# Role of the Outer Inflammatory Protein A/Cystine–Glutamate Transporter Pathway in Gastric Mucosal Injury Induced by *Helicobacter pylori*

Jie Du, PhD[1,2,3], Xiao-Hui Li, PhD[1], Fen Liu, PhD[4], Wen-Qun Li, PhD[1], Zhi-Cheng Gong, PhD[2,3] and Yuan-Jian Li, PhD[1]

INTRODUCTION: **_Helicobacter pylori_ infection is a major cause of gastrointestinal diseases. However, the pathogenesis of gastric mucosal injury by _H. pylori_ remains unclear. Exogenous glutamate supplementation protects against gastric mucosal injury caused by _H. pylori_. Previously, we showed that aspirin-induced gastric injury is associated with reduction in glutamate release by inhibition of cystine–glutamate transporter (xCT) activity. We hypothesized that the xCT pathway is involved in _H. pylori_-induced gastric mucosal injury. In this study, we tested the activity of xCT and evaluated the regulatory effect of outer inflammatory protein (Oip) A on xCT in _H. pylori_-induced gastric mucosal injury.**

METHODS: **In the _H. pylori_-infected mice and cell lines, the activity of xCT and the regulatory effect of microRNA on xCT were tested, and the effect of OipA from _H. pylori_ on xCT activity was observed.**

RESULTS: **The results of _in vivo_ and _in vitro_ experiments showed that _H. pylori_ infection induced gastric mucosal injury. This was accompanied by a reduction in xCT activity, which was attenuated by exogenous glutamate treatment. Furthermore, the expression of _miR-30b_ was upregulated, and miR-30b inhibitors significantly restored xCT activity and gastric mucosal injury caused by _H. pylori_ infection. The OipA, a virulence protein from _H. pylori_, significantly upregulated the expression levels of _miR-30b_ and inhibited xCT activity.**

DISCUSSION: **OipA plays a significant role in _H. pylori_-induced gastric mucosal injury, and the effects are mediated by micro30b/xCT pathway.**

**SUPPLEMENTARY MATERIAL** accompanies this paper at http://links.lww.com/CTG/A283

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## INTRODUCTION

The development of peptic ulcers involves a variety of factors, such as dietary, chemical (smoking, alcohol, and drugs), biological (*Helicobacter pylori*), mental, and environmental factors (1,2). *H. pylori* are considered as one of the most common pathogens causing chronic gastritis and gastric cancer (3,4). However, the mechanism of *H. pylori*-induced gastric mucosal injury has not yet been full elucidated.

*H. pylori*-induced gastric mucosal injury is related to changes in prostaglandins (5,6) and nitric oxide (7,8). Glutamate, as an extracellular signal mediator in peripheral tissues by autocrine and/or paracrine (9,10), might play a protective role in gastric mucosal injury from acute aspirin irritation and cold irritation (11,12). Exogenous glutamate supplementation might have beneficial effects on gastric function. For example, dietary glutamate supplementation improves gastric $^{13}C$ incorporation rates and nutrition management in postweaning pigs (13). Supplementing partial enteral nutrition with monosodium glutamate slows gastric emptying in preterm pigs (14). Moreover, an oral glutamate precursor (glutathione) and a glutamate supplementation drastically reduce *Helicobacter*-induced gastric pathologies (15,16). It is likely that alteration in endogenous glutamate transportation is also involved in gastric mucosal injury induced by multiple factors, such as aspirin irritation and cold stress.

Therefore, we explored endogenous glutamate transportation in *Helicobacter*-induced gastric pathologies. Extracellular levels of glutamate are regulated by the cystine–glutamate transporter (xCT), and its activity is affected by some inflammatory factors (17). Recently, we reported that aspirin-induced acute gastric mucosa is associated with a reduction in xCT activity (11). Thus,

[1]Department of Pharmacology, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, Hunan, China; [2]Department of Pharmacy, Xiangya Hospital of Central South University, Changsha, Hunan, China; [3]National Clinical Research Center for Geriatric Disorders (XIANGYA), Xiangya Hospital, Central South University, Changsha, Hunan, China; [4]Department of Digestion, The Third Xiangya Hospital of Central South University, Changsha, Hunan, China.
**Correspondence:** Yuan-jian Li, PhD. E-mail: yuan_jianli@sina.com.
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we hypothesized that the xCT/glutamate pathway is also involved in *H. pylori*-induced gastric mucosal injury.

MicroRNAs (miRNAs) are the regulators and biomarkers for human diseases on multiple levels (systemic, tissue, and cellular) (18–20). Some inflammatory factors affect miRNA profiles in stomach (21–25). Furthermore, differential expression of miRNA has been observed in *H. pylori*-induced gastritis *in vivo*, *in vitro*, and in clinical patients (20,26,27). Therefore, we speculated that *H. pylori* infection induces upregulation of certain miRNAs, which inhibits their target gene *xCT*, thereby reducing the glutamate transport activity. Three databases (TargetScan, Starbase, and miRanda) were used to predict the putative miRNAs targeting xCT. We screened for the expression of 4 gastro-enriched miRNAs in *H. pylori*-infected mouse models and cells, which showed upregulated *miR-30b* as a candidate miRNA marker. Therefore, the regulatory role of *miR-30b* on xCT was tested in a *H. pylori*-infected model.

*H. pylori*-induced gastric mucosal injury is associated with the secretion of multiple virulence proteins from *H. pylori*, such as, CagA oncoprotein, vacuolating cytotoxin A, and outer inflammatory protein (Oip) A (28,29). The *H. pylori* OipA (OipA, HopH, or OMP; 13–34 kDa), one of the most important virulence factors of *H. pylori*, was initially identified as a surface protein that promotes inflammatory cytokine secretion and heightens gastric inflammation *in vivo* (30–33). More recently, OipA is believed to affect intracellular signaling and modulate host signaling pathways (34,35). To our knowledge, miRNAs regulation by OipA has not been elucidated to date. Thus, we explored the regulatory effect of OipA on the miRNA/glutamate pathway in *H. pylori*-induced gastric mucosal injury.

## METHODS

### Animal and cell experiments

*H. pylori* Sydney strain (SS) 1 was purchased from the American Type Culture Collection and cultured on agar plates containing 10% sheep blood under microaerophilic conditions at 37°C. We used a rapid urease test, Gram staining, and polymerase chain reaction (PCR) amplification of specific urease genes for identification of certain virulence markers. Then, the purified bacteria were used to establish an experimentally infected model. The mice were perfused with *H. pylori* for 6 weeks. C57BL/6 male mice (8 weeks old, 19–22 g) were randomly divided into 4 groups (n = 12): (i) The control group was perfused with phosphate-buffered saline (PBS) daily. (ii) The *H. pylori*-infected group was perfused with *H. pylori* dissolved in PBS, $2 \times 10^9$ colony-forming units/mouse/d for 6 weeks, (iii) The L-glutamate group was perfused with *H. pylori* bacteria solution after 30 minutes of lavage with either of 2 different doses of glutamate (3 mg/kg/d or 6 mg/kg/d) and defined as L-glutamate (L) group and L-glutamate (H) group. The administration of L-glutamate began 2 weeks after the start of infection period. All animals were killed at 6-weeks postinfection. Age-matched uninfected mice were included as controls in all experiments.

Gastric tissues of all mice were separated by cutting along greater curvature of the stomach, washed, and photographed. *H. pylori* infection was confirmed using Giemsa staining and a polyclonal rabbit anti-*H. pylori* antibody. In addition, the expression of 23S rRNA was detected, which can be used to measure *H. pylori* colonization in the stomach. Samples of gastric tissue proteins were extracted for western blot assays. A portion of gastric tissue was fixed in 4% paraformaldehyde for morphological analysis as reported earlier (36).

Human gastric mucosa epithelial (GES-1) cells were serum starved before treatment for 24 hours in Dulbecco's Minimum Essential Medium containing 1% fetal bovine serum. Cells were prepared by seeding $2 \times 10^6$ cells on plates. We used rapid urease tests, Gram staining, and PCR amplification of specific urease genes for identification of certain virulence markers. To cause infection, bacteria were harvested in PBS (pH 7.4) and added to the host cells at a multiplicity of infection of 100 (37). The cell viability assay was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction assays and lactate dehydrogenase assays. To evaluate apoptosis, we used Hoechst staining and caspase-3 activity.

### RNA expression analysis

Total RNA was extracted according to the manufacturer's instructions, and 500 ng RNA was reverse transcribed using a reverse transcription-PCR kit according to the manufacturer's protocol (TaKaRa, Kusatsu, Japan). The expression levels of messenger RNAs (mRNAs) were measured using an ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA) with the SYBR Green PCR Master Mix (TOYOBO, Osaka, Japan). GAPDH was used as an internal control in quantitative analysis. The gene expression levels were normalized to GAPDH. For miRNA detection, *in situ* hybridization detection of miRNAs was performed using the miRCURY LNA miRNA ISH Kit (Qidgen, Germany).

### Protein detection

For western blot analysis, primary antibodies against xCT (1:1,000; Abcam, England), GAPDH (1:2,000, Abcam), and *Helicobacter* (1:2,000, Abcam) and a secondary HRP-anti-rabbit (1:5,000; Sangon, Shang hai) antibody were used. The relative optical density of each band was analyzed, and the results were expressed in relation to GAPDH levels. For immunofluorescence detection, cells were washed with PBS and fixed with paraformaldehyde for 15 minutes at room temperature (~25°C), before being incubated with 0.25% Triton X-100 in PBS at room temperature for 20 minutes. The cells were blocked with 1% bovine serum albumin at 37°C for 1 hour and rinsed 3 times with PBS before incubation with anti-xCT (1:200) antibody at 4°C overnight. This was followed by incubation with an anti-rabbit secondary antibody (1:200, FITC488) for 1 hour before immunofluorescence detection. Nuclei were stained with 4',6-diamidino-2-phenylindole 2HCI for 1 minute. Images were acquired using an immunofluorescence microscope (Olympus, Japan) equipped with a ×20 objective lens and were analyzed and processed with a Nikon camera with a SPOT image acquisition system. For immunohistochemistry staining, acetate buffer (pH 6.0) was used as the immersion solution for heat antigen pretreatment step. Rabbit polyclonal antibody and the Envision (DAKO, Germany) polymer detection system were used with diaminobenzidine acting as the chromogen.

### Dual luciferase reporter assay

GES-1 cells were transfected in 12-well plates with *Renilla* luciferase-based SLC7A11 (xCT) 3′UTR reporter constructs using Lipofectamine 2000 reagent (Invitrogen, Beijing). After 24 hours, the cells were supertransfected with miRNA mimics at 50 nM final concentration using Lipofectamine 2000. The cells were harvested 24 hours after reporter transfection and analyzed using a Dual-Luciferase Reporter Assay System (Promega). *Renilla* luciferase signal of each sample was normalized for differences in transfection efficiency using the activity of the cotransfected pCI-firefly reporter as control.

### Statistical analysis

TargetScan (http://www.targetscan.org/), Starbase (http://starbase.sysu.edu.cn/), miRanda (http://www.microrna.org/), and miRDB (http://www.mirdb.org/) databases were used to predict the putative miRNAs targeted to xCT (SLC7A11). All data are expressed as mean ± SE. Multiple mean comparisons were performed by ANOVA and Student-Newman-Keuls multiple comparison tests. Statistical analysis was performed using SPSS 17.0 software, and $P < 0.05$ was considered statistically significant.

## RESULTS

### Decreased xCT activity in gastric tissues of *H. pylori*-infected mice

*H. pylori* infection was confirmed using Giemsa staining and polyclonal rabbit anti-*H. pylori* antibody. *H. pylori* antigen positivity was intensely observed in the gastric mucosa of the *H. pylori*-infected group but was undetected in the control group. Giemsa staining showed similar results (see Supplementary Figure 1A, 1B, Supplementary Digital Content 1, http://links.lww.com/CTG/A283). The relative levels of 23S rRNA were increased up to 10-fold in *H. pylori* group (see Supplementary Figure 1C, Supplementary Digital Content 1, http://links.lww.com/CTG/A283).

After confirming the success of the model, we detected gastric mucosal injury in mice. Photographs of gastric mucosal lesions showed sporadic hemorrhagic spots in gastric mucosa. The hematoxylin-eosin staining showed gastric mucosal damage with dilation and exfoliation of gastric epithelial cells and disruption of mucosa in *H. pylori*-infected mice (Figure 1a). Meanwhile, the expression of *xCT* mRNA was significantly downregulated (Figure 1b), and a similar result was obtained for protein expression (Figure 1c, d). These results suggest that disturbances of glutamate transporter expression might be associated with the development of gastric ulcer caused by *H. pylori*.

### Effect of *H. pylori* on xCT activity in GES-1 cells

*In vitro*, we conducted experiments on the human gastric epithelial immortalized cell line (GES-1). When GES-1 cells were cocultured with bacteria for 24 hours, cavity lesions and prolonged degeneration could be seen in the cytoplasm of cells, and some cells gradually peeled from the surface and were suspended in the medium. Reduced cell viability and increased apoptosis were detected after exposure to *H. pylori* SS1 (Figure 2a, see Supplementary Figure 2, Supplementary Digital Content 1, http://links.lww.com/CTG/A283). These experimental results were consistent with previous results of *H. pylori*-infected cells (37).

In addition, we found that *xCT* mRNA levels changed over time when GES-1 cells were cocultured with *H. pylori* SS1 (Figure 2b). In agreement with the results of quantitative polymerase chain reaction, western blots and cell immunofluorescence results also showed a significant reduction in xCT protein levels (Figure 2c, d). $Xc^-$ system transports 1 molecule of cystine into the cell and simultaneously releases 1 molecule of glutamate from it. To further observe the activity of xCT, we tested glutamate concentration in the extracellular fluid (Figure 2e). These results suggested that the decreased release of endogenous glutamate might contribute to GES-1 apoptosis caused by *H. pylori* infection.

### Exogenous glutamate supplement attenuates *H. pylori*-induced gastric mucosal injury

To evaluate the effects of L-glutamate on *H. pylori*-associated gastric mucosal ulceration, animals were pretreated with glutamate at doses of 3 mg/kg/d or 6 mg/kg/d. In morphological studies, gastric

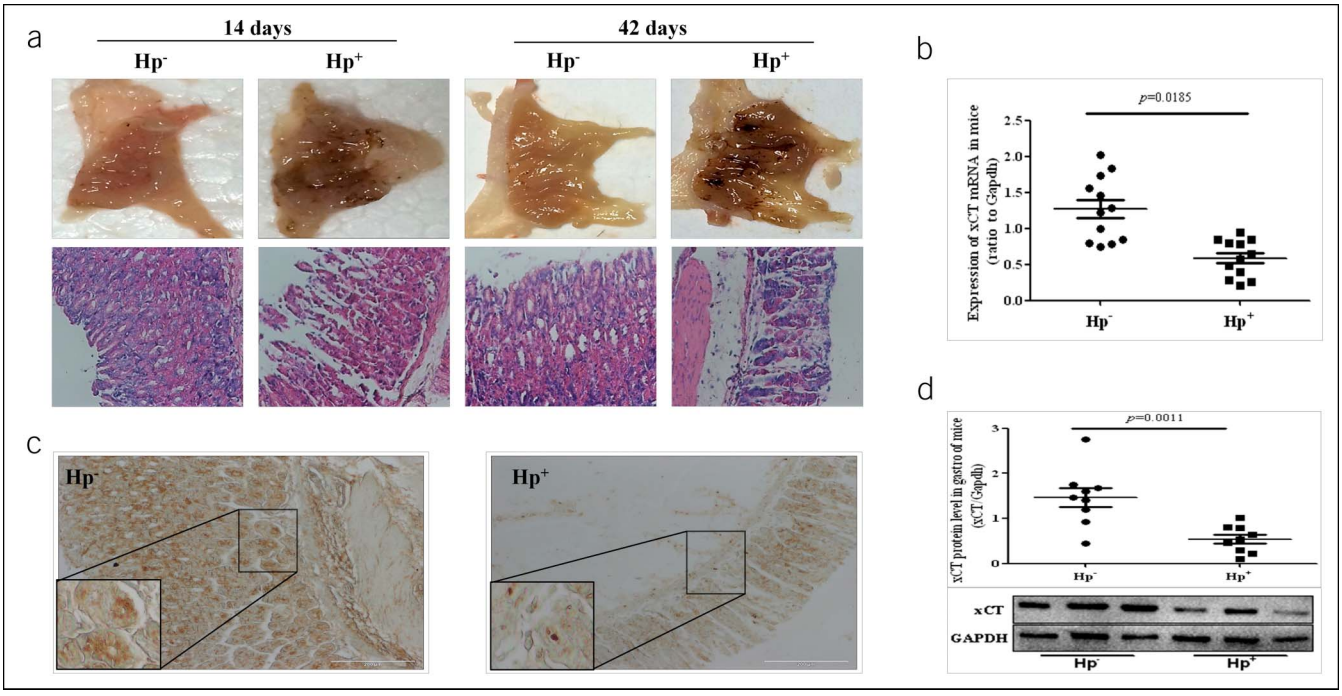


**Figure 1.** Decreased cystine-glutamate transporter (xCT) activity in gastric ulcer of *H. pylori*-infected mice. (**a**) Photographs of gastric mucosal lesions showed sporadic hemorrhagic spots in gastric mucosa. Hematoxylin-eosin staining showed gastric mucosal damage with dilation and exfoliation of gastric epithelial cells and disruption of mucosal in *H. pylori* -infected mice with an infiltration of inflammatory cells in the mucosa and submucosa. (**b**) Decreased expression of xCT mRNA in $Hp^+$ group was detected. (**c**) Immunohistochemical staining for xCT antigen in gastric tissue. (**d**) Western blot for xCT in gastric tissue. Data are means ± SEM, N = 8–12, $^*P < 0.05$ vs $Hp^-$, $^{**}P < 0.01$ vs $Hp^-$. GAPDH, glyceraldehyde phosphate dehydrogenase; *Hp, Helicobacter pylori*; mRNA, messenger RNA.

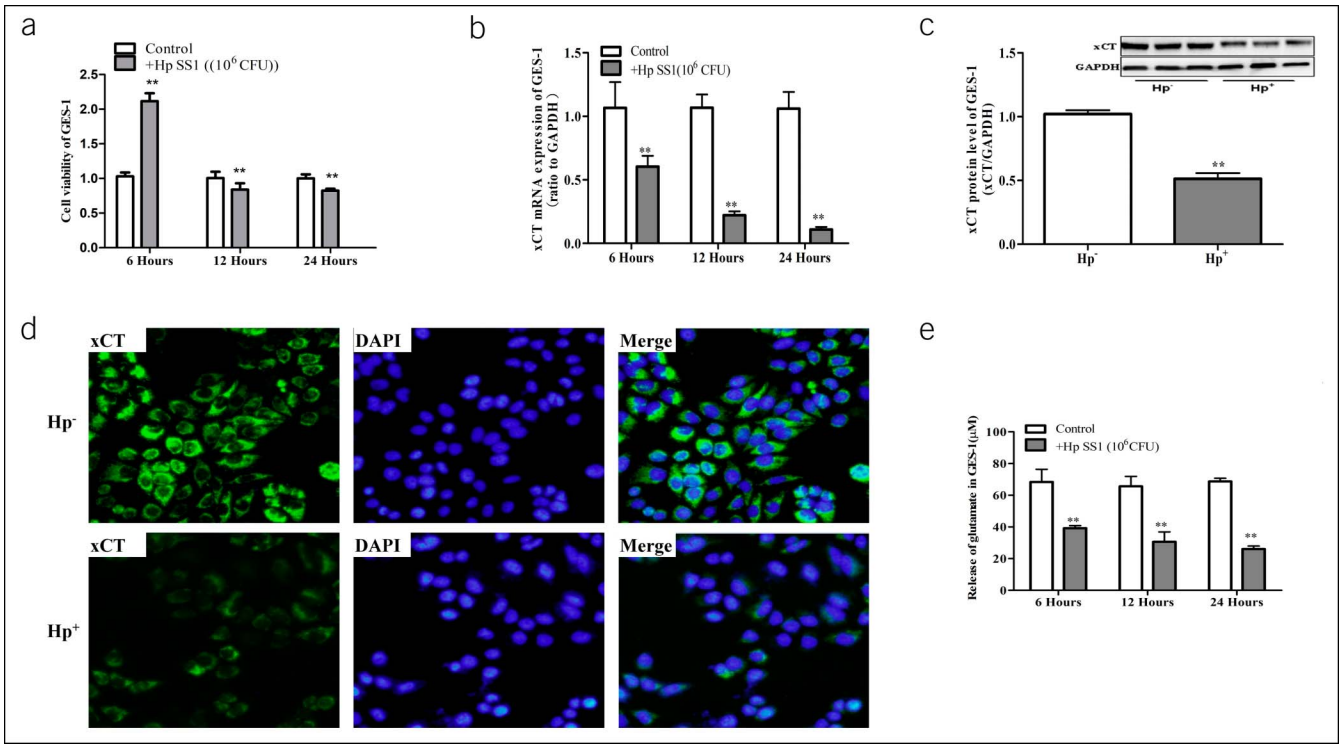


**Figure 2.** Effect of *H. pylori* on cystine-glutamate transporter (xCT) activity in GES-1 cells. (**a**) Cell viability of GES-1 were detected when coculturing with *H. pylori* SS1. (**b**) Decreased expression of xCT mRNA in *H. pylori* SS1 group. Western blot (**c**) and immunofluorescence (**d**) for xCT were detected in GES-1 cells. (**e**) The concentration of glutamate in cell culture supernatant of GES-1. Data are means ± SEM, N = 3, $*P < 0.05$ vs *H. pylori* control, $**P < 0.01$ vs control. CFU, colony-forming units; DPAI, 4′,6-diamidino-2-phenylindole 2HCI; GES-1, gastric mucosa epithelial; GAPDH, glyceraldehyde phosphate dehydrogenase; *Hp*, *Helicobacter pylori*; mRNA, messenger RNA; SS1, Sydney strain 1.

mucosal damage with dilation and exfoliation of gastric epithelial cells and disruption of mucosal layer were observed in the *H. pylori* group, whereas mice pretreated with glutamate had a smaller degree of loss of mucosal architecture and exfoliation than those observed in the *H. pylori* group (Figure 3a). In addition, lower caspase-3 activity in gastric tissues in the glutamate group was observed when compared with that in the *H. pylori* group (Figure 3b). Then, we verified these phenomena in cell-based experiments. We pretreated GES-1 cell line with L-glutamate. The results of caspase-3 activity and apoptosis staining suggested that glutamate treatment inhibited the process of apoptosis induced by *H. pylori* (Figure 3c, d). These results suggest that exogenous glutamate might be a potential supplementary therapeutic modality for the treatment of *H. pylori*-induced gastric ulcers.

### Overexpression of the *xCT* (*SLC7A11*) gene could reduce GES-1 apoptosis induced by *H. pylori*

After cloning *xCT* gene templates, PCR product sizes were examined by running 5 μL products on 3% agarose gel. The targeted band appeared between 1.5 kb and 2 kb, as expected (1,552 bp). Positive clones of the competent *Escherichia coli* transformed by granulosis virus core vector with *xCT* gene were confirmed by PCR, and the sequence alignment showed 100% alignment with the targeted gene.

After plasmid transfection, enhanced green fluorescent protein (Figure 4a) expression in GES-1 cells was detected. We transfected GES-1 with the help of a recombinant plasmid and cocultured it with *H. pylori*. The results showed that *xCT* overexpression restored xCT protein levels (Figure 4b) and increased endogenous glutamate release to a certain degree (Figure 4c), accompanied by reductions in cellular damage (Figure 4d) and apoptosis (Figure 4e). These results further confirmed that both endogenous and exogenous glutamate are involved in *H. pylori*-infected gastric ulcers.

### MiRNAs expression were altered in GES-1 and mice in response to *H. pylori* infection, and the xCT was regulated by miRNA-30b

The above-mentioned results have demonstrated that reduced glutamate was closely associated with downregulation of *xCT* expression and abnormal glutamate transport was associated with gastric toxicity in *H. pylori* infection. However, it remained unknown how *xCT* expression was altered under *H. pylori* infection conditions. We screened for the expression of miRNAs enriched in stomach, which showed upregulation of *miR-30b* levels in both stomach tissue and cell line (Figure 5a, b). *MiRNA-30b* expression has been linked to *xCT* using bioinformatics analysis and has been shown to be associated with *H. pylori* infection leading to gastritis (38,39).

To corroborate the hypothesis, we examined the expression levels of *miR-30b* in *H. pylori*-infected mice using *in situ* hybridization and confirmed that the levels of *miR-30b* were significantly elevated (Figure 5c). To validate *xCT* as a target of *miR-30b*, we constructed a luciferase expression vector containing the 3′-UTR segments of *xCT* along with the putative *miR-30b* binding sites. After cotransfection of miR-30b mimic and the *xCT* 3′-UTR expression vector, we found that the miR-30b mimic resulted in significant suppression of xCT luciferase activity, whereas mutating the putative miR-30b binding sites completely eliminated this inhibitory effect (Figure 5d). These data strongly indicated that upregulated *miR-30b* was indeed a target for *xCT* under *H. pylori* infection.

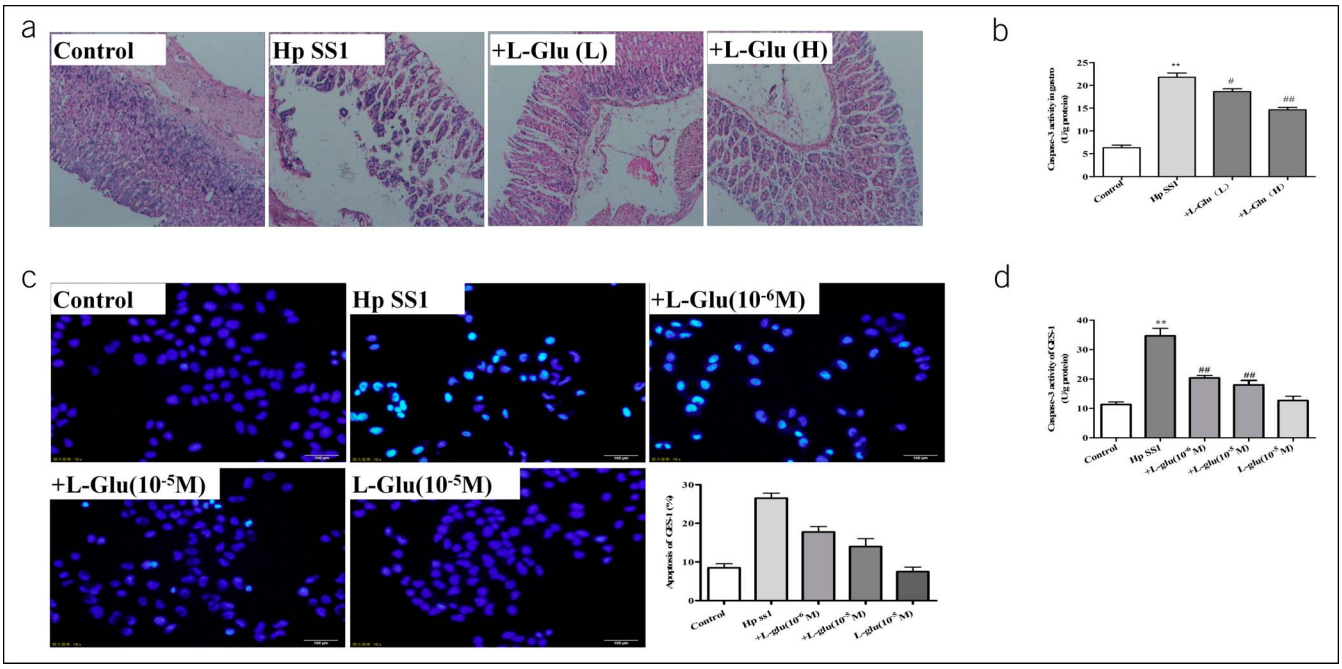


**Figure 3.** Exogenous glutamate supplement attenuates gastric mucosal injury induced by *H. pylori*. *In vivo* experiments: (**a**) Hematoxylin-eosin staining of gastric tissue showed the relieved dilation of gastric epithelial cells and less exfoliation of mucosal architecture in glutamate groups; (**b**) the effect of glutamate on apoptosis in gastric tissue was detected by Caspase-3 activity kit. N = 12, $*P < 0.05$ vs control, $**P < 0.01$ vs control, $\#P < 0.05$ vs *H. pylori* SS1; $\#\#P < 0.01$ vs *H. pylori* SS1. *In vitro* experiments: (**c**) Hoechst staining of GES-1; (**d**) Caspase-3 activity detection. Data are means ± SEM, N = 4. $**P < 0.01$ vs Control; $\#\#P < 0.01$ vs *H. pylori* SS1. GES-1, gastric mucosa epithelial; GFP, greenfluorescent protein; *Hp*, *Helicobacter pylori*; NC, numerical control; SS1, Sydney strain 1.

### The effect of *miRNA-30b* loss on cystine/glutamate transporter (xCT) activity and cell damage

As mentioned earlier, aberrant expression of *miR-30b* contributes to gastric ulcer through targeting xCT, so we explored the effect of miR-30b inhibitors in gastric ulcer. GES-1 was transfected with *miRNA-30b* inhibitor or a negative control (U22 inhibitor) and were exposed to *H. pylori* for 24 hours. MiRNA-30b inhibitor upregulated *xCT* mRNA expression (Figure 6a), which was accompanied by recovery of glutamate release (Figure 6b) and decreased damage of GES-1 (Figure 6c, d). These results showed that

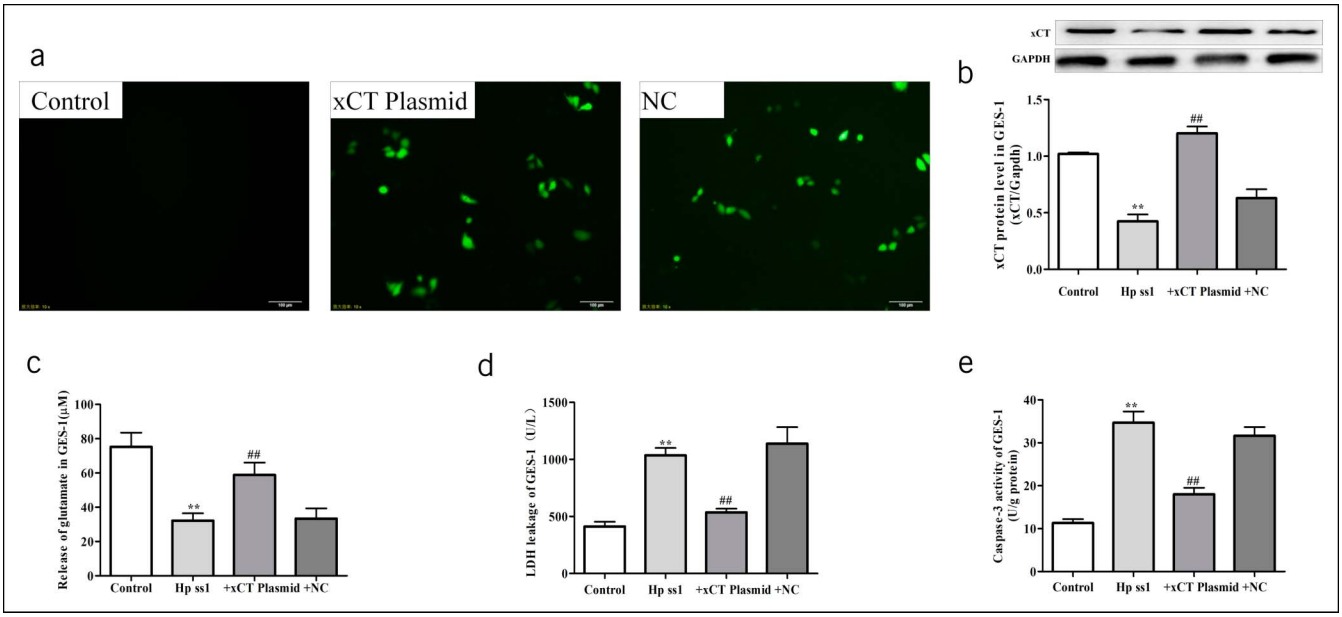


**Figure 4.** Overexpression of *xCT* (*SLC7A11*) gene could reduce the GES-1 apoptosis induced by *H. pylori*. (**a**) The expression of GFP was obviously induced in plasmid transgenic cell lines. (**b**) Western blot for xCT in GES-1. (**c**) LDH was assessed for cell damage. (**d**) Caspase-3 activity. Data are means ± SEM, N = 4, $*P < 0.05$ vs control, $**P < 0.01$ vs control. $\#\#P < 0.01$ vs *H. pylori* SS1. GES-1, gastric mucosa epithelial; *Hp*, *Helicobacter pylori*; LDH, lactic dehydrogenase; SS1, Sydney strain 1; xCT, cystine-glutamate transporter.

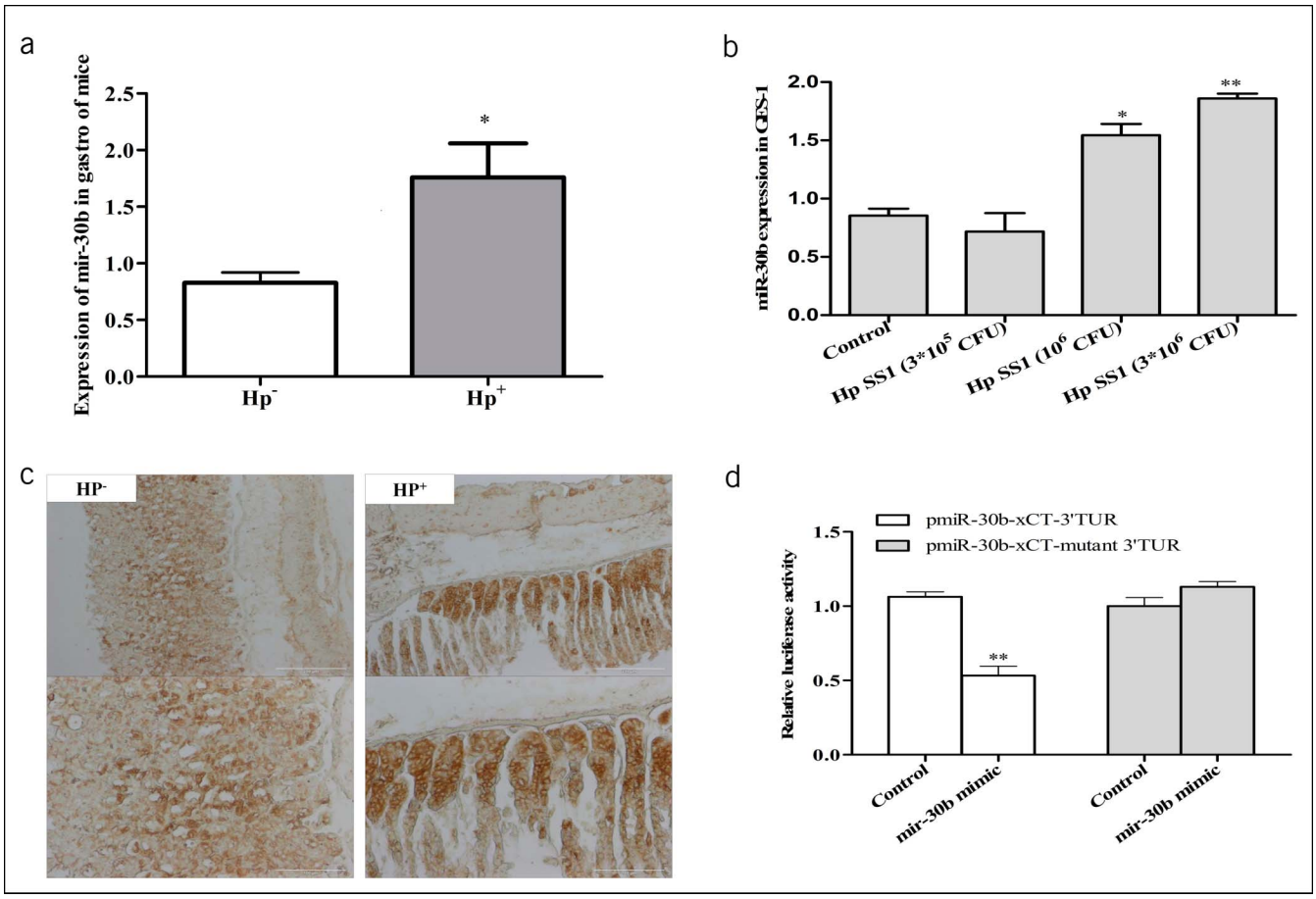


**Figure 5.** MicroRNAs (miRNAs) expression were regulated in GES-1 and mice under *H. pylori* infection, and the cystine-glutamate transporter (xCT) is regulated by miRNA-30b. (**a**) miR-30b expression in gastric tissue of mice, n = 10. (**b**) miR-30b expression in GES-1 cells, n = 4. (**c**) Identifying miR-30b in gastric tissue of mice using *in situ* hybridization. (**d**) The targeted regulating role of miRNA-30b on *xCT* gene was detected by dual luciferase reporter system. Data are means ± SEM, N = 4. $^{**}P < 0.01$ vs control. CFU, colony-forming units; GES-1, gastric mucosa epithelial; *Hp*, *Helicobacter pylori*; SS1, Sydney strain 1.

*miRNA-30b* loss reduced cell damage and apoptosis induced by *H. pylori* infection.

### OipA protein regulates the miR-30b/xCT pathway

Our findings suggested that the miR-30b/xCT pathway was involved in *H. pylori*-induced gastric mucosal injury. However, how the host miRNAs were regulated by the bacteria toxic components remained unknown. We transfected the recombinant OipA protein encoding pET28a plasmid (see Supplementary Figure 3, Supplementary Digital Content 1, http://links.lww.com/CTG/A283). Our results showed that recombinant OipA protein increased *miR-30b* levels significantly (Figure 7a) and the expression of *xCT* and glutamate concentration was inversely related to *miR-30b* levels (Figure 7b, c). These results supported a regulatory effect of OipA on the miR-30b/xCT pathway.

## DISCUSSION

Adequate levels of glutamate are crucial for cellular and tissue function. High concentrations of glutamate can exert toxicity effects in the brain (40); by contrast, excess glutamate can protect against gastric mucosal injury (11). Glutamate also protects against gastric mucosal injury induced by other factors, such as deoxynivalenol (41), aspirin (11), and cold stress (12). Our results confirmed earlier observations that, in cells and animal models, the exogenous glutamate pathway significantly reduces *H. pylori*-induced gastric mucosal injury (42). Taken together, exogenous glutamate has a protective effect on gastric mucosal injury induced by different factors. In stomach, glutamate plays protective roles as a substrate for various metabolic pathways, an energy source for intestinal mucosa, a mediator for cell signaling, and a regulator for oxidative reactions, and in immune responses.

Our earlier reports have shown that aspirin-induced gastric mucosal injury is related to reduction in endogenous glutamate levels and is accompanied by reduced activity of xCT. According to the inhibitory effect of some inflammatory factors on the xCT/glutamate pathway (43,44), we hypothesized that the xCT/glutamate pathway might be involved in *H. pylori*-induced gastric mucosal injury. The results of this study showed that *H. pylori* induced cell apoptosis with a decrease in glutamate release and xCT activity in cultured GES-1 cells, and these effects of *H. pylori* were attenuated by *xCT* (*SLC7A11*) overexpression. In mice, *H. pylori* infection induced gastric mucosal injury with down-regulation of *xCT* expression. These results supported the hypothesis that *H. pylori*-induced gastric mucosal injury might be associated with a reduction in glutamate release by inhibition of xCT.

MiRNAs mediate multiple responses, including inflammatory response (45). *H. pylori* induce alterations in some miRNAs

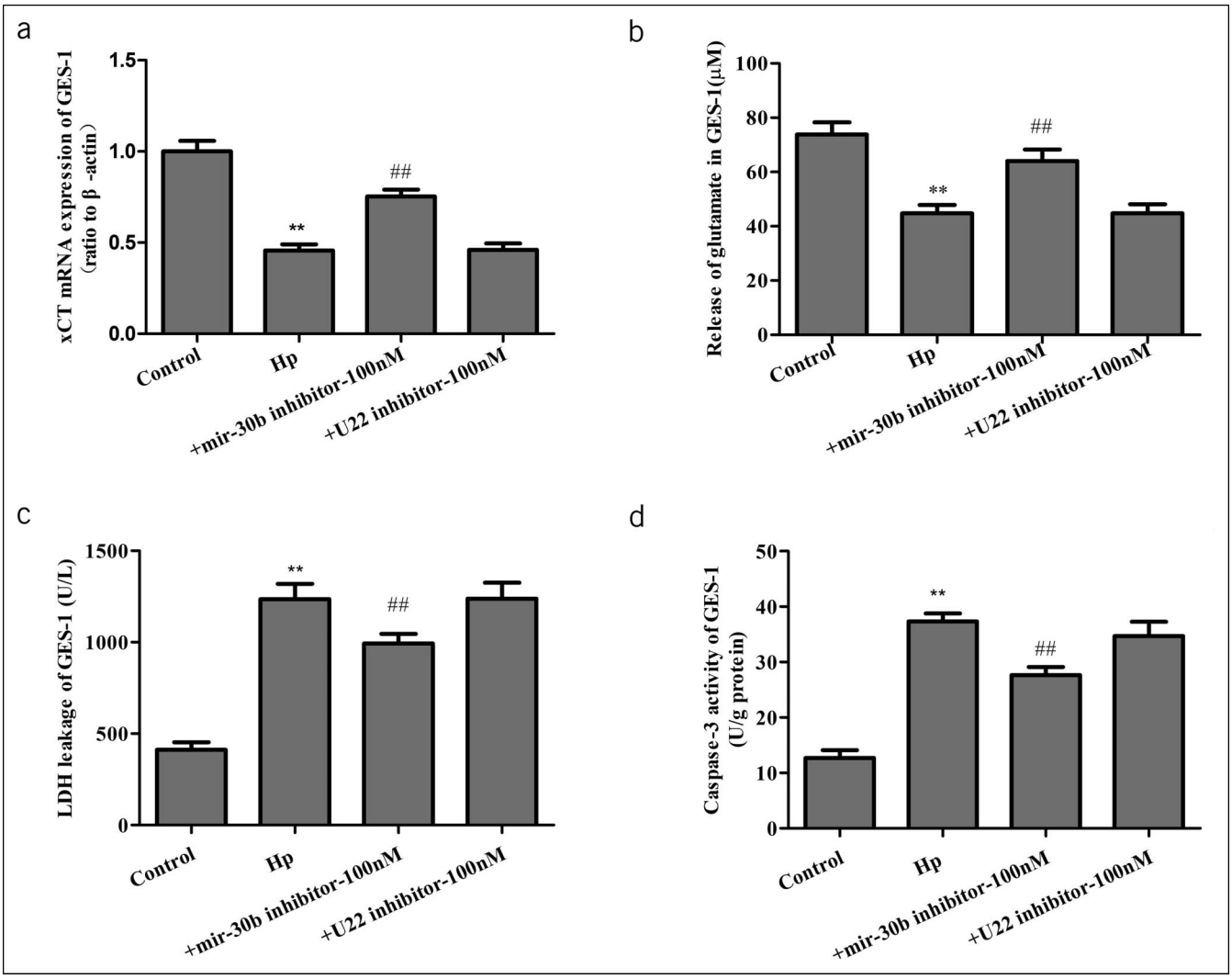


**Figure 6.** The effect of microRNA (miRNA)-30b loss on cystine-glutamate transporter (xCT) activity and cell damage. GES-1 was transfected with miRNA-30b or negative control (U22 inhibitor) and exposed to *H. pylori* for 24 hours. (**a**) xCT mRNA expression in GES-1. (**b**) The concentration of glutamate in cell culture supernatant of GES-1. (**c**) LDH was assessed for cell damage. (**d**) Caspase-3 activity. Data are means ± SEM, N = 4. $^{**}P < 0.01$ vs control; $\#\#P < 0.01$ vs *Hp*. GES-1, gastric mucosa epithelial; *Hp, Helicobacter pylori*; LDH, lactic dehydrogenase; mRNA, messenger RNA.

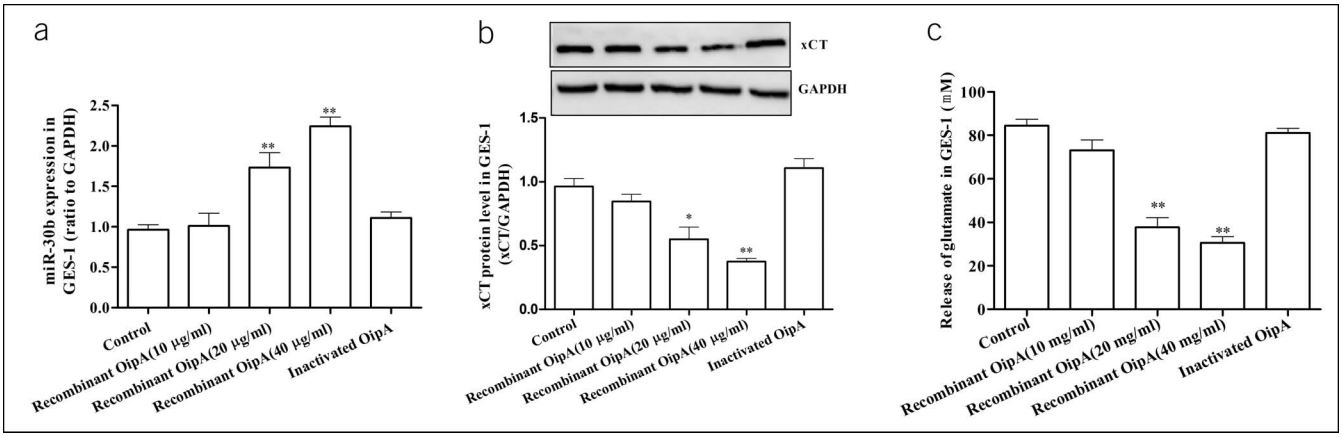


**Figure 7.** The regulatory of OipA on miR-30b/xCT pathway. The GES-1 cells were incubated with different concentrations of recombinant OipA protein for 24 hours. (**a**) The effect of recombinant OipA protein on microRNA-30b expression in GES-1. (**b**) The effect of recombinant OipA protein on xCT expression in GES-1. (**c**) The concentration of glutamate in cell culture supernatant of GES-1. Data are means ± SEM, N = 4, $^{*}P < 0.05$ vs control, $^{**}P < 0.01$ vs control. GAPDH, glyceraldehyde phosphate dehydrogenase; GES-1, gastric mucosa epithelial; OipA, outer inflammatory protein A.

(46,47). Interestingly, some miRNAs regulate *xCT* expression (48). It is likely that the reduction in xCT activity by *H. pylori* might involve specific miRNA pathways. Our results based on *in vivo* and *in vitro* systems have shown that *H. pylori*-induced gastric mucosal injury led to an increase in *miR-30b* expression. *MiR-30b* is closely related to *H. pylori*-induced gastric ulcers (38). In this study, we found that miR-30b inhibitors attenuated the reduction in xCT activity caused by *H. pylori*, thus supporting the hypothesis that the regulatory effect of *miR-30b* on xCT activity plays an important role in *H. pylori*-induced gastric mucosal injury.

OipA is a virulence protein from *H. pylori*, which promotes inflammatory cytokines secretion and heightens gastric inflammation (49,50). OipA, in addition to inducing injury directly, can also weaken the protective effects of gastric tissue. Thus, the effect of OipA on the miRNA/glutamate pathway in *H. pylori*-induced mucosal injury was explored. The recombinant OipA protein increased *miR-30b* levels, accompanied by a reduction in xCT activity. Taken together, a reduction in xCT activity by *H. pylori* might involve the OipA/miRNA pathway.

In conclusion, decreased glutamate levels caused by reduction in xCT activity play an important role in *H. pylori*-induced gastric mucosal injury. Furthermore, OipA reduced the protective effects of the xCT/glutamate pathway on gastric mucosa by regulating *miRNA-30b*.

### CONFLICTS OF INTEREST

**Guarantor of the article:** Yuan-Jian Li, PhD.

**Specific author contributions:** J.D., X.-H.L., and Y.-J.L. designed this experiment. J.D., F.L., and W.-Q.L. performed the experiment. J.D., X.-H.L., Z.-C.G., and Y.-J.L. analyzed the data and wrote this manuscript. All authors edited and approved the final manuscript.

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**Potential competing interests:** None to report.

## Study Highlights

### WHAT IS KNOWN

✓ Exogenous glutamate protected against gastric mucosal injury.

### WHAT IS NEW HERE

✓ OipA plays a key role in *H. pylori* infection, and the effects are mediated by micro30b/xCT pathway.

### TRANSLATIONAL IMPACT

✓ Our study provide a new potential target for the treatment of *H. pylori* infection.

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