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Expression Profile Analysis and ceRNA Network Prediction of the lncRNA OIP5-AS1/miR-145-5p/DNAJA3 Axis in *Helicobacter pylori* Infection

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection disrupts mitochondrial homeostasis in gastric epithelial cells. However, the lncRNA-mediated stress responses during early infection remain unclear. Objective: To investigate the expression dynamics and putative competing endogenous RNA (ceRNA) network of lncRNA OIP5-AS1, miR-145-5p, and DNAJA3 during *H. pylori* infection. Methods: We utilized starBase and TargetScan for interaction predictions. A time-gradient *in vitro H. pylori* infection model using GES-1 cells was established. Expression levels were quantified via RT-qPCR and Western blotting. Results: Bioinformatics revealed conserved miR-145-5p binding sites on OIP5-AS1 and DNAJA3. *In vitro*, *H. pylori* infection time-dependently upregulated OIP5-AS1 and DNAJA3 mRNA ($P < 0.0001$), with a concurrent downregulation of miR-145-5p. Western blotting confirmed a significant increase in DNAJA3 protein levels at 24 h post-infection. Conclusion: *H. pylori* infection significantly alters the OIP5-AS1/miR-145-5p/DNAJA3 expression profile. We candidly acknowledge that this study is strictly an expression profile analysis. Lacking direct physical binding and functional phenotypic evidence, the proposed ceRNA regulatory network serves merely as a preliminary hypothesis requiring extensive future validation.

Keywords

Helicobacter pylori, lncRNA OIP5-AS1, DNAJA3, Expression Profile Analysis, ceRNA Network

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that specifically colonizes the human gastric mucosal epithelium. It is recognized as a Group I carcinogen by the World Health Organization and is a primary environmental driver for the development of chronic gastritis, peptic ulcers, and gastric adenocarcinoma [1]. Despite the effectiveness of current eradication therapies, the global burden of *H. pylori*-associated gastric diseases remains high, underscoring the urgent need to elucidate the early molecular events of infection. During pathogenesis, *H. pylori* disrupts host cell mitochondrial integrity via secreted virulence factors (such as VacA and CagA), triggering severe intracellular stress responses, ATP depletion, and subsequent epithelial damage [2] [3].

In recent years, transcriptomic studies have revealed the central role of long non-coding RNAs (lncRNAs) in host-pathogen interactions and epigenetic reprogramming [4] [5]. OIP5-AS1 (OIP5 antisense RNA 1) is an lncRNA that has been shown to be dysregulated in various malignancies, including gastric cancer [6]. Mechanistically, its promoter region is rich in hypoxia-response elements and can be directly transcriptionally activated by hypoxia-inducible factor-1α (HIF-1α) [7]. Given that *H. pylori* colonization can induce local microcirculatory disturbances and stabilize HIF-1α in the gastric microenvironment [8], we hypothesized that OIP5-AS1 might act as a key stress response factor initiated by host cells to cope with bacterial invasion.

According to the competing endogenous RNA (ceRNA) hypothesis, lncRNAs can act as “molecular sponges” to adsorb miRNAs, thereby regulating the stability of downstream target mRNAs. In the present study, multiple bioinformatics predictions indicated that the OIP5-AS1 sequence contains potential binding sites for hsa-miR-145-5p. Previous studies have consistently demonstrated that miR-145-5p acts as a crucial tumor suppressor in gastric cancer, actively inhibiting cell proliferation, migration, and invasiveness [9] [10]. Interestingly, one of the predicted downstream targets of miR-145-5p is the mitochondrial chaperone protein gene DNAJA3 (also known as Tid1) [11]. DNAJA3 synergizes with mtHsp70 to maintain mitochondrial protein folding, clearance of misfolded proteins, and membrane potential stability [12].

Based on this framework, the present study combined bioinformatics predictions with an *in vitro* cell infection model to map the dynamic expression profiles of OIP5-AS1, miR-145-5p, and DNAJA3 during *H. pylori* infection. By investigating this novel regulatory axis, we aim to provide new insights into the host non-coding RNA stress response mechanisms.

2. Materials and Methods

2.1. Bioinformatics Analysis

The starBase v3.0 database [13] was utilized to predict the binding sites between lncRNA OIP5-AS1 (ENSG00000247556) and hsa-miR-145-5p, with the following search parameters: species set to Homo sapiens (hg38), CLIP-Data threshold ≥ 1, Degradome-Data ≥ 0, and Pan-Cancer ≥ 0. The predicted interaction (8mer site, chr15:41301483-41301503[+]) was supported by Ago CLIP-seq data with a TDMD

Score of 0.9194 and phyloP conservation score of 0.509.

The TargetScan Human database [14] was used to predict the targeted binding sites of hsa-miR-145-5p within the 3'UTR of DNAJA3 (ENST0000262375.6, 1243 bp). The search was restricted to broadly conserved sites among vertebrates, identifying a 7mer-A1 binding site (position 25 - 31) with a Context++ score of −0.03 (percentile 63) and a weighted context++ score of −0.03.

2.2. Cell Culture and Bacterial Strains

The human immortalized gastric mucosal epithelial cell line (GES-1) was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin. Cells were incubated in a humidified incubator at 37˚C with 5% CO_2.

The *H. pylori* standard virulent strain (CagA+/VacA+, ATCC 43504) was inoculated onto Columbia agar plates containing 5% defibrinated sheep blood and cultured for 72 hours in a microaerophilic environment (85% N_2, 10% CO_2, 5% O_2) before harvesting.

2.3. Cell Infection Model

GES-1 cells were seeded into 6-well plates and cultured to 70% - 80% confluence. Prior to infection, the culture medium containing penicillin/streptomycin was removed, and cells were gently washed three times with sterile PBS to eliminate residual antibiotics, followed by replacement with antibiotic-free infection medium. The *H. pylori* suspension was then added to the cells at a multiplicity of infection (MOI) of 100:1. Cell samples were collected at 0 h (Control), 6 h, 12 h, and 24 h post-co-culture for RNA extraction. For protein extraction, to coincide with the peak transcriptional changes observed in initial assays, Control and *H. pylori*-infected cells were harvested specifically at 24 h post-infection.

2.4. Quantitative Real-Time

Total cellular RNA was extracted using TRIzol reagent. For mRNA and lncRNA detection, RNA was reverse transcribed into cDNA using a reverse transcription kit according to the manufacturer's instructions. For miRNA detection, cDNA was synthesized using the All-in-One™ miRNA First-Strand cDNA Synthesis Kit (Sangon Biotech, Shanghai, China) with stem-loop RT primers specific for miR-145-5p and U6 provided in the kit. qPCR reactions were performed using SYBR Green Master Mix. The primer sequences for OIP5-AS1, DNAJA3, and GAPDH (internal control for lncRNA/mRNA) are listed in **Table 1**. For miR-145-5p, the forward primer sequence is listed in **Table 1**, and the reverse primer was the universal primer supplied with the All-in-One™ Kit. U6 snRNA served as the internal control for miRNA quantification. All experiments were performed with three independent biological replicates (n = 3), with each sample run in technical triplicate (three qPCR replicates per sample). Relative expression levels were calculated using the 2-ΔΔCt method.

Table 1. Primer sequence.

Gene	Primer sequence (5’-3’)
GAPDH	F: TCGGAGTGAACGGATTTGGC
	R: TGACAAGCTTCCCGTTCTCC
OIP5-AS1	F: TGGGAGCTGGCAATGTCTAAA
	R: TTACTGTGCGCTACGTTGTTG
DNAJA3	F: TTTGCGTCTTCCCTGACCTC
	R: TCGTGTGGAAGGAGGCAGTA
U6	F: TGGCTGAGGCAGAAAAGTGC
	R: CCTGGTCAGAAATCTCGCGG
miR-145-5p	GTCCAGTTTTCCCAGGAATCC

2.5. Western Blotting

Total cellular proteins were extracted using RIPA lysis buffer containing PMSF protease inhibitors. After determining the protein concentration via the BCA method, equal amounts of protein (30 μg) were separated by 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% non-fat milk for 1 hour, the membranes were incubated with specific primary antibodies: Anti-DNAJA3 (1:3000, Proteintech) and Anti-Tubulin (1:5000, Proteintech) overnight at 4˚C. The following day, membranes were incubated with HRP-conjugated secondary antibodies (1:3000, Proteintech). Protein bands were visualized using an ECL chemiluminescence kit, and densitometry analysis was performed using ImageJ software.

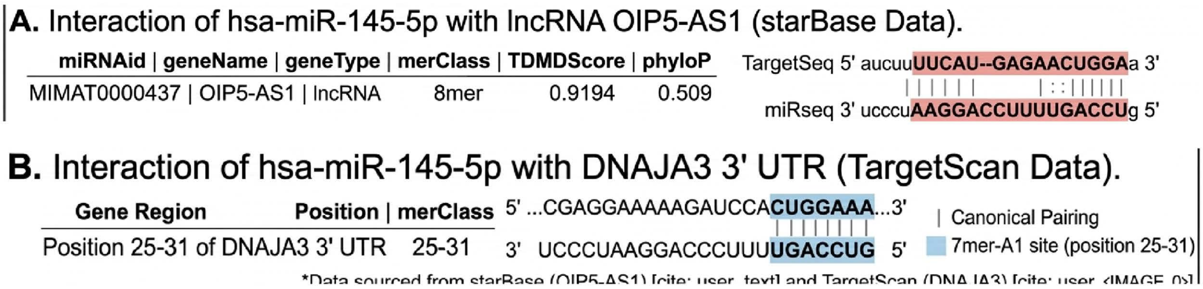
2.6. Statistical Analysis

All experiments were independently repeated at least three times. Quantitative data were presented as Mean ± SD. Statistical analysis was performed using GraphPad Prism 8.0 software. Comparisons between two groups were conducted using Student’s t-test, and comparisons among multiple groups were performed using One-way ANOVA followed by Tukey’s post hoc test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Bioinformatics Predictions Suggest a Putative OIP5-AS1/miR-145-5p/DNAJA3 Regulatory Network

Based on bioinformatics predictions, we identified potential interactions among OIP5-AS1, miR-145-5p, and DNAJA3. Data obtained from the starBase database indicated that the OIP5-AS1 sequence contains an 8mer binding site (TargetSeq 5’ auccuuUUCAUGAGAACUGGAa 3’) highly complementary to the hsa-miR-145-5p sequence (miRseq 3’ ucccuAAGGACCUUUGACCUg 5’) (**Figure 1(A)**). Furthermore, TargetScan data revealed that the 3’ UTR of DNAJA3 mRNA (Position 25-31) contains a canonical 7mer-A1 site (...CUGGAAA...) that binds to hsa-miR-145-5p (Figure 1(B)). This provides a structural basis for OIP5-AS1 to act as an miRNA sponge for miR-145-5p, thereby potentially regulating DNAJA3.



A. Interaction of hsa-miR-145-5p with lncRNA OIP5-AS1 (starBase Data).

miRNAid | geneName | geneType	merClass	TDMDScore	phyloP
MIMAT0000437 | OIP5-AS1 | lncRNA	8mer	0.9194	0.509

TargetSeq 5' aucuu**UUCAU--GAGAACUGGA**a 3'

miRseq 3' ucccu**AAGGACCUUUUGACCU**g 5'

B. Interaction of hsa-miR-145-5p with DNAJA3 3' UTR (TargetScan Data).

Gene Region	Position | merClass
Position 25-31 of DNAJA3 3' UTR	25-31

5' ...CGAGGAAAAAGAUCCA**CUGGAAA**...3'

3' UCCCUAAGGACCCUUU**UGACCUG** 5'

| Canonical Pairing

🟦 7mer-A1 site (position 25-31)

*Data sourced from starBase (OIP5-AS1) [cite: user text] and TargetScan (DNAJA3) [cite: user <IMAGE 0>]

Figure 1. Bioinformatics prediction of binding sites in the putative OIP5-AS1/miR-145-5p/DNAJA3 regulatory relationship (A) Interaction and predicted binding sequence between hsa-miR-145-5p and lncRNA OIP5-AS1 based on starBase data. (B) Interaction and canonical pairing sequence between hsa-miR-145-5p and the DNAJA3 3' UTR region based on TargetScan data.

3.2. *H. pylori* Infection Alters the Expression Profiles of the ceRNA Network Components in GES-1 Cells

To investigate whether this predicted network is activated during *H. pylori* infection, we examined the expression dynamics in GES-1 cells across a time gradient (Control, 6 h, 12 h, 24 h). RT-qPCR results demonstrated that *H. pylori* infection significantly induced OIP5-AS1 expression in a time-dependent manner, peaking at 24 h post-infection (approximately 2.0-fold that of the control group, $P < 0.0001$) (Figure 2, left).

Conversely, the relative expression of miR-145-5p decreased progressively over time, reaching its lowest point at 24 h (approximately 0.5-fold compared to control, $P < 0.0001$) (Figure 2, middle). Concurrently, the relative mRNA expression of DNAJA3 exhibited a continuous upward trend, similar to OIP5-AS1, reaching approximately 1.8-fold at 24 h ($P < 0.0001$) (Figure 2, right). These highly correlated expression trends strongly support the existence of the OIP5-AS1/miR-145-5p ceRNA interaction *in vitro*.

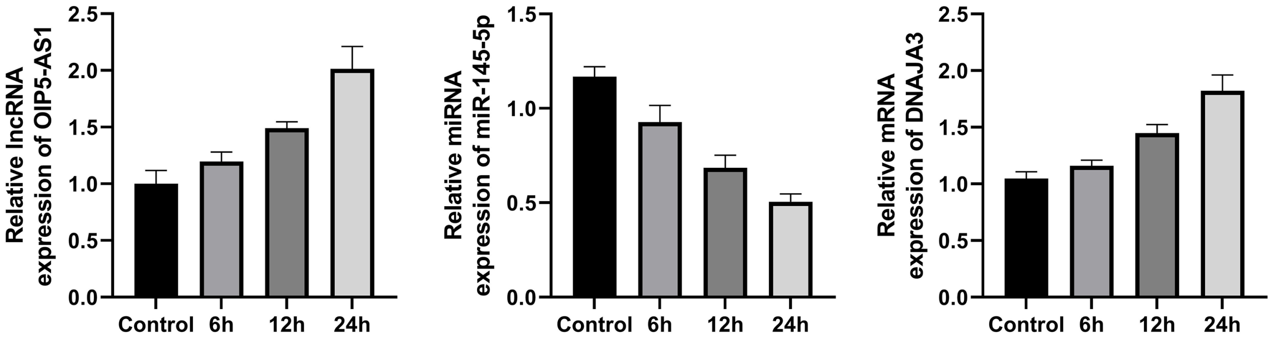


Figure 2. *H. pylori* infection alters the expression profiles in GES-1 cells over time. RT-qPCR analysis showing the relative expression levels of lncRNA OIP5-AS1 (left), miR-145-5p (middle), and DNAJA3 mRNA (right) at control, 6 h, 12 h, and 24 h post-infection. Data are presented as Mean ± SD (n = 3 biological replicates). $^{**}P < 0.01$ compared to the Control group (One-way ANOVA with Tukey's post hoc test).

3.3. *H. pylori* Infection Induces High Protein Expression of DNAJA3

To confirm whether the changes at the transcriptional level translated to the protein level, we performed Western blot analysis. Based on the peak mRNA expression at 24 h, we assessed protein levels at the identical time point (24 h post-infection). Results showed that compared to the uninfected control group, the protein level of DNAJA3 (40 kDa) was visibly elevated in the *H. pylori* infection group, with Tubulin (55 kDa) serving as a stable loading control (**Figure 3(A)**). Densitometric quantification further confirmed that the relative protein expression of DNAJA3 was significantly upregulated following *H. pylori* infection (**Figure 3(B)**). This aligns with the reduction of the inhibitory miR-145-5p and the increase in OIP5-AS1, supporting the preliminary correlation logic of the network.

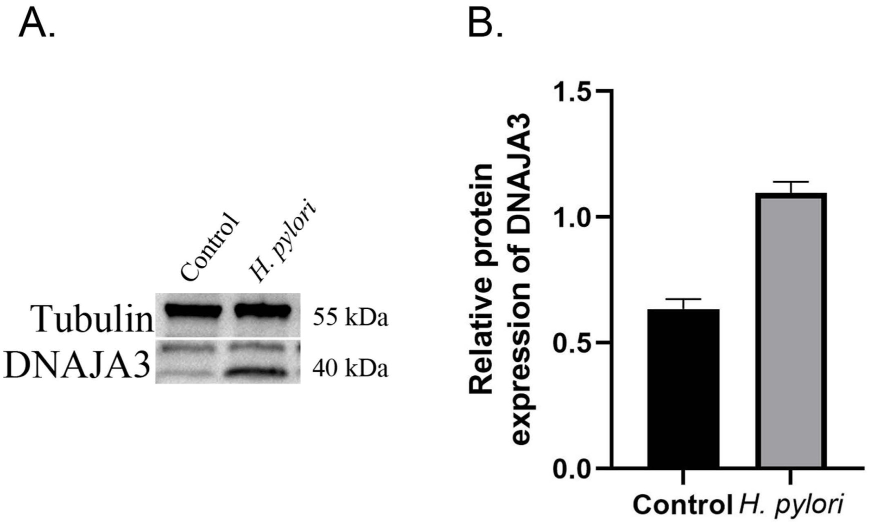


Figure 3. *H. pylori* infection induces high protein expression of DNAJA3. (A) Representative Western blot images showing the protein expression of DNAJA3 (40 kDa) and the internal loading control Tubulin (55 kDa) in control and *H. pylori*-infected groups (harvested at 24 h post-infection). (B) Densitometric quantitative analysis of DNAJA3 relative protein expression. Data are presented as Mean ± SD (n = 3 biological replicates).

4. Discussion

Through a time-course *in vitro* infection model, we observed that *H. pylori* induced OIP5-AS1 expression coincident with decreased miR-145-5p and increased DNAJA3. These patterns are consistent with a putative OIP5-AS1/miR-145-5p/DNAJA3 axis participating in early host responses, though mechanistic validation is pending.

Our findings align with recent evidence that *H. pylori* extensively rewires ceRNA networks, including axes such as HOXA-AS2/miR-509-3p/MMD2 [15] AEG-1/miR-375-3p/JAK2 [16], and circ_0002669/miR-223-3p/ARID1A [17]. OIP5-AS1 has been implicated in gastric cancer via miR-367-3p/HMGA2 [6] and hypoxia pathways [18], while miR-145-5p functions as a tumor suppressor targeting N-cadherin, ZEB2 [9], and ANGPT2 [10]. We suggest *H. pylori* may exploit ceRNA crosstalk to disrupt epithelial homeostasis.

DNAJA3 upregulation implies a compensatory response to *H. pylori*-induced mitochondrial stress [19]. As a mitochondrial co-chaperone, DNAJA3 maintains proteostasis [20] and modulates NF-*κ*B responses [21]. We postulate that OIP5-AS1 may competitively bind miR-145-5p, relieving DNAJA3 repression to preserve mitochondrial integrity during acute infection, though this remains speculative.

Study limitations must be acknowledged: This work is observational, relying on bioinformatics predictions without direct binding evidence (luciferase, RIP) or functional validation (mitochondrial assays, rescue experiments). The GES-1 cell line model lacks primary tissue complexity. Thus, the proposed ceRNA network represents a preliminary hypothesis rather than a confirmed mechanism.

Future work should validate direct RNA interactions and extend to primary organoids and *in vivo* models to establish physiological relevance in *H. pylori* pathogenesis.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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