Helicobacter pylori promotes gastric cancer progression by upregulating semaphorin 5A expression via ERK/MMP9 signaling

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INTRODUCTION

Gastric cancer is one of the most common malignant tumors and seriously threatens human health. Based on global cancer statistics in 2018, an estimated 1,030,000 new stomach cancer cases were diagnosed, and 782,685 deaths occurred.1 Although great achievements have been made in gastric cancer research and clinical treatment in recent years, patients with advanced gastric cancer have a poor prognosis, with a 5-year survival rate lower than 20%.2,3 Therefore, the identification of risk factors is of great clinical value to understand their pathogenic effects.

Helicobacter pylori (H. pylori) is an important pathogen contributing to the occurrence and progression of gastric cancer. Chronic infection with H. pylori leads to atrophic gastritis, along with the development of intestinal metaplasia, dysplasia, and gastric cancer. Therefore, H. pylori has been defined as a class I carcinogenic factor in the human stomach by the World Health Organization (WHO) since 1994.4–6 However, the mechanism underlying the H. pylori-induced pathogenesis of gastric cancer remains to be fully elucidated.

Semaphorin 5A, a class 5 semaphorin, is an integral membrane protein with seven characteristic thrombospondin-specific repeats (TSP-1). Initially, the gene was identified to play a crucial role in the guidance of growing axons to their targets during the development of the central nervous system.7–11 As shown in our previous studies, semaphorin 5A is overexpressed in gastric cancer tissue, which may be closely related to the tumorigenesis and metastasis of gastric cancer.12,14 In our recent study, semaphorin 5A expression was identified as a potentially important contributor to the occurrence and development of H. pylori-related gastric precancerous lesions.1 However, researchers have not yet determined whether semaphorin 5A is associated with H. pylori-related gastric carcinoma. In the present study, we investigated the role of semaphorin 5A in H. pylori-induced gastric carcinogenesis and identified a novel intracellular signaling pathway involving semaphorin 5A in H. pylori-mediated pathogenesis.

RESULTS

H. pylori infection is related to the clinicopathological features of gastric cancer

We first observed H. pylori colonization in 200 gastric cancer samples to explore the relationship between H. pylori infection and the...
Semaphorin 5A expression in gastric cancer tissues.

(A) Semaphorin 5A expression in H. pylori-positive gastric cancer tissues. (B) Semaphorin 5A expression in H. pylori-negative gastric cancer tissues. The red arrow indicates positive staining. Scale bars, 100 μm. Note: Samples were obtained from 200 patients who underwent surgical resection for gastric cancer with no preoperative chemotherapy or radiotherapy between March 2019 and July 2020 at the Department of Surgery in the First Affiliated Hospital of Kunming Medical University (P.R. China). By observing the status of H. pylori infection, the 200 cases were divided into an infection-positive group and an infection-negative group. All samples underwent Sema5A IHC staining and semiquantitative scoring. Semaphorin 5A expression was associated with H. pylori infection (p < 0.001) (Table 2).

5A upregulation in H. pylori-infected gastric cancer. After 0, 12, and 24 h of incubation with H. pylori, differential upregulation of the semaphorin 5A mRNA was observed in SGC7901 and MKN28 cells (Figure 2A). To evaluate whether H. pylori-mediated upregulation of semaphorin 5A also occurred at the protein level, we measured semaphorin 5A expression at 12 and 24 h in H. pylori-stimulated or untreated cells using western blotting (WB) (Figure 2B). H. pylori significantly increased levels of the semaphorin 5A protein in both gastric cancer cell lines. These data further show that H. pylori upregulates semaphorin 5A expression.

**H. pylori infection upregulates semaphorin 5A expression in an experimental rat gastric H. pylori infection model**

A Sprague-Dawley rat model colonized with H. pylori was established to further assess the relationship between semaphorin 5A expression and H. pylori infection. The rapid urease test results confirmed that rats in the infection group were infected with H. pylori (Figure S1). The gastric mucosa of the rats in the infection group showed chronic inflammatory cell infiltration (Figure 3B), but marked histological changes were not observed in the control group (Figure 3A). Modified Giemsa and Warthin-Starry staining revealed a number of bacteria that colonized the mucosa of the rats in the infection group.

**Table 1. Correlations of H. pylori infection and clinicopathological features of gastric cancer patients**

| Clinicopathological factors | H. pylori positive (n = 101) | H. pylori negative (n = 99) | p     |
|-----------------------------|-----------------------------|-----------------------------|-------|
| Sex                         |                             |                             |       |
| Female                      | 98                          | 53                          |       |
| Male                        | 102                         | 48                          | 0.197 |
| Age (years)                 |                             |                             |       |
| ≥65                         | 110                         | 54                          | 0.383 |
| <65                         | 90                          | 47                          |       |
| Lauren classification       |                             |                             |       |
| Intestinal                  | 100                         | 63                          | <0.001|
| Diffuse                     | 100                         | 38                          |       |
| Depth of invasion           |                             |                             | <0.001|
| T1–T2                      | 103                         | 72                          |       |
| T3–T4                      | 97                          | 29                          |       |
| Lymph metastasis            |                             |                             |       |
| Negative                    | 131                         | 54                          | <0.001|
| Positive                    | 69                          | 47                          |       |
| TNM stage                   |                             |                             | <0.001|
| I–II                        | 124                         | 46                          |       |
| III–IV                      | 76                          | 55                          |       |

Semaphorin 5A expression in gastric cancer tissues correlates with the H. pylori infection status

We first investigated semaphorin 5A expression in H. pylori-infected gastric cancer tissues to explore the mechanism underlying H. pylori-induced gastric carcinogenesis. The expression of the semaphorin 5A protein was evaluated in 200 human gastric cancer specimens using immunohistochemistry (IHC). IHC staining of gastric carcinoma specimens showed that the mean staining intensity of semaphorin 5A was higher in H. pylori-positive gastric cancer than in H. pylori-negative tissues (Figures 1A and 1B; Table 2). Our results suggest that H. pylori infection upregulates semaphorin 5A expression, which might play a crucial role in H. pylori-induced gastric cancer progression.

**Effect of H. pylori on semaphorin 5A mRNA and protein levels in SGC7901 and MKN28 cells**

We investigated the effect of H. pylori infection on semaphorin 5A expression in gastric cancer cells to further confirm semaphorin expression.

**Table 2. Semaphorin 5A expression is associated with H. pylori infection**

| Variable                   | Semaphorin 5A expression | 0  | +   | ++  | +++ | Total | χ²  | p   |
|----------------------------|--------------------------|----|-----|-----|-----|-------|-----|-----|
| H. pylori-positive group   |                          | 6  | 18  | 34  | 43  | 101   | 71.51| <0.001|
| H. pylori-negative group   |                          | 17 | 63  | 16  | 3   | 99    |     |     |
and Transwell assays were performed
cells via its effects on semaphorin 5A
H. pylori
on the migration and invasion of SGC7901, SGC7901-siSema 5A cells. As illustrated in Figures 4C and 4D, semaphorin 5A depletion resulted in decreased cell proliferation. Similarly, the colony-formation assay indicated that semaphorin 5A knockdown inhibited the invasive abilities of SGC7901- and SGC7901-siScrambled cells were higher than those of SGC7901-siSema 5A cells. Taken together, the ERK signaling pathway may be responsible for H. pylori-induced gastric cancer progression through a semaphorin 5A-dependent mechanism.

**MMP9 was involved in H. pylori-induced gastric cancer progression mediated by semaphorin 5A**
Elevated levels of matrix metalloproteinase 9 (MMP9) in tumors are generally associated with cancer cell invasiveness and metastasis. Here, we explored whether MMP9 was involved in H. pylori-induced gastric cancer progression mediated by semaphorin 5A. First, the effects of H. pylori on the expression level of MMP9 were examined in SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells after a co-incubation with H. pylori for 24 h. As shown in Figure 6A, the induction of MMP9 expression in SGC7901 and SGC7901-siScrambled cells was more noticeable than that in SGC7901-siSema 5A cells. Next, we performed an invasion assay to examine the effect of an MMP9 antibody (Ab) on the invasion of SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells. As illustrated in Figure 6B, treatment with the MMP9 Ab inhibited the H. pylori-induced invasive activities of these cells. The inhibition rates caused by the MMP9 Ab in SGC7901 and SGC7901-siScrambled cells were higher than those in SGC7901-siSema 5A cells. Taken together, our findings suggest that MMP9 is involved in H. pylori-induced gastric cancer progression mediated by semaphorin 5A.

**H. pylori infection induces MMP9 expression via the semaphorin 5A-mediated activation of the ERK signaling pathway**
According to our previous study, semaphorin 5A upregulates MMP9 expression in gastric cancer cells by activating the ERK signaling pathway.

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**H. pylori infection enhances the biofunctions of gastric cancer cells via its effects on semaphorin 5A in vitro**
As shown above, semaphorin 5A is overexpressed in gastric cancer tissue and gastric cancer cells. However, we have not clearly determined whether semaphorin 5A is involved in H. pylori-mediated gastric cancer progression. As shown in Figure 4A, semaphorin 5A knockdown inhibited the H. pylori-induced increase in gastric cancer cell proliferation. Similarly, the colony-formation assay indicated that semaphorin 5A knockdown led to a decreased number of colonies after 14 days of coincubation with H. pylori (Figure 4B). Next, scratch and Transwell assays were performed in vitro to evaluate the effect of H. pylori on the migration and invasion of SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells. As illustrated in Figures 4C and 4D, semaphorin 5A depletion resulted in decreased cell migration and invasion induced by H. pylori. Together, these data indicate that H. pylori enhances the biological function of gastric cancer cells via its effects on semaphorin 5A in vitro.

**The ERK signaling pathway is responsible for H. pylori-induced gastric cancer progression mediated by semaphorin 5A**
As reported previously, semaphorin 5A promotes the invasion and migration of gastric cancer cells via activation of the extracellular regulated protein kinase (ERK) signaling pathway. Here, we further investigated whether the ERK signaling pathway was involved in H. pylori-induced gastric cancer progression mediated by semaphorin 5A. First, we measured the effect of H. pylori on the levels of phosphorylated ERK1/2 in SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells after coinfection with H. pylori for 24 h. As shown in Figure 5A, the level of phosphorylated ERK1/2 (pERK) in H. pylori-stimulated SGC7901- and SGC7901-siScrambled cells was higher than that in SGC7901-siSema 5A cells, whereas the levels of the total ERK1/2 (tERK) proteins were not altered. Next, we used a Matrigel invasion chamber to determine whether an ERK inhibitor attenuates gastric cancer cell invasion induced by H. pylori. SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells were incubated with H. pylori in the presence or absence of the ERK inhibitor PD98059. As illustrated in Figure 5B, the ERK inhibitor PD98059 significantly reduced the invasion of H. pylori-stimulated cells. The inhibition rates of PD98059 on the invasive abilities of SGC7901- and SGC7901-siScrambled cells were higher than those of SGC7901-siSema 5A cells. Taken together, the ERK signaling pathway may be responsible for H. pylori-induced gastric cancer progression through a semaphorin 5A-dependent mechanism.
pathway. To investigate whether activation of MMP9 by *H. pylori* infection was dependent on semaphorin 5A-mediated ERK signaling, SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells were cocultured with *H. pylori* in the presence or absence of the ERK inhibitor PD98059 to investigate whether the induction of MMP9 expression by *H. pylori* infection depended on semaphorin 5A-mediated activation of ERK signaling. As shown in Figure 7, PD98059 reduced levels of both the MMP9 protein and transcript in gastric cancer cells infected with *H. pylori*. These alterations were more significant in SGC7901 and SGC7901-siScrambled cells than in SGC7901-siSema 5A cells. Based on these data, *H. pylori* increased the expression of MMP9 by modulating semaphorin 5A expression and the subsequent activation of the ERK signaling pathway.

**MMP9 and semaphorin 5A expression in gastric cancer tissues with *H. pylori* infection**

We randomly collected 30 gastric cancer specimens and examined the expression of MMP9 and semaphorin 5A protein using IHC to investigate whether these phenomena occurred in *vivo*; 15 samples were *H. pylori* positive and the other 15 were *H. pylori* negative. As shown in Figures 8A–8E, the intensity of semaphorin 5A and MMP9 immunostaining was stronger in *H. pylori*-positive samples than in *H. pylori*-negative tissue specimens.

**DISCUSSION**

*H. pylori* is one of the main factors contributing to the occurrence and development of gastric carcinoma. In our study, *H. pylori* infection was associated with the progression of gastric cancer *in vivo* and promoted the migration and invasion of gastric cancer cells *in vitro*. Although a number of studies have been performed to investigate *H. pylori* pathogenesis and carcinogenesis, the detailed molecular mechanism remains largely elusive. In the present study, we provide the first evidence that *H. pylori* promotes the invasion and metastasis of gastric cancer by upregulating semaphorin 5A expression via the ERK/MMP9 signaling pathway.

Semaphorin 5A, a membrane-bound protein with seven characteristic thrombospondin-specific repeats (TSP-1), contributes to the development of the central nervous system. In our previous studies, we found that semaphorin 5A plays a crucial role in the development and progression of gastric carcinoma, and that *H. pylori* infection upregulates semaphorin 5A expression in gastric precancerous lesions and normal gastric GES-1 cells. However, we had not yet completely characterized whether semaphorin 5A is involved in *H. pylori*-related gastric cancer.

In this study, we examined the effect of *H. pylori* infection on semaphorin 5A expression in gastric cancer tissue and gastric cancer cells. Significantly higher semaphorin 5A expression was detected in *H. pylori*-positive gastric cancer tissues than in *H. pylori*-negative gastric cancer tissues, and *H. pylori* infection increased the levels of the semaphorin 5A mRNA and protein in gastric cancer cells in a time-dependent manner. In addition, we established a Sprague-Dawley rat model colonized with *H. pylori* and observed higher semaphorin 5A expression in the gastric mucosa of the control group than in the control group, indicating that semaphorin 5A expression is involved in *H. pylori* infection. Together, these observations show that semaphorin 5A activation may be involved in *H. pylori* pathogenesis. However, the mechanism underlying the biological function of semaphorin 5A in *H. pylori*-mediated gastric cancer progression remains to be determined.

In the present study, we performed *in vitro* experiments using gastric cancer cells cocultured with *H. pylori*, which may contribute to the
Figure 4. *H. pylori* infection increases the proliferation, growth, migration, and invasion of gastric cancer cells via semaphorin 5A.

SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells were treated with *H. pylori* at a ratio of 1:100 for 24 h. (A) Cell proliferation was detected using MTT assays and is shown in a line chart. (B) Cells were incubated for 14 days in colony-formation assays before colonies were fixed and stained with 0.1% crystal violet. (C) Wound-healing assays were conducted to evaluate the migration of cells after *H. pylori* infection *in vitro*. Photos were captured at 0 and 36 h at 100× magnification. (D) The invasion of gastric cancer cells was evaluated by performing a Matrigel invasion assay. Penetrating cells were stained with hematoxylin, and pictures were captured at 200× magnification. The data shown in the histogram are a summary of three independent experiments. *p < 0.05.
carcinogenic process. Knockdown of semaphorin 5A attenuated the 
*H. pylori*-induced proliferation, growth, invasion, and migration of 
gastric cancer cells. These results suggest that semaphorin 5A may 
play an important role in the development and progression of 
*H. pylori*-related gastric cancer. However, the exact mechanism(s) re-
main(s) unclear.

Matrix degradation by MMPs is critical for tumor cell invasion and 
metastasis. In this study, *H. pylori* induced MMP9 expression in 
SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells. In 
addition, the *in vitro* assay showed that the blockade of MMP9 
with an antibody partially reversed the effect of *H. pylori* on 
increasing the invasion of SGC7901, SGC7901-siScrambled, and 
SGC7901-siSema 5A cells. Therefore, MMP9 was involved in 
*H. pylori*-induced gastric cancer progression mediated by 
semaphorin 5A.

The ERK signaling pathway has been shown to be an important 
mediator of tumor cell invasion and migration. Therefore, we 
worried whether the ERK signaling pathway was respon-
sible for *H. pylori*-mediated gastric cancer progression. Using 
the human gastric cancer cell lines SGC7901, SGC7901-siScrambled, 
and SGC7901-siSema 5A, we found that *H. pylori* increased ERK1/2 phosphorylation, but the addition of 
PD98059 reduced the ability of *H. pylori* to induce MMP9 
expression, migration, and invasion. Consistent with the results 
obtained from gastric cancer cells, MMP9 expression positively 
correlated with semaphorin 5A expression in gastric cancer 
tissues.

Taken together, *H. pylori* promotes the migration and invasion of gastric cancer by upregulating 
semaphorin 5A expression via the ERK/ MMP9 signaling pathway.

To the best of our knowledge, this study is the first to document the functional roles of sema-
phorin 5A and its molecular mechanism in *H. pylori*-mediated gastric cancer progression, 
which not only reveals a novel function of semaphorin 5A outside the nervous system but also 
improves our knowledge of semaphorin 5A. Moreover, *H. pylori* is 
deemed to be an important contributing factor to the development of gastric cancer. Therefore, this study may provide useful information 
for the prevention and effective treatment strategies for gastric 
cancer.

**Materials and Methods**

**Human gastric cancer tissue specimens**

Tissue specimens were collected from patients with gastric cancer be-
tween March 2019 and May 2020 at the Department of Pathology, the 
First Affiliated Hospital, Kunming Medical University, Kunming, 
China. The patients were diagnosed by a pathological examination 
and had not received chemotheraphy and radiation therapy before tu-
mor resection. They all provided consent for the use of their tissue 
specimens for clinical research, which was approved by our Univer-
sity Ethics Committee. The pathological diagnosis was classified 
based on the diagnostic criteria formulated by the WHO. Of the pa-
tients, 101 were confirmed to be *H. pylori*-positive by hematoxylin-
eosin staining and IHC.

**Cell culture**

The human gastric cancer cell lines MKN28, SGC7901, and 
SGC7901-siScrambled, which was transfected with the scrambled 
plasmid stably expressing semaphorin 5A, and SGC7901-siSema 
5A, which was transfected with siRNA plasmid stably expressing 
semaphorin 5A siRNA, were cultured in DMEM (Gibco, Carlsbad, CA, 
USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, 
Carlsbad, CA, USA), 100 U/mL penicillin, and 100 μg/mL strepto-
mycin in a humidified atmosphere of 5% CO2 and 95% air at 37°C.
**H. pylori culture and infection of gastric cancer cells**

*H. pylori* bacteria were grown on Columbia agar plates (Oxoid, Basingstoke Hampshire, UK) containing 5% sheep blood and incubated at 37°C under microaerophilic conditions. After 72 h, *H. pylori* were harvested and suspended in phosphate-buffered saline (PBS). The *H. pylori* densities were adjusted by measuring the optical density (OD) at 660 nm, and OD$_{660} = 1 \times 10^8$ colony-forming units (CFUs)/mL. *H. pylori* was then coincubated with MKN28, SGC7901, SGC7901-siScrambled, and SGC7901-siSema5A $1:100$ for up to 24 h in the medium.

**IHC analysis**

Paraffin-embedded gastric tissue samples were cut into 4 μm sections, which were deparaffinized and subjected to antigen retrieval. Sections were incubated with a primary antibody against semaphorin 5A (GeneTex, GTX117160, Irvine, CA, USA), followed by a secondary antibody (GK500705, DAKO A/S, China). Semaphorin 5A expression was determined by scoring the percentage of positive tumor cells (scale, 0%–100%) with a staining intensity from 0–3+. The percentage of cells with positive expression was determined by counting the number of immunopositive cells in three areas randomly chosen in the tissue with a total of 300 cells (0 = 0%, 1 = 1%–25%, 2 = 26%–35%, 3 = 36%–45%, 4 = 46%–65%, and 5 = 66%–100%). The staining intensity was scored (0 = no detectable cell membrane and cytoplasmic staining, 1 = low cell membrane and cytoplasmic staining, 2 = moderate cell membrane and cytoplasmic staining, and 3 = substantial cell membrane and cytoplasmic staining). The two scores were then summed to obtain a final score ranging from 0–8 points. Semaphorin 5A expression levels were divided into 4 groups: 0 (total score = 0), + (total score = 2–3), ++ (total score = 4–5), and +++ (total score = 6–8).

**RT-PCR and qRT-PCR analysis**

Total RNA was extracted from samples using TRIzol reagent (Invitrogen). After quantification, RNA was reverse transcribed into cDNAs according to the manufacturer’s guidelines. The following primers were used for RT-PCR: semaphorin 5A-f, 5’-AAGATCCAGTACGGTAGAAGAG-3’, and semaphorin 5A-r, 5’-GGCCACTTGTCGCGATAAGG-3’, and MMP9-f, 5’-CACTGTCCACCTCAGACG-3’, and GAPDH-f, 5’-GACCACCTTGTCGCCATGAAGG-3’, and β-actin-f, 5’-GGCCACTTGTCGCGATAAGG-3’, and β-actin-r, 5’-CACTGTCCACCTCAGACG-3’; MMP9-r, 5’-BACAGGTCATCAGACG-3’, and GAPDH-r, 5’-GACCACCTTGTCGCCATGAAGG-3’.

**Western blot analysis**

The cells were homogenized in lysis buffer. Equal amounts of protein were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes, which
were blocked with 5% non-fat milk and then incubated with primary antibodies against semaphorin 5A, MMP9, pERK1/2, tERK1/2, and β-actin at 4°C overnight. After three washes for 15 min with Tris-buffered saline (TBS) supplemented with 0.1% Tween 20 (TBST), the membranes were incubated with a horseradish peroxidase-conjugated rabbit anti-mouse secondary antibody, followed by enhanced chemiluminescence detection (KPL, Gaithersburg, MD, USA). β-actin served as an internal control.

Cell proliferation and colony-formation assays
Five thousand cells were seeded in each well of 96-well plates and incubated for 0, 24, 48, 72, 96, and 120 h. Then, the MTT (Sigma) stock solution was added to the medium and incubated for an additional 4 h at 37°C in a cell incubator; subsequently, 200 µL of dimethyl sulfoxide (DMSO) was added to each well. The plate was shaken on a rotary platform for 8 min, and then values were measured using a microplate reader at a wavelength of 490 nm to evaluate cell growth. For the colony-formation assay, 2,000 cells from each group were plated in 60 mm dishes. After 14 days of coculture with *H. pylori*, cells were fixed with 10% formaldehyde and stained with 0.1% crystal violet. Colony numbers were measured under a microscope, and photos were acquired.

Wound-healing assay
A wound-healing assay was performed to evaluate gastric cancer cell mobility. A total of 50,000 of each cell line were plated on a six-well plate coated with fibronectin. When cells reached more than 90% confluence after culture, scratch wounds were carefully generated using a sterile pipette tip, and any cellular debris was removed by washing the wells with PBS. Pictures were captured at 0 and 36 h after wounding.

Invasion assay
The examined cells were seeded in Transwell chambers coated with Matrigel to evaluate invasion. The medium in the upper compartment lacked serum, while the medium in the lower compartment contained 10% bovine serum. In some inhibitor experiments, inhibitors and antibodies were also added to the upper chambers. After culture for 24 h at 37°C in a humidified 5% CO₂ incubator, the cells that migrated through the Matrigel were fixed with methanol and stained with hematoxylin. Invasion was

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**Figure 7.** *H. pylori* infection induces MMP9 expression via the ERK signaling pathway in a semaphorin 5A-dependent manner.
SGC7901, SGC7901-siScrambled, and SGC7901-siSema 5A cells were cocultured with *H. pylori* in the presence or absence of the ERK inhibitor PD98059. MMP9 expression at the mRNA and protein levels was detected using RT-PCR (A) and western blot (B), respectively. Data shown in the histogram are a summary of three independent experiments. *p < 0.05.

**Figure 8.** IHC staining for MMP9 and semaphorin 5A in gastric cancer tissues.
The intensity of semaphorin 5A and MMP9 expression was increased in *H. pylori*-positive tissue (A and C) compared with *H. pylori*-negative tissues (B and D). Scale bars, 100 µm. Data shown in the histogram are a summary of three independent experiments (E). *p < 0.05.
determined by counting the cells that penetrated the Matrigel matrix under a microscope.

**Statistical analysis**
The results are presented as the means ± SDs from three independent experiments. The data were analyzed using ANOVA. The statistical analysis was performed using SPSS 13.0 software (SPSS), and p < 0.05 was considered significant.

**SUPPLEMENTAL INFORMATION**
Supplemental information can be found online at https://doi.org/10.1016/j.omto.2021.06.002.

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**AUTHOR CONTRIBUTIONS**
G.P., X.W., and Y.W. conceived the study, and S.L., Y.L., R.L., G.L., and Y.H. participated in its design and coordination. L.W. and L.Z. helped to draft the manuscript. All authors read and approved the final manuscript.

**DECLARATION OF INTERESTS**
The authors declare no competing interests.

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