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Molecular Mechanism of lncRNA NR2F2-AS1 Regulating CTGF in Helicobacter pylori-Induced Gastric Epithelial Cell Injury

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Abstract:Objective: To investigate the role of lncRNA NR2F2-AS1 and its regulation of CTGF in *Helicobacter pylori*-induced gastric epithelial injury.. **Methods:** GES-1 cells were infected with *H. pylori* (MOIs 50–200). Cell viability, proliferation, and migration were assessed alongside the expression of NR2F2-AS1, CTGF, and pro-inflammatory cytokines (IL-6, IL-8). The regulatory link was verified using stable NR2F2-AS1 overexpression. **Results:** *H. pylori* infection significantly upregulated NR2F2-AS1 and CTGF, leading to dose-dependent decreases in cell viability, suppressed proliferation, and impaired migration ($p < 0.01$). NR2F2-AS1 overexpression further boosted CTGF levels and exacerbated inflammatory responses. FISH localized NR2F2-AS1 primarily to the cytoplasm, suggesting post-transcriptional regulation. **Conclusion:** NR2F2-AS1 promotes *H. pylori*-induced injury by positively regulating CTGF. This axis represents a potential therapeutic target for mitigating gastric mucosal damage.

Keywords: *Helicobacter pylori*; NR2F2-AS1; CTGF; GES-1; Inflammatory response

1. Introduction

Helicobacter pylori (H. pylori) is a prevalent gastric pathogen fundamentally linked to various gastrointestinal disorders, including chronic gastritis, peptic ulcers, and gastric adenocarcinoma [1]. Classified as a Group 1 carcinogen, its pathogenicity primarily stems from its ability to disrupt gastric mucosal integrity and trigger persistent inflammatory responses. While several virulence factors have been identified, the precise molecular mechanisms by which *H. pylori* subverts host cell homeostasis and impairs mucosal repair remain a subject of intense investigation[2,3].

Long non-coding RNAs (lncRNAs) represent a diverse class of RNA molecules exceeding 200 nucleotides in length [4,5]. Cumulative evidence suggests that *H. pylori* infection can modulate lncRNA expression to influence the initiation and progression of gastric diseases. For instance, specific lncRNAs have been shown to act as molecular decoys or scaffolds that regulate oncogenic signaling[6,7]. However, the full spectrum of lncRNAs involved in *H. pylori*-mediated epithelial injury is not yet fully characterized.

Connective Tissue Growth Factor (CTGF, also known as CCN2) is a matricellular protein critical for cell adhesion, migration, and extracellular matrix remodeling[8]. Previous studies have indicated that *H. pylori* infection correlates with upregulated CTGF expression, which is closely associated with mucosal remodeling and gastric cancer progression[9,10]. Despite its importance in gastric pathophysiology, the upstream non-coding RNA regulators governing CTGF expression during infection remain poorly understood.

In this study, we focused on lncRNA NR2F2-AS1 (NR2F2 antisense RNA 1). The rationale for selecting NR2F2-AS1 is twofold: first, it has recently been implicated in the progression of several gastrointestinal malignancies[11]; second, our preliminary screening identified NR2F2-AS1 as one of the most significantly upregulated lncRNAs in gastric epithelial cells following *H. pylori* infection.

This study aims to elucidate the functional impact of the NR2F2-AS1/CTGF axis on gastric epithelial cells during *H. pylori* infection and its underlying molecular mechanism.

2. Materials and Methods

2.1 Cell Culture and Bacterial Strains

The human gastric epithelial cell line, GES-1, was cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO_2. *H. pylori* strain 266695 was maintained on Columbia agar plates containing 10% calf serum under microaerophilic conditions (85% N_2, 10% CO_2, and 5% O_2).

2.2 *H. pylori* Infection Model

GES-1 cells (70–80% confluence) were infected with *H. pylori* at various multiplicities of infection (MOI = 50, 100, 150, and 200) for 24 h. Cell morphology was observed using inverted phase-contrast microscopy.

2.3 Cell Viability, Proliferation, and Migration Assays

Cell viability was assessed using the CCK-8 assay (10 μL reagent per well, 1.5 h incubation) at 450 nm. For proliferation,

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a colony formation assay was performed by seeding 500 cells/well and culturing for 10–14 days post-infection; colonies >50 cells were quantified after crystal violet staining. Cellular migration was evaluated via a wound-healing assay, with scratch closure monitored at 0, 24, and 48 h and analyzed using ImageJ.

2.6 Gene and Protein Expression Analysis

Total RNA was extracted and reverse-transcribed for RT-qPCR using the 2^−ΔΔCt method, with Tubulin as the internal control. For protein analysis, lysates were subjected to SDS-PAGE and Western blotting with antibodies against CTGF and Tubulin (loading control). Pro-inflammatory cytokines (IL-6 and IL-8) in the supernatants were determined via an automated chemiluminescent immunoassay (CLIA) according to the manufacturer's instructions.

2.7 Construction of Stable NR2F2-AS1 Overexpression Cell Line

GES-1 cells were infected with lentiviruses carrying OE-NR2F2-AS1 (Hanbio Biotechnology) in the presence of 4 μg/mL polybrene. Stable clones were selected with 8 μg/mL puromycin for 14 days and validated by RT-qPCR.

2.8 FISH

The subcellular localization of NR2F2-AS1 was determined using a FISH kit. Cells were fixed, permeabilized, and hybridized with Cy3-labeled NR2F2-AS1-specific probes. Nuclei were counterstained with DAPI, and images were captured using laser scanning confocal microscopy.

2.9 Statistical Analysis

Data are presented as mean ± SD from three independent experiments. Statistical significance was determined using Student's t-test or one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism 9.0). $P < 0.05$ was considered statistically significant.

3. Results

3.1 *H. pylori* Infection Impairs the Morphological and Functional Integrity of GES-1 Cells

To evaluate the impact of *H. pylori* on gastric epithelial health, we first assessed cellular morphology and survival. Following infection at an MOI of 200 for 24 h, GES-1 cells lost their typical "pavement-stone" epithelial appearance, transitioning to a pleomorphic, shrunken state with increased cell detachment (Figure 1A). CCK-8 assays demonstrated that *H. pylori* exerted a dose-dependent inhibitory effect on cell viability, with significant reductions observed across all infection groups compared to the control (Figure 1B, $p < 0.001$).

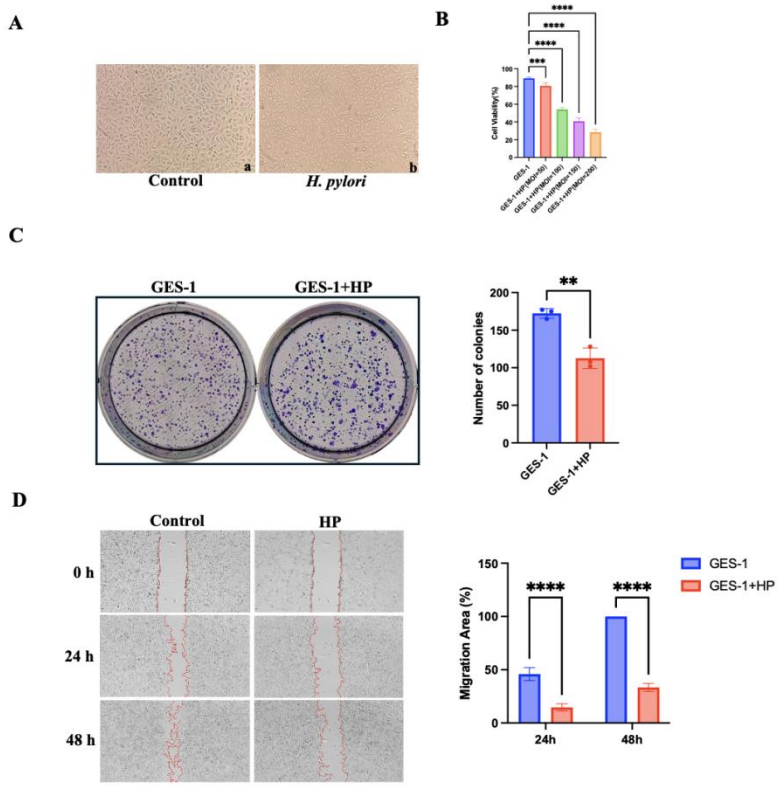

Figure 1. *H. pylori* infection compromises the morphological and functional integrity of GES-1 cells.
(A) Representative optical microscopy images showing morphological alterations in GES-1 cells following *H.pylori* infection (MOI = 200, 24 h). (B) Dose-dependent effects of *H. pylori* infection on cell viability, as measured by CCK-8 assay across various multiplicities of infection (MOIs). (C) Colony formation assay evaluating the long-term proliferative capacity of GES-1 cells post-infection. (D) Wound healing assay images (left) and quantitative analysis (right) illustrating the inhibitory effect of *H. pylori* on cellular migration at 0, 24, and 48 h. Data are presented as mean ± SD, n=3. **p < 0.01 ,***p < 0.001 , ****p < 0.0001 .

Furthermore, we examined long-term proliferative and migratory capacities. The colony formation assay showed a significant decrease in the number of colonies in the infected group (Figure 1C, $p < 0.01$), indicating suppressed cell renewal. Similarly, wound healing assays revealed that *H. pylori* markedly delayed the closure of the scratch area at both 24 and 48 h compared to uninfected cells (Figure 1D, $p < 0.0001$).
In summary, these data confirm that *H. pylori* infection causes acute cytotoxicity and leads to a sustained impairment of growth and repair mechanisms in gastric epithelial cells.

3.2 *H. pylori* Infection Triggers Inflammatory Responses and Upregulates the NR2F2-AS1/CTGF Axis

Inflammation is a primary pathological feature of *H. pylori* infection. Our analysis showed that *H. pylori* significantly upregulated the mRNA levels of pro-inflammatory cytokines IL-6 and IL-8 (Figure 2A-B, $p < 0.01$). Consistent with the transcriptional data, protein secretion levels of both cytokines in the culture supernatants were substantially elevated, as determined by CLIA (Figure 2C-D, $p < 0.001$).
To explore the underlying molecular mechanisms, we examined the expression profiles of lncRNA NR2F2-AS1 and CTGF. RT-qPCR results revealed that both NR2F2-AS1 and CTGF mRNA levels were significantly increased post-infection, exhibiting a synchronized upward trend (Figure 2E-F, $p < 0.01$). Western blot analysis further confirmed that *H. pylori* infection markedly increased CTGF protein levels (Figure 2G, $p < 0.001$).
Together, these findings validate that *H. pylori* infection activates a robust inflammatory response and synchronously induces the NR2F2-AS1/CTGF regulatory axis, suggesting a potential link between this lncRNA and mucosal injury.

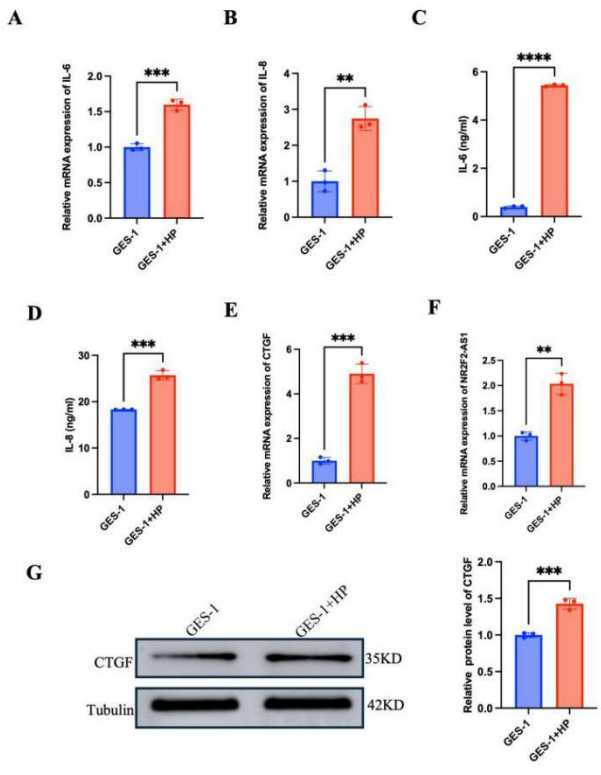

Figure 2. *H. pylori* infection triggers pro-inflammatory responses and activates the NR2F2-AS1/CTGF axis. (A–D) Relative mRNA expression (A, B) and secreted protein levels (C, D) of pro-inflammatory cytokines IL-6 and IL-8 in GES-1 cells following *H. pylori* infection. (E–F) RT-qPCR analysis of CTGF (E) and lncRNA NR2F2-AS1 (F) mRNA levels in response to infection. (G) Representative Western blot images (left) and quantitative analysis (right) of CTGF protein levels, with Tubulin used as the loading control. Data are presented as mean ± SD. **p < 0.01, ***p < 0.001, ****p < 0.0001.

3.3 NR2F2-AS1 Overexpression Promotes CTGF Expression in GES-1 Cells

To directly verify the regulatory effect of NR2F2-AS1 on CTGF, we constructed a stable NR2F2-AS1-overexpressing cell line. Fluorescence microscopy confirmed successful lentiviral transduction (Figure 3A), and RT-qPCR validated a significant increase in NR2F2-AS1 levels compared to the control group (Figure 3B; $P < 0.0001$). Furthermore, NR2F2-AS1 overexpression led to a marked increase in CTGF mRNA levels (Figure 3C; $P < 0.001$) and CTGF protein levels (Figure 3D; $P < 0.01$). These data provide direct evidence that NR2F2-AS1 positively regulates CTGF expression in gastric epithelial cells, supporting our hypothesis that *H. pylori* infection promotes CTGF-mediated injury through the upregulation of NR2F2-AS1.

These results provide direct evidence that NR2F2-AS1 positively regulates CTGF expression, supporting the hypothesis that this lncRNA acts as an upstream driver of CTGF-mediated injury.

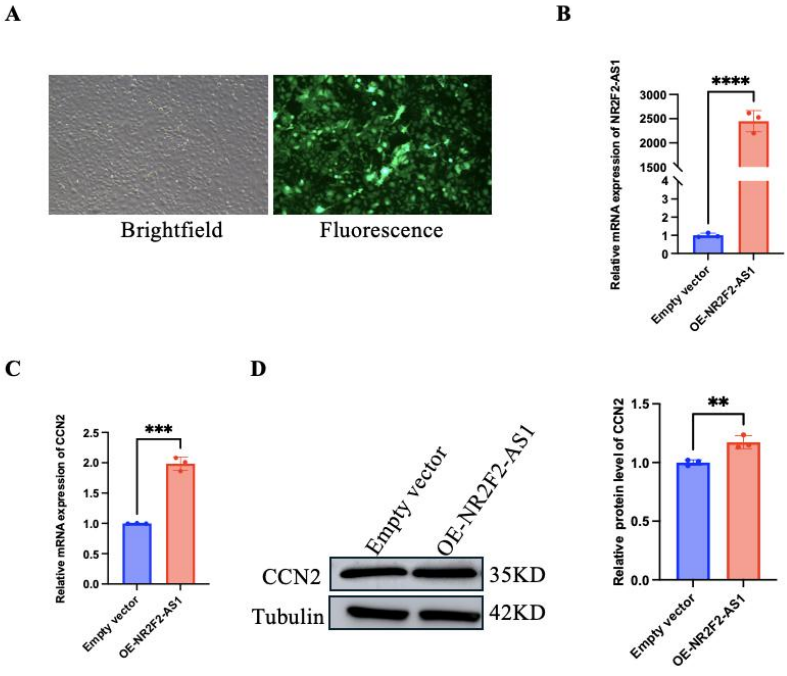

Figure 3. NR2F2-AS1 overexpression promotes CTGF expression in GES-1 cells. (A) Fluorescence microscopy validation of lentiviral transduction. (B) RT-qPCR validation of NR2F2-AS1 overexpression efficiency. (C-D) mRNA and protein levels of CTGF (CCN2) in cells overexpressing NR2F2-AS1 compared to vector control. Data are presented as mean ± SD. **p < 0.01, ***p < 0.001, ****p < 0.0001.

3.4 NR2F2-AS1 is Predominantly Localized in the Cytoplasm

The subcellular localization of an lncRNA is often indicative of its functional mechanism. FISH analysis revealed that in normal GES-1 cells, NR2F2-AS1 signals were primarily distributed within the cytoplasm, with weak signals observed in the nuclei. Following *H. pylori* infection, the cytoplasmic fluorescence intensity of NR2F2-AS1 increased markedly, with a small fraction of the signal translocating to the nucleus (Figure 4). The predominantly cytoplasmic localization of NR2F2-AS1 suggests it may regulate CTGF expression through post-transcriptional mechanisms, such as acting as a competitive endogenous RNA (ceRNA) to modulate mRNA stability or interacting with proteins to influence translation efficiency.

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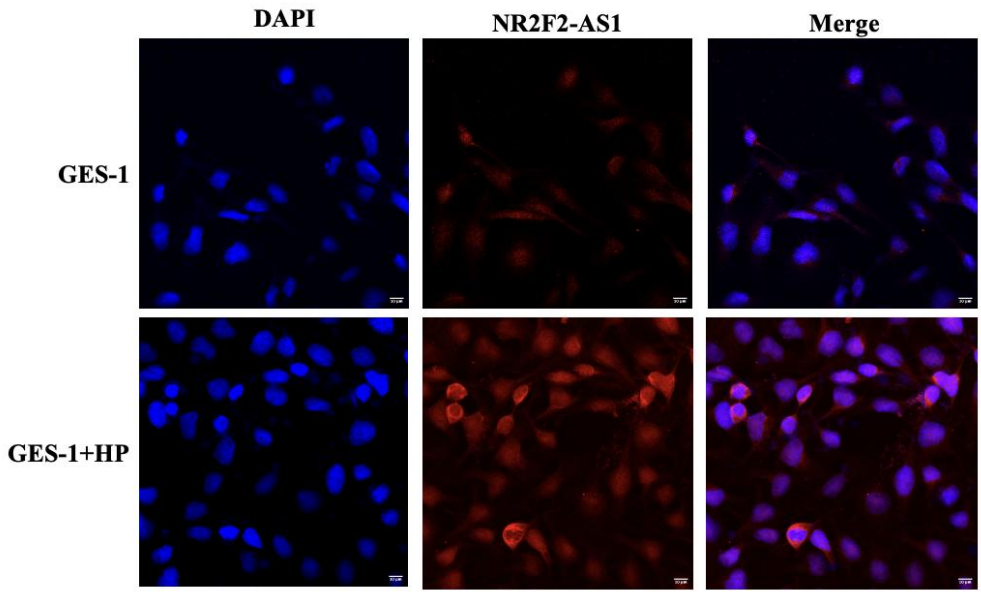

Figure 4. Subcellular localization of NR2F2-AS1 in GES-1 cells detected by FISH. Fluorescence in situ hybridization (FISH) demonstrates that NR2F2-AS1 (red) is predominantly localized in the cytoplasm of both uninfected and H. pylori-infected GES-1 cells. Nuclei were counterstained with DAPI (blue). H. pylori infection increased the abundance of the NR2F2-AS1 signal in the cytoplasm, with a minor fraction observed translocating to the nucleus. Scale bar = 10 μm.

4. Discussion

The present study identifies the long non-coding RNA (lncRNA) NR2F2-AS1 as a critical regulator of *Helicobacter pylori*-induced gastric epithelial injury. Our findings demonstrate that *H. pylori* infection significantly upregulates NR2F2-AS1, which in turn promotes the expression of Connective Tissue Growth Factor (CTGF), leading to exacerbated inflammatory responses, growth inhibition, and migratory dysfunction in GES-1 cells. This NR2F2-AS1/CTGF axis represents a novel regulatory node through which *H. pylori* disrupts cellular homeostasis.

The pathogenic mechanism of *H. pylori* involves a complex interplay between bacterial virulence factors and host cell responses. Beyond acute cytotoxicity, our data from colony formation and wound healing assays suggest that *H. pylori* infection significantly impairs the long-term proliferative and migratory repair capacities of the gastric epithelium. This likely contributes to the persistent nature of mucosal damage observed in chronic gastritis[12,13]. The concurrent upregulation of IL-6 and IL-8 further confirms the activation of pro-inflammatory signaling pathways, which are essential mediators of *H. pylori*-associated mucosal injury [14].

A key contribution of this study is the elucidation of a potential hierarchical regulatory role for NR2F2-AS1. FISH analysis localized NR2F2-AS1 primarily to the cytoplasm. In the context of lncRNA biology, cytoplasmic localization strongly suggests post-transcriptional regulatory mechanisms. We propose that NR2F2-AS1 may function as a competitive endogenous RNA (ceRNA), effectively "sponging" microRNAs that would otherwise target and degrade CTGF mRNA[5,15]. Given that CTGF is a well-recognized mediator of tissue remodeling and inflammation capable of activating the NF-κB signaling pathway, its NR2F2-AS1-dependent stabilization creates a self-sustaining inflammatory cycle that hinders mucosal recovery[16,17,18].

From a clinical perspective, the identification of the NR2F2-AS1/CTGF axis offers a new perspective on why some *H. pylori* infections lead to protracted mucosal damage and fibrosis. CTGF is known to drive extracellular matrix deposition and epithelial barrier disruption[19]. Therefore, the persistent activation of this axis might serve as a molecular bridge between early chronic inflammation and long-term complications such as gastric atrophy or intestinal metaplasia. Targeting this lncRNA-mediated pathway may provide a promising therapeutic strategy to mitigate gastric inflammation and promote mucosal healing.

Despite these insights, several limitations must be acknowledged. First, while we established a clear correlation and functional link between NR2F2-AS1 and CTGF, the specific microRNAs involved in this "molecular bridge" remain to be identified through dual-luciferase reporter assays. Second, our results are based on *in vitro* experiments using the GES-1 cell line. The complex *in vivo* environment, involving interactions with immune cells and various stromal components, was not fully represented. Future studies utilizing NR2F2-AS1 knockout mice or clinical biopsy samples are necessary to confirm the therapeutic relevance of this axis in human patients.

5. Conclusions

In summary, this study demonstrates that *H. pylori* infection significantly upregulates lncRNA NR2F2-AS1, which positively regulates CTGF expression to exacerbate cellular injury and inflammatory responses in gastric epithelial cells. The identification of the NR2F2-AS1/CTGF axis provides a novel molecular perspective on *H. pylori* pathogenesis. Future research should focus on validating this axis *in vivo* and exploring its potential as a diagnostic biomarker or therapeutic target for the clinical management of *H. pylori*-associated gastric diseases.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Acknowledgments

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Abbreviations:

GES-1	Human Gastric Epithelial Cell Line-1
NR2F2-AS1	NR2F2 antisense RNA 1
CTGF	Connective Tissue Growth Factor
IL-6	Interleukin-6
IL-8	Interleukin-8
CCK-8	Cell Counting Kit-8
FISH	Fluorescence In Situ Hybridization
CLIA	Chemiluminescent Immunoassay
FBS	fetal bovine serum
PBS	phosphate-buffered saline
MOI	multiplicities of infection