR-30a-3p and miR-30a-5p were decreased in H. pylori-infected gastric cancer tissues and H. pylori-infected tumor-adjacent tissues (Figure 1C, D). Real-time qPCR further demonstrated that the miR-30a-3p and miR-30a-5p were decreased in gastric cancer tissues and tumor-adjacent tissues while NEAT1 was overexpressed (Figure 1E, F).

3.2 NEAT1 promotes the proliferative, migratory, and invasive abilities of gastric cancer cells

In our preliminary study, we found NEAT1 was expressed in gastric cancer cell lines including MKN45 and SGC-7901. Here, we found that NEAT1 expression was greatly elevated in MKN45 and SGC-7901 cells upon H. pylori filtrate treatment for 24 h (Figure 2A), demonstrating that H. pylori promoted NEAT1 expression. However, the detailed function of NEAT1 in gastric cancer is still unclear. Both CCK-8 and the colony formation assay demonstrated that upregulation of NEAT1 facilitated the proliferation of MKN45 and SGC-7901 cells, while downregulation of NEAT1 inhibited MKN45 and SGC-7901 cell proliferation (Figure 2B, C). Additionally, both the wound healing test and transwell assay determined that aberrant expression of NEAT1 influenced the migratory and invasive abilities of MKN45 and SGC-7901 cells. We found both the migration and invasion of MKN45 and SGC-7901 cells were promoted by the upregulation of NEAT1 and suppressed by the downregulation of NEAT1 (Figure 2D, E).

3.3 NEAT1 directly targets miR-30a

Bioinformatic analysis from RNAbhybrid and starBase v.2.0 revealed that lncRNA NEAT1 could effectively bind to the two strands of...
miR-30a (miR-30a-3p and miR-30a-5p). The predicted binding sites of NEAT1 to miR-30a-3p or miR-30a-5p are shown in Figure 3A,B. In MKN45 and SGC-7901 cells, the dual-luciferase assay validated that miR-30a-3p mimics and miR-30a-5p mimics suppressed the expression of a reporter plasmid carrying the wild-type gene sequence, but not the mutant sequence, of NEAT1 in MKN45 and SGC-7901 cells. In addition, either the miR-30a-3p inhibitor or the miR-30a-5p inhibitor slightly increased the expression of a reporter plasmid carrying the wild-type gene sequence of NEAT1, though the difference was not statistically significant (Figure 3C). Overexpression of NEAT1 obviously decreased the expression of miR-30a-3p and miR-30a-5p in MKN45 cells and SGC-7901 cells, whereas silencing NEAT1 significantly increased miR-30a-3p or miR-30a-5p expression in MKN45 and SGC-7901 cells (Figure 3D,E).

3.4 Effect of NEAT1 on miR-30a and the downstream COX-2/BCL9 pathway

In a previously reported study, we demonstrated that the downstream mRNA targets of miR-30a-3p and miR-30a-5p were the 3′-UTR of COX-2 and BCL9, respectively. We subsequently identified that the expression of COX-2 and BCL9 proteins positively correlated with NEAT1. In MKN45 and SGC-7901 cells, expression of both COX-2 and BCL9 proteins was promoted by transfection of p-NEAT1 but was decreased by transfection with si-NEAT1 (Figure 4A). Compared with the NC group, co-transfection of miR-30a-3p inhibitor and si-NEAT1 or co-transfection of miR-30a-3p mimics and p-NEAT1 did not change the expression of COX-2 significantly (Figure 4B,C). Moreover, co-transfection of miR-30a-5p...
**FIGURE 2** LncRNA NEAT1 enhances the proliferative, colony-forming, migratory, and invasive abilities of gastric cancer cells. (A) NEAT1 expression was elevated upon *H. pylori* filtrate-infected MKN45 and SGC-7901 cells for 24 h. (B) CCK-8 assay showed that NEAT1 promoted the proliferation of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. (C) Colony formation assay showed that NEAT1 promoted the colony-forming ability of MKN45 and SGC-7901. (D) Wound healing test showed that the migration of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells was promoted by the upregulation of NEAT1 and suppressed by the downregulation of NEAT1. (E) Transwell test showed that the invasion and migration of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells were promoted by the upregulation of NEAT1 and suppressed by the downregulation of NEAT1. All experiments were repeated three times, *p < 0.05, **p < 0.001 compared with the NC group.
inhibitor and si-NEAT1 or co-transfection of miR-30a-5p mimics and p-NEAT1 did not change the expression of BCL9 significantly (Figure 4B, C).

3.5 | miR-30a reverses the effect of NEAT1 on the proliferation, invasion, and migration of *H. pylori* filtrate-infected gastric cancer cells

Since p-NEAT1 promoted the proliferation of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells, while si-NEAT1 inhibited it, we explored if miR-30a affected the regulatory effect of NEAT1 on gastric cancer cell proliferation, migration, and invasion. The *H. pylori* filtrate-infected MKN45 and SGC-7901 cells were divided into seven groups based on transfections: empty pcDNA3.1 plasmid; p-NEAT1; si-NEAT1; co-transfection of mir-30a (either miR-30a-3p or miR-30a-5p) mimics and p-NEAT1; and co-transfection of miR-30a (either miR-30a-3p or miR-30a-5p) inhibitor and si-NEAT1. The CCK-8 assay showed that neither co-transfection of miR-30a (miR-30a-3p, miR-30a-5p) mimics and p-NEAT1 nor co-transfection of miR-30a (miR-30a-3p, miR-30a-5p) inhibitor and si-NEAT1 changed the proliferation of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells significantly (Figure 5A). Similarly, the transwell assay also showed that co-transfection of miR-30a (miR-30a-3p, miR-30a-5p) mimics and p-NEAT1 slightly reduced the promoting effect of p-NEAT1 on *H. pylori* filtrate-infected MKN45 and SGC-7901 cell invasion and migration, while co-transfection of miR-30a (miR-30a-3p, miR-30a-5p) inhibitor and si-NEAT1 slightly reduced the inhibitory effect of si-NEAT1 on *H. pylori* filtrate-infected MKN45 and SGC-7901 cells invasion and migration (Figure 5B-E).

3.6 | Immunohistochemical detection of COX-2/BCL9 protein in gastric cancer tissues

Using the gastric cancer tissues with or without *H. pylori* infection, as well as the adjacent tumor tissues, we evaluated the expression of COX-2 and BCL9 protein by IHC staining. Positive COX-2/BCL9 immunostaining was mainly localized in the cytoplasm of gastric cancer tissue cells. According to the positive expression area, compared with non-*H. pylori* infection gastric cancer tissue, expression of both COX-2 and BCL9 proteins was increased in the *H. pylori*-infected gastric cancer tissues (Figure 6A, B). All of the above findings suggest that NEAT1 may accelerate tumorigenesis in *H. pylori* gastric cancer, by sponging miR-30a (miR-30a-3p, miR-30a-5p) to regulate COX-2/BCL9 pathway (Figure 6C).
FIGURE 4  NEAT1 regulates the downstream COX-2/BCL9 pathway by miR-30a. (A-B) Protein expression of COX-2 and BCL9 was increased by upregulation of NEAT1 and decreased by downregulation of NEAT1 in *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. (C) Protein expression of COX-2 was negatively regulated by miR-30a-3p, co-transfection of miR-30a-3p mimics and p-NEAT1 or co-transfection of miR-30a-3p inhibitor and si-NEAT1 did not change the protein expression of COX-2 significantly in *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. (D) Protein expression of BCL-9 was negatively regulated by miR-30a-5p; co-transfection of miR-30a-5p mimics and p-NEAT1 or co-transfection of miR-30a-5p inhibitor and si-NEAT1 did not change the protein expression of BCL-9 significantly in *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. All experiments were repeated three times, *p < 0.05, **p < 0.001 compared with the NC group.

FIGURE 5  (A) CCK-8 assay showed that NEAT1 promoted the proliferation of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells, but miR-30a suppressed the effect of NEAT1 on the proliferation of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. (B-E) Transwell assay showed that NEAT1 promoted the invasion and migration of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells, but miR-30a suppressed the effect of NEAT1 on the invasion and migration of *H. pylori* filtrate-infected MKN45 and SGC-7901 cells. All experiments were repeated three times, *p < 0.05, **p < 0.001 compared with the NC group.
The pathological mechanisms by which *H. pylori* promotes gastric cancer development are not precisely well defined. Evidence has established that *H. pylori* genotypes are related to gastric cancer risk. The *H. pylori* strains that contain the cag pathogenicity island (PAI) are more likely to induce gastric cancer, and CagA thread through the host cell membrane into cytosol, and exert multiple effects on the host cell.
promoting cell proliferation, migration, and invasion through relative gene activation, such as COX-2,23 and stimulating signaling pathways such as the β-catenin/WNT pathways.24-26 Thus, we chose the H. pylori strain NCTC11637 (containing cacA and caga gene) for this study.

lncRNAs participate in transcriptional regulation processes, and miRNAs are an important link between lncRNAs and mRNA. lncRNAs compete with miRNAs, which is known as “miRNA sponging.”27 miRNAs negatively regulate mRNA expression by targeting mRNA for directing translational inhibition,28 and each miRNA is able to target multiple genes. This reciprocal regulatory mechanism is involved in many biological processes, including cancer development.29-31

Previous study showed that miR-30a inhibited H. pylori-induced gastric cancer.10 We then explored the mechanism of how H. pylori downregulates miR-30a. Researches found that NEAT1 promotes gastric cancer progression by targeting multiple miRNA related pathways, such as miR-365a-3p/ABCC4,32 miR-17-5p,33 miR-335-5p/ ROCK1,34 and miR-506/STAT3.35 Here, we observed the tumor-promoting effect of NEAT1 in H. pylori gastric cancer by sponging miR-30a to regulate the COX-2/BCL9 pathway.

We determined the expression of NEAT1 in the H. pylori-infected gastric cancer tumor tissues and adjacent tissues of patients by FISH and RT-qPCR. The results showed that NEAT1 was up-regulated in gastric cancer tumor tissues as compared to tumor-adjacent tissues, which is consistent with previous studies,32,35,36 and the expression of NEAT1 significantly increased in H. pylori-infected tumor tissues. Unexpectedly, the expression level of NEAT1 in a small portion in H. pylori-infected gastric cancer tumor tissues was lower than the non–H. pylori-infected specimens, and we hypothesized that it largely resulted from individual differences. We also found the expression of NEAT1 was inversely correlated with expression of miR-30a (miR-30a-3p and miR-30a-5p).

Subsequent studies showed that the expression of NEAT1 was significantly elevated after H. pylori filtrate-infected MKN45 and SGC-7901 cells. Overexpression of NEAT1 facilitated the proliferation, migration, and invasion of H. pylori filtrate-infected MKN45 and SGC-7901 cells while downregulation of NEAT1 inhibited these abilities. Downregulation of miR-30a in gastric cancer specimens corresponded to the upregulation of NEAT1 as predicted by bioinformatics analysis, it also be verified by the dual-luciferase reporter assay. Moreover, transfection of si-NEAT1 increased the expression of miR-30a (miR-30a-3p and miR-30a-5p), while transfection of p-NEAT1 decreased the expression of miR-30a (miR-30a-3p and miR-30a-5p).

Some previous studies have reported that NEAT1 is related to the expression of COX-2. NEAT1 repressed COX-2 via modulating miR-342-3p in macrophages.37 Silencing NEAT1 reduced the expression of COX-2 in epilepsy cell by sponging miR-129-5p38; NEAT1 was also reported to regulate expression of COX2 via its role in competing with endogenous RNAs.39 Our previous study revealed that miR-30a regulates the COX-2/BCL9 pathway, and this current study verified that NEAT1 indirectly regulates the protein expression of COX-2 and BCL9 in a miR-30a-dependent manner. To further investigate whether miR-30a influences the regulatory effect of NEAT1 in H. pylori filtrate-infected gastric cancer cell, we found co-transfection of p-NEAT1 and miR-30a (miR-30a-3p and miR-30a-5p) mimics or co-transfection of si-NEAT1 and miR-30a (miR-30a-3p and miR-30a-5p) inhibitor did not significantly change the cell proliferation, invasion, and migration of H. pylori-infected MKN45 and SGC-7901 cells, which indicated that miR-30a (miR-30a-3p and miR-30a-5p) was negatively associated with the biological function of NEAT1 in H. pylori-infected gastric cancer cell. Additionally, the IHC staining positive expression area showed that the protein expression of COX-2 and BCL9 in H. pylori-infected gastric cancer tissues was increased compared with non–H. pylori-infected gastric cancer tissues. NEAT1 regulates various biological processes, and we found that it promotes tumorigenesis in H. pylori gastric cancer by sponging miR-30a to regulate COX-2/BCL9 pathway.

5 | CONCLUSION

Our results indicate that lncRNA NEAT1 is elevated in gastric cancer tissues, especially in H. pylori-infected gastric cancer tissues. Aberrant expression of NEAT1 in H. pylori-infected gastric cancer cells affects the proliferation, migration, and invasion of gastric cancer cells. The tumor-promoting activity of NEAT1 is achieved mainly by sponging miR-30a and subsequently upregulating the expression of COX-2 and BCL9.

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Not applicable.

CONFLICT OF INTEREST

All other authors declare no conflicts of interest.

AUTHORS’ CONTRIBUTIONS

QL, QJ, XL, CH, and HJG supervised the project. XWR and NNL performed the experiments. RJ, YYF, and ZZZ analyzed the experiments. GH, NLS, RC, and ZYW provided clinical samples. LHZ wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All datasets supporting the conclusion for this study are included in the article.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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