Helicobacter pylori-induced YAP1 nuclear translocation promotes gastric carcinogenesis by enhancing IL-1β expression

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Abstract
Gastric cancer (GC) is one of the most common and malignant pathologies, and a significant portion of GC incidences develops from Helicobacter pylori (Hp)-induced chronic gastritis. Although the exact mechanisms of GC are complex and poorly understood, gastric carcinogenesis is a good model to investigate how inflammation and infection collaboratively promote tumorigenesis. Yes-associated protein 1 (YAP1) is the key effector of the Hippo pathway, which is silenced in most human cancers. Herein, we verified the tumor-promoting effect of YAP1 in vitro, in vivo, and in human specimens. We revealed that YAP1 displays nuclear translocation and works with TEAD to activate transcription of the crucial inflammatory cytokine IL-1β in gastric cells infected with Hp. As IL-1β accounts for inflammation-associated tumorigenesis, this process can lead to gastric carcinogenesis. Thus, in addition to activating proliferation genes, YAP1 also plays a major role in inflammation amplification by activating inflammatory cytokine genes. Excitingly, our research demonstrates that transfection of mutant plasmid YAP-5SA/S94A or addition of the drug verteporfin, both of which are thought to disrupt the YAP1-TEAD interaction, can arrest the carcinogenesis process. These findings can provide new approaches to GC treatment.

KEYWORDS
gastric cancer, Helicobacter pylori, IL-1β, TEAD, YAP1

1 | INTRODUCTION

With one of the five highest morbidities among all cancer types, gastric cancer (GC) is also one of the top five causes of cancer death and led to 951,600 new cases and approximately 723,100 deaths in 2012.1 Helicobacter pylori (Hp) infection plays a triggering role in the process of inflammation-associated gastric carcinogenesis.2 Hp is such a successful pathogenic microorganism that approximately 50% of all human stomachs have been colonized.3 Recent studies have shown that Hp chronic infection accounts for approximately 90% of new noncardia GC cases worldwide, ranking it the most dangerous risk factor for GC. Interactions between Hp and its hosts are rather complicated. Hp uses its bacterial type IV secretion system (T4SS) to inject the toxin CagA into its host's stomach epithelial cells, triggering a vast range of downstream signaling cascades and ultimately accelerating gastric carcinogenesis.4 Other toxins, such as VacA and CagL, as
as \textit{Hp} components, such as lipopolysaccharides (LPS), also contribute to this malignant transformation.

Yes-associated protein 1 (YAP1) is the key terminal effector of the Hippo pathway, an old, conserved pathway that was first found to regulate organ size and cell fate that responds to density signals outside the membrane.\textsuperscript{5,6} YAP1 is normally inactive in the cytoplasm due to the phosphorylation of serine 127 by the upstream kinase LATS.\textsuperscript{7} When extracellular signals silence the Hippo cascade, as the terminal effector, YAP1 is translocated into the nucleus and becomes a transcription co-activator, cooperating with transcription factors, such as TEAD family members, to initiate the transcription of multiple oncogenes.\textsuperscript{8,9} YAP1 overexpression has been reported in various human cancers, including breast, lung, colorectal, ovarian, pancreatic, gastric, and liver cancer.\textsuperscript{10} In GC, elevated YAP1 and its nuclear accumulation are associated with poor prognosis.\textsuperscript{13-15} YAP1 has been shown to promote proliferation and metastasis as well as to induce apoptosis.\textsuperscript{16} However, the molecular mechanisms underlying how YAP functions in gastric carcinogenesis require further elucidation.

\textit{IL-1}\textbeta is a multifunctional proinflammatory cytokine that has profound inflammatory and immune effects,\textsuperscript{17} and it plays a crucial role in the initiation and development of a wide range of inflammation-associated cancers, especially GC.\textsuperscript{17,18} IL-1\textbeta is widely reported to promote gastric carcinogenesis and is associated with poor prognosis.\textsuperscript{21-24} IL-1\textbeta polymorphisms (IL-1b-511T and IL-1b-31C) can promote GC by boosting IL-1\textbeta production and increasing circulating cytokine levels,\textsuperscript{17,18} and \textit{Hp} infection can induce IL-1\textbeta expression.

This study aimed to prove that YAP1 plays a tumor-promoting role in \textit{Hp}-induced gastric carcinogenesis via activating the key cancer-related inflammatory cytokine IL-1\textbeta and thereby provide a new drug target for GC treatment.

2 | MATERIALS AND METHODS

2.1 | Cell lines and culture

The immortal gastric epithelium cell line GES-1 and the GC cell lines AGS and BGC-823 were used in this study. Cells were cultured in RPMI-1640 medium (Gibco) with 10% fetal bovine serum. All cells were incubated at 37°C in a humidified 5% CO2 atmosphere.

2.2 | Clinical samples and datasets

Twenty-eight human AG samples and thirteen GC samples were obtained from surgery patients. The specimens were collected immediately after surgery and stored in formalin. The diagnosis of GC for all patients was confirmed by histological examination. Eighty-two AG samples were obtained from a gastro-endoscope room. All patients had pathology reports from the hospital.

The TCGA Research Network is available at http://cancer.gov. GTEx dataset analysis is available at https://www.gtexportal.org. Kaplan-Meier plotter is available at http://kmplot.com.\textsuperscript{25}

2.3 | \textit{Helicobacter pylori (Hp)} culture

\textit{Hp} strains were cultured in Brucella broth with 5% fetal bovine serum under microaerobic conditions (5% O2, 10% CO2, and 85% N2) at 37°C. Bacteria were harvested and centrifuged and then added to gastric cell lines at varying multiplicity of infection (MOI) ratios.

2.4 | Animal experiments

Seven (four weeks old) male nude mice were purchased from QING ZI LAN Animal Company (Nanjing, China). One week after their arrival, seven five-week-old nude mice were used for xenograft experiments with both control and shYAP1 BGC-823 cells. In total, $4 \times 10^5$ BGC-823 cells were subcutaneously injected for each treatment. Tumors could be observed beginning on day 8, and relative parameters were recorded to construct the tumor growth curve. All the mice were sacrificed on day 22, and their tumors were collected for both mRNA extraction and IHC.

2.5 | siRNA, transfections, and lentivirus infection

YAP1, IL-1\textbeta, and negative control (nc) siRNAs were purchased from the RiboBio company (the si-h-YAP1 and si-h-IL-1\textbeta kits each included three target sequences). We transfected AGS, BGC-823, and GES-1 cells with 20 nmol/L siRNA in antibiotic-free Opti-MEM (Gibco) for 72 hours using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The shYAP1 lentivirus was produced by the GENECHEM company using the siYAP1 sequences 5’-GGUGAUACUAUCAACAAATdTdT-3’ and 3’-dTdTCCACUAUGAUAGUUGGUUU-5’.

2.6 | Plasmids

pcDNA4/HisMaxB-YAP1-S127A (Addgene plasmid #18988), pcDNA4/HisMaxB-YAP1 (Addgene plasmid #18978), and pCMV-flag YAP2-5SA (Addgene plasmid #27371) were obtained from Addgene. The pGL3 Basic luciferase reporter vector was purchased from Promega.
2.7 | Colony formation assay

We transfected AGS, BGC-823, and GES-1 cells with siRNA (siYAP1 and siIL-1β) or plasmids. Seventy-two hours after siRNA transfection or forty-eight hours after plasmid transfection, the cells were seeded in 6-well plates. For AGS cells, seven days were needed for the colonies to grow, while 14 days were needed for the BGC-823 and GES-1 cells. After 7-14 days of incubation at 37°C in a humidified 5% CO2 atmosphere, the colonies were fixed and stained with methanol and Giemsa buffer, respectively, and the colony numbers were counted.

2.8 | Western blotting

Cell pellets from the different treatment groups were lysed with 1 × Laemmli sample lysis buffer (62.5 mmol/L Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 0.002% bromophenol blue, and 100 mmol/L DTT), and a spectrophotometer was used to determine the protein concentrations. We used the Bio-Rad system to run electrophoresis and transfer proteins to PVDF membranes. After 1.5 hours of blocking (blocking buffer: 5% milk diluted in 1 × TBST buffer), primary antibodies were added, and the membranes were incubated at 4°C overnight with gentle shaking. The secondary antibodies were horseradish peroxidase-labeled, and Millipore ECL reagents were used to detect the proteins on the blots.

2.9 | Antibodies

YAP (D8H1X) XP® rabbit mAb (#14074) and phospho-YAP (Ser127) (D9W2I) rabbit mAb (#13008) were purchased from Cell Signaling Technology (CST), and the IL-1β antibody (16806-1-AP) was purchased from Proteintech. The anti-CagA antibody was purchased from Abcam, and the β-Actin antibody (C4) (sc-47778) was purchased from Santa Cruz Biotechnology.

2.10 | Immunofluorescence (IF) staining

Cells were initially seeded on coverslips. After treatment and incubation in the cell culture incubator, the cells were fixed in 4% paraformaldehyde and incubated with primary antibodies at 4°C overnight. Anti-rabbit IgG (H + L) and the F(ab')2 fragment (CST) were used as secondary antibodies, and DAPI (4-6-diamidino-2-phenylindole dihydrochloride) was used to detect nuclei.

2.11 | Edu assay

The Cell-Light™ Edu Apollo®488 In Vitro Imaging Kit was used to detect cell proliferation according to the manufacturer’s instructions (Guangzhou RiboBio).

2.12 | Immunohistochemistry (IHC) staining

Tissues from patients and mice were embedded in paraffin. After deparaffination, dehydration, epitope retrieval, and H2O2 treatment, the sections were blocked in 5% normal goat serum for 30 min and incubated with primary antibodies at 4°C overnight. After incubation with the secondary antibody, the DAB Staining Kit (Vector Laboratories) was used for staining.

2.13 | RNA extraction and quantitative RT-PCR

Total RNA was extracted from harvested cells using TRIzol reagent according to the manufacturer's instructions, and the RevertAid First Strand DNA Synthesis Kit (Fermentas) was used to reverse transcribe the total RNA into cDNA. The sequences of primers used in the qPCR assays were as follows: β-actin: 5’-AGTTGCGTGTAACCCCTCTTG-3' and 5’-CACCTTCACCGTTCAGTTTTT-3'; and YAP1-168: 5’-CATGGCAGAAAGACTGAAATAAC-3' and 5’-GAGGATAAAAATCCACCTGAGCAC-3'.

2.14 | Dual luciferase assay

Human IL-1β promoter fragments were cloned from human genomic DNA by PCR. Primers containing the KpnI and HindIII restriction sites were designed in the fragment sequences to construct luciferase reporter plasmids. The primers were as follows: IL-1-1 (motif A) F: 5’-GGTACCCCCCAGCCAAGAAAGGTCA-3', R: 5’-AAGCTTTGGAAGGGCAAGGAGTAGC-3'; and IL-1-2 (motif B) F: 5’-5’-GGTACCACAACAGGCTGCTCTGGGATT-3', R: 5’-AAGCTTTGGAAGGGCAAGGAGTAGC-3'. The fragments were cloned into the pGL3 Basic luciferase reporter vector (Promega). The Luciferase Assay System (Promega) was used to detect firefly and Renilla luminescence, and the Renilla signal was used for normalization.

2.15 | Chromatin immunoprecipitation (ChIP) assay

A Chromatin Immunoprecipitation (ChIP) Assay Kit (Millipore) was used to perform the ChIP assay. Sonicated chromatin fragments were incubated overnight at 4°C with the anti-YAP1 antibody, and bound DNA precipitants were analyzed using PCR. The primers used for PCR detection were as follows: IL-1-1 (motif A) F: 5’-GGTACCCCCCAGCCAAGAAAGGTCA-3' and R: 5’-AAGCTTTGGAAGGGCAAGGAGTAGC-3'; and IL-1-2 (motif B) F: 5’-GGTACCACAACAGGCTGCTCTGGGATT-3' and R: 5’-AAGCTTTGGAAGGGCAAGGAGTAGC-3'.

2.16 | Immunofluorescence (IF) staining

Cells were initially seeded on coverslips. After treatment and incubation in the cell culture incubator, the cells were fixed in 4% paraformaldehyde and incubated with primary antibodies at 4°C overnight. Anti-rabbit IgG (H + L) and the F(ab')2 fragment (CST) were used as secondary antibodies, and DAPI (4-6-diamidino-2-phenylindole dihydrochloride) was used to detect nuclei.

2.17 | Edu assay

The Cell-Light™ Edu Apollo®488 In Vitro Imaging Kit was used to detect cell proliferation according to the manufacturer’s instructions (Guangzhou RiboBio).
2.16 Statistical analysis

Experimental quantitative data were obtained in biological replicates and shown as means (±SD). Student's t tests, \( \chi^2 \) tests, and log-rank test were used to analyze the data. \( P < 0.05 \) was considered statistically significant.

3 RESULTS

3.1 YAP1 is upregulated in human gastric specimens and gastric cell lines

To clarify the association between YAP1 and GC, we first collected data from the TCGA (The Cancer Genome Atlas) and GTEx (Genotype-Tissue Expression) database and compared the YAP1 mRNA expression levels among normal gastric tissues (\( N = 211 \)) and cancerous gastric tissues (\( T = 408 \))\(^{26} \) (Figure 1A). YAP1 was expressed at significantly higher levels in stomach adenocarcinoma (STAD) than in normal gastric mucosa. Kaplan-Meier analysis revealed that high YAP1 expression was significantly associated with shorter patient overall survival in GC (Figure 1B). We next stained YAP1 in paired nontumorous and cancerous gastric tissues using immunohistochemistry (IHC) (Figure 1C), revealing that both the positivity and intensity of the YAP1 signals were enhanced in GC. We detected YAP1 expression levels in 28 human atrophic gastritis (AG) samples and 13 GC samples by quantitative real-time PCR (qPCR), revealing obviously higher YAP1 expression in GC tissues compared to that in AG tissues (Figure 1D). YAP1 expression in GC reached a new level, which emphasized the role of YAP1 in AG-GC malignant transformations. We next divided 82 AG tissue samples into \( Hp^+ \)–positive and \( Hp^- \)–negative groups. We performed qPCR and IHC staining and found high YAP1 expression levels in \( Hp^+ \)-positive patients, which implied the role of YAP1 in \( Hp^+ \)-induced gastric malignancy progression (Figure 1E and Figure S1A). Higher levels of YAP1 expression were detected in the GC cell lines AGS and BGC-823 compared with that in the gastric epithelial cell line GES-1 (Figure 2A). These results illustrated that YAP1 is overexpressed in human gastric tumor tissues and might be positively correlated with \( Hp \)-induced GC progression.

3.2 Helicobacter pylori promotes YAP1 expression and nuclear translocation

In our studies, the fact that \( Hp \) might be associated with YAP1 overexpression aroused our attention. Additionally, we found an interesting phenomenon that \( Hp \) infection can promote YAP1 expression and nuclear translocation. We added the \( Hp \) standard strain \( hp26695 \) to the gastric epithelial cell lines AGS and BGC-823 and the human gastric epithelial cell line GES-1 and harvested these cells at 0, 2, 4, 6, and 8 hours. We then detected the protein expression of YAP1 and YAP1 serine 127 phosphorylation (YAP1S127) by Western blot (Figure 2A-C). YAP1S127 is a phosphorylated form of YAP1 that cannot be transferred into the nucleus. With \( Hp26695 \) infection, YAP1 protein expression in AGS, BGC-823, and GES-1 cells was upregulated in a time-dependent manner, while YAP1S127 protein expression showed the opposite trend, indicating a potential increase in YAP1 nuclear translocation. To investigate whether the \( Hp26695 \) and CagA stimuli could trigger YAP1 nuclear translocation, we conducted immunofluorescence (IF) staining after infecting AGS and BGC-823 cells with the CagA+ and CagA− \( Hp \) strains. \( Hp \) promoted YAP1 nuclear translocation only when CagA was present, as the CagA− \( Hp \) infection did not show similar effects (Figure 2D,E). We then transfected AGS cells with the CagA plasmid, and the results were consistent (Figure 2F). To investigate the role of YAP1 in \( Hp^+ \)-infected GC cells, we knocked down YAP1 using YAP1 small interfering RNA (siRNA). Among three parallel YAP1 siRNAs, siYAP1.3 (si3) had the highest knockdown efficiency (Figure S2B) and was thus used to perform all knockdown experiments in this study. Seventy-two hours after the YAP1 siRNA was transfected, AGS and BGC-823 cells were infected with \( hp26695 \) for 6 hours, and the colony formation assay was then performed (Figure 2G). In both cell lines, \( Hp26695 \) infection significantly increased the colony formation ability, and this effect was inhibited by YAP1 knockdown. These results proved that \( Hp \) promotes GC cell

**FIGURE 1** YAP1 expression in gastric cancer and its association with Helicobacter pylori infection. (A) TCGA (https://cancer.gov) and GTEx (https://www.gtexportal.org/home/) data analysis of YAP1 mRNA levels in normal gastric mucosa tissues and stomach adenocarcinoma (STAD) tissues (Stomach Adenocarcinoma (TCGA, Provisional) January 2017). Normal gastric mucosa has lower YAP1 expression. (B) http://kmplot.com/ data analysis showed higher YAP1 expression is a predictor for shorter overall survival in GC patients. The median cutoff was used to categorize patients into low and high YAP1 expression groups. Mantel-Cox test was used in the survival analysis. Hazard ratio (HR) was presented in each panel, respectively. (C) Immunohistochemical staining was performed on nontumorous gastric samples and gastric tumor samples. Quantification of the data shows that YAP1 is highly expressed in gastric tumor tissues (data are presented as the mean ± SD, ***\( P < 0.001 \)). (D) Relative YAP1 mRNA levels were detected by qPCR in atrophic gastritis (AG, \( n = 28 \)) and gastric cancer (GC, \( n = 13 \)) patient samples (data are presented as the mean ± SD, ****\( P < 0.0001 \), unpaired \( t \) test). (E) Atrophic gastritis (AG) patient samples were divided into \( Hp^+ (n = 20) \) and \( Hp^- (n = 62) \) groups based on Helicobacter pylori (Hp) infection. YAP1 mRNA levels were detected by qPCR (data are presented as the mean ± SD, *\( P < 0.05 \), **\( P < 0.01 \), unpaired \( t \) test)
proliferation partially by regulating YAP1. *Hp* functions not only by upregulating YAP1 expression but also by promoting YAP1 nuclear translocation. *Hp* enhanced YAP1 expression and nuclear translocation by injecting its toxin protein CagA into gastric epithelial cells.

### 3.3 YAP contributes to GC cell proliferation in vitro

To further investigate the role of YAP1 in GC cell proliferation, we induced YAP1 siRNA in the AGS and BGC-823 cell lines and performed colony formation assays. Both AGS and BGC-823 cells had concordant results; cells of the siYAP1 group formed much fewer colonies than those of the nc group (Figure 3A). The YAPS127 plasmid provides sustained overexpression of the serine 127 mutant form of YAP1. This YAP1 mutant cannot be phosphorylated at its serine 127 position and thus can continuously translocate into the nucleus. We transfected AGS and BGC-823 cells with the YAPS127 mutant plasmid and performed the colony formation assay (Figure 3B). Both AGS and BGC-823 control group cells formed fewer colonies than the YAPS127 group, indicating that activated YAP1 plays a key role in GC cell proliferation. The efficiency of the plasmid was confirmed by Western blotting (Figure S2C, D). We also collected YAP1 knockdown and YAP1 activated cells to test their proliferation capacities using the Edu assay. In accordance with the colony formation assays, the Edu assays also provided evidence of the promotional effect of YAP1 on proliferation in vitro (Figure 3C-F), suggesting that YAP1 can transform non-tumorigenic cells in vitro. These results collectively prove that YAP1 can contribute to GC cell proliferation and might promote the malignant transformation of nontumorigenic gastric epithelial cells.

### 3.4 YAP1 can induce gastric tumor growth in vivo

To confirm the oncogenic role of YAP1 in gastric epithelial cells in vivo, we constructed a lentivirus-infected BGC-823 cell line stably expressing shYAP1. The YAP1 knockdown efficiency was verified at the protein level (Figure 4A). Both negative control (con) and shYAP1 BGC-823 cells were used for xenograft experiments with subcutaneous infection. Tumors could be observed beginning on day 8, and relative parameters were recorded to construct the tumor growth curve (Figure 4B,D). All of the mice were sacrificed on day 22, and their tumors were excised (Figure 4C). According to the photographs shown in Figure 4C, the shYAP1 group formed smaller and fewer (con/n = 7, shYAP1/n = 5) tumors than the negative control group. Tumors from the shYAP1 group also had reduced volumes and weights compared to those of the control group, which was in accordance with the result in Figure 4C (Figure 4D,E). The tumors were collected for both RNA extraction and paraffin sectioning. YAP1 mRNA expression was significantly downregulated in the shYAP1 group (Figure 4F), and IHC analysis indicated a similar trend (Figure 4G). Moreover, decreased expression of IL-1β, a crucial inflammatory cytokine, was also proven by IHC analysis (Figure 4G). Hematoxylin and eosin (HE) stain showed that more lymphocytes were observed in the negative control group than shYAP1 group, indicating reduced inflammation in the xenografts with YAP1 knockdown (Figure S1B). The collected data confirmed YAP1’s role in the initiation and growth of tumors generated from GC cells in vivo.

### 3.5 YAP1 regulates IL-1β transcription directly in cooperation with TEAD

IHC staining of mouse tumor tissues demonstrated decreased IL-1β expression in the shYAP1 group (Figure 4G). A time-dependent increase in IL-1β expression was also observed in the *Hp* infection experiment, following the trend of YAP1 (Figure 2A-C). We knocked down YAP1 expression in the GC cell lines AGS and BGC-823 using YAP1 siRNA and detected IL-1β mRNA expression by qPCR, observing that IL-1β mRNA expression was decreased by approximately 50% after YAP1 knockdown (Figure 5A). Similar YAP1 knockdown results were obtained in BGC-823 cells at the protein level using both siYAP1 and shYAP1 (Figure 5B). Because numerous published studies have reported that YAP1 can activate downstream genes as a transcriptional co-activator by interacting with TEAD family transcription factors, we proposed that YAP1 regulates IL-1β transcription directly by binding to IL-1β’s promoter region in cooperation with TEAD. According to T Mizuno’s publication in Oncogene in 2012,8 we searched the classic putative TEAD recognition motif on IL-1β’s promoter region and found three TEAD DNA-binding sites.
within 1000-bp upstream of the TSS of IL-1β (Figure 5C). Since binding sites 1 and 2 were close to each other, we regarded the two sites together as motif A. Motif B was located between Motif A and the TSS. Motifs A and B were constructed into two plasmids for dual luciferase assays, which were conducted to verify that YAP1 functions to activate IL-1β gene expression. The luciferase signals in the siYAP1-transfected group were weaker than those in the nc group in both AGS and BGC-823 cells (Figure 5D), and the luciferase activity was stronger in both cell lines transfected with the high YAP1 expression plasmid YAP1up and the serine 127 mutant plasmid YAP1S127 compared with that in the control group (Figure 5E). These findings were then validated with a chromatin immunoprecipitation (ChIP) assay. DNA immunoprecipitated by a YAPI antibody contained the proximal promoter with two putative TEAD binding motifs on the IL-1β gene, which was confirmed by PCR (Figure 5F). These results indicated that YAPI can bind the IL-1β promoter as a transcription co-factor and thus regulate IL-1β production.

YAPI-5SA and YAPI-S94A are mutant forms of YAPI. YAPI-5SA expresses constitutively active YAPI-5SA, which is resistant to phosphorylation at multiple sites by Mst2 and Lats2. YAPI-S94A can disrupt the YAPI-TEAD interaction but does not impair its general transcriptional activity. Here, we introduced the YAPI-5SA/S94A mutant plasmid, which is a combination of YAPI-5SA and YAPI-S94A, to explore the role of TEAD in IL-1β activation 27 (TEAD mediates YAPI-dependent gene induction and growth control). We transfected GES-1 cells with the YAPI-127A and YAPI-5SA/S94A mutant plasmids. Colony formation and Edu assays were performed after 72 hours, revealing that YAPI-5SA/S94A could reverse the positive effect of YAPI-127A on proliferation (Figure 6A,C). These results showed that when the YAPI-TEAD interaction was disrupted, GES-1 cells had a decreased proliferation ability even if the active form of YAPI was continuously overexpressed, which indicated that TEAD plays a key role in assisting YAPI’s activity on the IL-1β promoter.

Verteporfin (VP), a drug that was first reported to treat neovascular age-related macular degeneration that is capable of disrupting YAPI-TEAD binding, 28-30 was utilized to reveal the function of YAPI-TEAD in initiating IL-1β transcriptional expression. VP was dissolved in DMSO and added at 10 μmol/L. 24 hours after BGC-823 cells were transfected with the YAPI-127A plasmid. The colony formation assay showed that VP compromised the ability of YAPI-127A to promote proliferation (Figure 6B). Similarly, cells treated with VP had a weaker luciferase signal than those transfected with the YAPI-127A plasmid (Figure 6D), suggesting that VP slows GC cell proliferation by disrupting the YAPI-TEAD interaction.

These findings confirmed YAPI’s transcription co-activator role in the regulation of IL-1β and elucidated the mechanism of this process. Transfection of mutant plasmids or addition of the drug VP, which disrupts the YAPI-TEAD interaction, can arrest the carcinogenesis process. Our findings might provide a new drug target for GC treatment.

3.6 | Deleting IL-1β partially neutralized YAPI’s ability to promote proliferation

To confirm the role of IL-1β in YAPI-regulated GC development, we introduced IL-1β siRNA, and the knockdown efficiency was verified at the mRNA level (Figure S2E). IL-1β si1 had the highest efficiency and was thus applied in the subsequent experiments. IL-1β was knocked down in AGS and BGC-823 cells after overexpressing YAPI-127A, and the colony formation assay was performed. IL-1β knockdown compromised the positive effect of YAPI-127A on colony formation (Figure 6E). These results illustrated the important role of IL-1β as a key downstream effector of YAPI in GC cell proliferation. The Hs-YAPI-IL-1β pathway may serve as a novel drug target in GC treatment (Figure 7A).

4 | DISCUSSION

GC progression is a complex process that combines uncontrolled proliferation and unhealed inflammation, and the mechanism by which Hs infection induces gastric carcinogenesis is not fully elucidated. In this study, we demonstrate that Hs promotes tumor-accelerating inflammation by enhancing YAPI expression and nuclear translocation. YAPI functions as a transcriptional co-activator working together with TEAD. The two-molecule complex binds the IL-1β promoter and thereby increases IL-1β expression. The abundant IL-1β expression assists GC development by stimulating cancer cell proliferation. Taken together, Hs plays an important role in GC progression by regulating YAPI and downstream IL-1β.

The facts that higher incidence rates of GC occur in developing countries, such as China, and GC often develops...
from severe forms of gastritis indicates that hygienic standards, eating habits, and microbiota colonization are closely correlated with GC development. Interactions between Hp and its hosts are rather complicated. Hp uses its bacterial type IV secretion system (T4SS) to inject the toxin CagA into its host’s stomach epithelial cells, triggering a vast range of downstream signaling cascades and ultimately accelerating gastric carcinogenesis. Other toxins, such as VacA and CagL, as well as Hp components, such as LPS, also contribute to this malignant transformation. Recently, a group reported that Hp can promote GC by activating gastric stem cells. The gastric carcinogenesis initiated by Hp can be partially attributed to changes in signaling pathways, and the most studied pathways are toll-like receptor-4 (TLR-4) and nuclear factor-kB (NF-kB) signaling. Activation of TLR-4 triggers a cascade of downstream signaling pathways, including phosphorylation of the mitogen-activated protein kinase (MAPK) pathway, which contains the components extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38. Phosphorylation of the MAK pathway can further activate several key transcription factors and lead to uncontrolled growth in GC. Moreover, activation of the NF-kB pathway also plays a critical role in maintaining cell proliferation and protecting cells from apoptosis in GC. Although not all mechanisms underlying the tumor-promoting role of Hp have been well illustrated, Hp clearly and cleverly manipulates routine pathways in host cells as an external stimulus to ensure its survival and proliferation, as these pathways react sensitively to external signals. The Hippo cascade is a pathway that is sensitive to environmental signal perturbation. We observed an unreported phenomenon that the Hippo cascade key effector YAP1 exhibited increased nuclear translocation after gastric cells were infected with Hp, which aroused our interests to research new mechanisms underlying the role of YAP1 in Hp-induced gastric carcinogenesis.

Despite the ability of inflammation to resist infection, the occasional unstoppable activation of inflammatory responses and activators creates a persistent inflammatory microenvironment that facilitates tumor-promoting inflammation, which contributes to unresolved inflammation and is recognized as a new cancer hallmark. As in some patients, Hp infection of the stomach can first cause chronic superficial gastritis, then chronic AG with intestinal metaplasia, then gastric dysplasia, and eventually GC. studying Hp-induced gastric carcinogenesis is sufficient to illustrate how chronic inflammation and infection collaboratively trigger tumors. In our study, we attempted to elucidate the mechanism by which Hp infection triggers GC and how GC cells transform infection signals into constant inflammation, revealing that YAP1 serves as the link. YAP1 transcriptionally activates IL-1β in GC cells, which is beyond the classic mechanism involving immune cell responses, and creates a complete inflammatory environment wherein the infection occurs.

Although the mechanisms by which inflammation promotes carcinogenesis remain unclear, some consensus has been reached among researchers regarding the inflammatory factors influencing carcinogenesis. Both IL-1β and IL-18 are proinflammatory cytokines that require inflammasomes to cleave unprocessed IL-1β (pro IL-1β) and unprocessed IL-18 (pro IL-18) into their biologically active forms. Once activated, with the help of caspase-1, the mature IL-1β and IL-18 cause pyroptosis, a type of inflammatory cell death. In contrast to the constitutive expression of IL-18, transcriptional activation of the pro IL-1β gene is the foundation of IL-1β activation, which is mainly mediated by NF-kB. Activated IL-1β provides positive feed-forward stimulation for inflammatory cytokines, thereby amplifying inflammation, which can result in some diseases, including autoimmune diseases and cancer.

Numerous studies have shown the positive role of IL-1β in the progression of various cancers, including breast cancer, lung cancer, and hematological malignancies. Moreover, IL-1β has been found to be the most relevant to GC development. Abnormally high IL1β expression is one of the most important factors accelerating GC progression. In our animal experiment, we observed reduced IL-1β levels in the YAP1 knockdown group, which had an impaired tumorigenesis ability. In the Hp infection study, both YAP1 and IL-1β expression showed an infection time-dependent trend, which provided a clue into the novel mechanism. We also checked the expression of other inflammatory factors (IL-6, IL8, IL-10, and TNFα) in negative control and YAP1 knockdown groups in AGS and BGC. However, we did not find accordant results showing that they were YAP1 direct downstream effectors (Figure S2f).

TEADs are DNA-binding proteins that can be activated by YAP1 and work as transcription factors. YAP1 has been demonstrated to promote tumorigenesis by activating...
TEAD in breast cancers, cholangiocarcinoma, and renal cell carcinoma. Moreover, TEADs were shown to be overexpressed in GC. In our study, disrupting the YAP1-TEAD interaction interfered with the gastric tumor development process. The YAP1-TEAD interaction can be disrupted by either adding the drug VP or transfecting mutant plasmids. In addition, an increasing number of studies have shown that VP has an anti-proliferation effect on various cancers, including melanoma, prostate, liver, esophageal, and lung cancer. These findings indicate that YAP1 might become a new drug target in GC treatment, but further investigation is needed for clinical transformation.

**FIGURE 5** YAP1 induces IL-1β expression by binding the IL-1β promoter region. (A) Loss of YAP1 reduced IL-1β mRNA expression in AGS and BGC-823 cells (data are presented as the mean ± SD of three independent biological experiments, *=P < 0.05, **=P < 0.001, unpaired t test). (B) IL-1β was downregulated when YAP1 was knocked down in YAP1 siRNA-transfected and stable shYAP1 BGC-823 cells. (C) TEAD binding sites on the IL-1β promoter region. Three TEAD binding sites were divided into two motifs, which were inserted into a backbone plasmid for the construction of a dual luciferase assay vector. (D) Decreased luciferase activity (motif A and motif B) was observed upon YAP1 knockdown in both AGS and BGC-823 cells (data are presented as the mean ± SD, **=P < 0.01, ***=P < 0.001, ****=P < 0.0001). (E) Increased luciferase activity (motif A and motif B) was observed in AGS and BGC-823 cells transfected with the YAP1S127A and YAP1up plasmids (data are presented as the mean ± SD, **=P < 0.01, ***=P < 0.001, ****=P < 0.0001). (F) ChIP analysis confirming that YAP1 can bind two regions of the IL-1β promoter in BGC-823 cells.

**FIGURE 6** YAP1 functions on IL-1β promoter via interaction with the transcriptional factor TEAD. (A) GES-1 cells transfected with the YAP5SA/S94A plasmid formed fewer colonies than the YAP1S127A-transfected and control groups (data are presented as the mean ± SD, ***=P < 0.001, unpaired t test). (B) Verteporfin (VP, 10 μmol/L) reversed the promotional proliferation effect of YAP1 on BGC-823 cells (data are presented as the mean ± SD, **=P < 0.01, ***=P < 0.001, unpaired t test). (C) Edu assay showing that the YAP5SA/S94A plasmid can compromise the promotional proliferation effect of YAP1 on GES-1 cells. (D) Dual luciferase assay showing that VP (10 μmol/L) disrupted the regulation of YAP1 on the IL-1β promoter region (data are presented as the mean ± SD, *=P < 0.05, **=P < 0.01, ***=P < 0.001). (E) Loss of IL-1β partially compromises the pro-proliferative effect of YAP1. Complementary experiments were performed in AGS and BGC-823 cells by transfecting the YAP1S127A plasmid into IL-1β knockout cells. IL-1β knockout compromised the positive effect of YAP1s127A on colony formation (data are presented as the mean ± SD, **=P < 0.01, ***=P < 0.001, ****=P < 0.0001).
In summary, our findings sketch an outline of the \textit{Hp}-YAP1-IL-1β pathway. This axis connects \textit{Hp} infection to vast tissue inflammation and tumorigenesis and thereby plays an important role in GC initiation and development.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Jihui Jia, Jingya Yu and Yujiao Wu designed the study; Yujiao Wu, Jingya Yu, Xiuming Liang, Li Shen, Shuyan Li, and Lixin Zheng performed the study; Lin Ma, Tongyu Li, Han Yu and Hillary Chan analyzed the data; Chunyan Chen and Jihui Jia supervised the study; and Yujiao Wu, Jingya Yu and Jihui Jia wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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