DNA AND CELL BIOLOGY
Volume 38, Number 3, 2019
Mary Ann Liebert, Inc.
Pp. 272–280
DOI: 10.1089/dna.2018.4487

# *Helicobacter pylori*-Induced DNA Damage Is a Potential Driver for Human Gastric Cancer AGS Cells

Yanyan Shi,[1] Pan Wang,[2] Yanlei Guo,[3] Xiaoling Liang,[2] Yuan Li,[3] and Shigang Ding[3]

*Helicobacter pylori* is a major cause of gastric cancer. This study was aimed to explore the characteristic of DNA damage induced by *H. pylori* infection in gastric cancer AGS cells. After infection with *H. pylori*, the reactive oxygen species (ROS) levels in AGS cells were significantly higher than those in the uninfected cells. Cells with longer comet tails were detected after infection with *H. pylori*. The number of apurinic/apyrimidinic endonuclease 1- and phosphorylated H2AX-positive cells was significantly increased compared with the number of negative control cells. The expression of pChk1 and pChk2 was significantly upregulated by *H. pylori* infection. Cell growth was inhibited after *H. pylori* infection. All these results were dose dependent. The cell alterations were more significant upon infection with *H. pylori* at a multiplicity of infection (MOI) of 100:1 than at an MOI of 50:1. *H. pylori* infection can induce DNA single-strand breaks, DNA double-strand breaks, and cell cycle checkpoint activation after ROS generation in the gastric cancer cell line AGS, which is a potential driver for gastric cancer.

**Keywords:** *Helicobacter pylori*, gastric cancer, cell, DNA strand breaks, cell cycle checkpoint activation

## Introduction

Gastric cancer is one of the most common cancers in the world (Tan and Yeoh, 2015). Despite a steady decline in gastric cancer incidence and mortality rates observed in developed countries for the past 50 years, gastric cancer is still a considerable global health burden (Torre *et al.*, 2015; Ajani *et al.*, 2017). The incidence rate of gastric cancer is highest in Eastern Asia, including China (Torre *et al.*, 2015). China contributes ~40% of new cases of gastric cancer around the world every year (Torre *et al.*, 2016). Primary prevention is considered to be a particularly effective way to fight against cancer. Investigation of the mechanisms of risk factors is extremely important for appropriate prevention strategies and prioritization of cancer-control planning (Vineis and Wild, 2014).

*Helicobacter pylori* as an infectious agent is a major risk factor for gastric cancer and is the most important infectious agent worldwide (Plummer *et al.*, 2016). Approximately 75% of gastric cancer cases are induced by *H. pylori* infection (Plummer *et al.*, 2015). The International Agency for Research on Cancer has classified *H. pylori* as a group I carcinogen that causes gastric cancer in humans (1994). *H. pylori* eradication is beneficial for prevention of gastric cancer (Choi *et al.*, 2018; Mera *et al.*, 2018). *H. pylori* is a gram-negative spiral organism that colonizes gastric surface mucous cells and resides in the mucous layer (Steer, 1985). *H. pylori* infection causes inflammatory responses in the host that lead to chronic gastritis and the development of peptic ulcer disease and gastric cancer (Graham and Fischbach, 2010). The risk of gastric cancer is three to six times higher in individuals infected with *H. pylori* than in uninfected individuals (Kim *et al.*, 2011). The discovery of *H. pylori* has revolutionized the practice of gastroenterology, and Correa's multistep cascade theory is a leading factor (Wroblewski and Peek, 2007; Plottel and Blaser, 2011; Rugge *et al.*, 2016). Our previous studies have also shown an association between *H. pylori* infection and development of gastric cancer (Shi *et al.*, 2013; Liu *et al.*, 2015). However, the underlying mechanisms of *H. pylori*'s role in the development of gastric cancer are complex and unclear.

Gastric tissue is particularly sensitive to reactive oxygen species (ROS) (Chaturvedi *et al.*, 2015). The research on ROS started around 1956 with the finding that 2% of the oxygen used by the respiratory chain can be released and

[1]Research Center of Clinical Epidemiology, Peking University Third Hospital, Beijing, P.R. China.
Departments of [2]Obstetrics and Gynecology and [3]Gastroenterology, Peking University Third Hospital, Beijing, P.R. China.

transformed into a superoxide radical anion $O_2^{\bullet -}$ (Harman, 1956). Endogenously generated ROS can be eliminated to achieve homeostasis. Additional stimuli induce excessive production of ROS that can exceed the scavenging capacity and cause ROS to accumulate in the human body. Studies indicate that ROS affect various signaling pathways and control many cellular processes. ROS can increase metabolic, survival, and proliferation signaling and can induce oxidative DNA damage (Kruk and Aboul-Enein, 2017; Gwangwa *et al.*, 2018). DNA damage has been demonstrated to be the culprit for tumorigenesis (Gorgoulis *et al.*, 2005). Oxidative DNA damage is repaired to a certain extent to maintain genome integrity by the DNA repair systems of the cell, but the damage may also avoid the repair systems (Davalli *et al.*, 2018). DNA double-strand breaks (DSBs) are the most deleterious of all types of DNA damage. Continuous formation of DNA DSBs may contribute to genomic instability and consequently to tumorigenesis (Halazonetis *et al.*, 2008).

In this study, we investigate *H. pylori* infection-induced DNA damage response, including SSBs, DSBs, and the activation of cell cycle checkpoint in the *H. pylori*-infected gastric cancer cell line. A real-time cell proliferation assay was used to estimate cell growth rate after *H. pylori* infection. Therefore, the aim of this study is to comprehensively assess the characteristics of *H. pylori*-induced DNA damage in association with the ROS levels; the results may help to develop early intervention and targeted therapy in *H. pylori*-related gastric mucosal diseases, especially in gastric cancer.

## Materials and Methods

### H. pylori *culture*

*H. pylori* strain ATCC 26695 used for this study was preserved in the Key laboratory for *H. pylori* infection and upper gastrointestinal diseases in Peking University Third Hospital. *H. pylori* ATCC 26695 was cultured on blood agar plates containing 39 g/L Columbia solid culture medium (Oxiod), 5% (v/v) sheep blood (Curtin Matheson, Jessup, MD), and the following antibiotics: 4 μg/mL amphotericin B (Life Tech, Carlsbad, CA), 4 μg/mL trimethoprim, and 4 μg/mL vancomycin. The plates were incubated at 37°C for 3 or 5 days in a microaerobic environment [5% (v/v) $O_2$, 10% (v/v) $CO_2$, and 85% (v/v) $N_2$]. Before harvesting, the *H. pylori* cultures were examined using urease tests and Gram staining. Oxidase and catalase tests were also used to ensure that the strains were not contaminated.

### *Cell culture, culture conditions, and coculture assays*

AGS cells were cultured in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum (HyClone, Logan, UT). AGS cells were cultured at 37°C in a humidified incubator at 5% (v/v) $CO_2$. After the bacterial cultures had been resuscitated on blood agar plates, *H. pylori* 26695 bacteria were harvested, washed three times with phosphate-buffered saline (PBS), resuspended in the cell growth medium, and diluted to a final concentration of $1\times10^8$ CFU/mL. AGS cells were plated 1 day before *H. pylori* treatment. For coculture of the cells with bacteria, cells were rinsed once with PBS and fresh growth medium was added. The bacterial strains were then added to the cell medium at multiplicity of infection (MOI) of 50:1 and 100:1 for 24 h.

### *Measurement of intracellular ROS*

Intracellular ROS levels were measured using a cell-permeable fluorogenic probe. AGS cells were seeded in 6-well plates (at a density of $2\times10^5$ cells). After coculture of the cells with *H. pylori* at an MOI of 50:1 or 100:1 for 24 h, cells were washed with PBS for three times, and then ROS levels were monitored using a 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA) molecular probe (Beyotime, Shanghai, China). The DCF fluorescence distribution in the cells was observed under a fluorescence microscope (Olympus, Japan) at 200× magnification. The DCF fluorescence was measured using a Bio-Rad 680 multilabel counter with the excitation source at 488 nm and emission at 525 nm (Bio-Rad, CA) and data were presented as fold of control.

### *Comet assay*

Single-cell gel electrophoretic comet assay was performed under neutral conditions to detect DSBs as described previously (Jin *et al.*, 2016). Intact AGS cells infected with *H. pylori* were collected and rinsed twice with ice-cold PBS; $2\times10^4$ cells/mL were combined with 1% LMAgarose at 40°C at a ratio of 1:3 (v/v) and immediately pipetted onto the slides. For cellular lysis, the slides were immersed in a neutral lysis solution (2% sarkosyl, 0.5 M $Na_2EDTA$, 0.5 mg/mL proteinase K, pH 8.0) overnight at 37°C in the dark, followed by washing in the rinse buffer (90 mM Tris-buffer, 90 mM boric acid, 2 mM $Na_2EDTA$, pH 8.5) for 30 min. The slides were then subjected to electrophoresis at 20 V (0.6 V/cm) for 25 min and stained in 2.5 μg/mL propidium iodide for 20 min. Images were taken with a fluorescence microscope (Olympus, Japan) at 400× magnification and analyzed by the Comet Assay IV software.

### *Immunofluorescence microscopy*

Immunofluorescence was performed as described previously (Ma *et al.*, 2013). In brief, $4\times10^4$ cells per well were seeded into 24-well culture plates and incubated for 24 h at 37°C in 5% (v/v) $CO_2$; the cells were then treated with the *H. pylori* strain at MOI of 50:1 or 100:1 for 24 h. PBS was used to wash the cells three times. The cells were then fixed in 4% paraformaldehyde in PBS (pH 7.4) at room temperature for 30 min. After permeabilization with 0.1% Triton X-100 at room temperature for 30 min, cells were blocked in 1% BSA-supplemented PBS for 1 h and incubated overnight at 4°C with antibodies to apurinic/apyrimidinic endonuclease 1 (APE1) and phosphorylated H2AX (γH2AX). After washing three times in PBS containing 0.1% Tween-20 and 0.01% Triton X-100 for 5 min each, the cells were labeled with 1:500 FITC-conjugated IgG or Rho-conjugated IgG for 1 h at room temperature. After washing in PBS containing 0.1% Tween 20 and 0.01% Triton X-100, the cells were co-stained. Finally, the cells were examined with a fluorescence microscope (Olympus, Japan) at 400× magnification.

### *Western blot analysis*

Proteins related to DNA damage were detected by western blot analysis. Cells were harvested and suspended in the cell lysis buffer containing a protease inhibitor mixture and shaken on ice for 30 min. The cell lysate was centrifuged at $15{,}000 \times g$ at 4°C for 10 min, and the supernatant was collected. The total protein concentration was measured by the Bradford method using a BCA protein assay kit. Proteins were separated by 12% (w/v) sodium dodecylsulfate polyacrylamide gel electrophoresis and electrophoretically transferred onto polyvinylidene fluoride membranes. The membranes were blocked in 5% (w/v) fat-free milk in Tris-buffered saline containing 0.5% (v/v) Tween-20 at room temperature for 1 h and incubated overnight at 4°C with antibodies against pChk1, pChk2, Chk1, Chk2, or β-actin. After three washes in PBS supplemented with 0.1% (v/v) Tween-20 for 15 min, the membranes were incubated with a secondary antibody for 1 h at room temperature. Proteins were identified by scanning the membranes using an Odyssey Imager (LI-COR Biosciences).

### *Cell growth analysis*

A real-time cell proliferation assay was conducted using the ACEA RT-CES microelectronic cell sensor system (ACEA Biosciences) to measure the numbers of living cells. This system works by measuring electrical impedance of sensor electrodes integrated on the bottom of microtiter E-plates. In brief, after coculture with *H. pylori* for 24 h at MOI of 50:1 or 100:1, $1 \times 10^4$ cells per well were seeded in E-Plate 96 and allowed to attach for 12 h. A unitless parameter termed the cell index was derived and used to represent the cell numbers based on the measured relative changes in electrical impedance that occurred in the presence and absence of the cells in the wells. The cell index was normalized to the baseline reading at time point 0 after the attachment (Zaid *et al.*, 2013). Cellular impedance was measured periodically every 5 min. The electronic sensors provided a continuous and quantitative measurement of the cell index (which depends on the number of attached cells and the shape of the cells) in each well. Cell proliferation measured using the cell index was monitored for 72 h.

### *Statistical analysis*

Differences between two groups were analyzed using Student's *t*-test. Comparison between multiple sample sets and the control were performed using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls (SNK) test. Data are presented as the mean ± SD of three independent experiments. All statistical analyses were performed using the SPSS 22.0 computer software. *p*-Values that were <0.05 were considered statistically significant.

## Results

### H. pylori*-induced intracellular ROS in gastric cancer AGS cells*

To gain insight into the events underlying the pathogenic mechanisms of *H. pylori* in gastric mucosa, we assayed intracellular ROS in the gastric cancer AGS cells after *H. pylori* treatment. Cells were infected with *H. pylori* strain 26695 at an MOI of 50:1 or 100:1. ROS levels were measured after treatment with the DCF-DA molecular probe. As shown in Figure 1, after infection at MOIs of 50:1 and 100:1, the ROS levels in the AGS cells were significantly higher than those in the uninfected cells ($p < 0.05$). The ROS levels were higher at an MOI of 100:1 than at an MOI of 50:1 ($p < 0.05$).

### H. pylori*-induced DNA single-strand breaks and DNA DSBs in AGS cells*

To study whether ROS induce oxidative DNA damage in the *H. pylori*-infected cells, we performed the neutral comet assay to examine DNA damage. As shown in Figure 2, cells with longer comet tails were detected among the cells infected with *H. pylori*, but longer tails were not detected in the negative control cells, indicating that DNA damage was induced by *H. pylori* infection ($p < 0.05$). Compared with the cells infected at an MOI of 50:1, the tail moment was longer in the cells infected at an MOI of 100:1 ($p < 0.05$). To further confirm the presence of DNA damage, we examined a surrogate marker of DNA single-strand breaks (SSBs), APE1, and a surrogate marker of DNA DSBs, γH2AX, using immunofluorescence microscopy. The data in Figure 3 indicate that the fractions of APE1- and γH2AX-positive cells significantly increased after *H. pylori* infection versus the negative control ($p < 0.05$). Positive cells infected at MOI of 100:1 had higher levels than cells infected at MOI of 50:1 ($p < 0.05$).

### H. pylori*-activated cell cycle checkpoint in AGS cells*

To study whether the cell cycle checkpoint was activated due to persistent DNA damage, western blot analysis was used to monitor the active status of checkpoint kinase 1 (Chk1) and checkpoint kinase 2 (Chk2) with phosphoprotein-specific antibodies. As shown in Figure 4, no active Chk1 and Chk2 was detected in the control cells. In the cells infected with *H. pylori*, Chk1 and Chk2 were activated. The expression of pChk1 and pChk2 was significantly upregulated by *H. pylori* infection. The levels of kinases were higher in the cells infected at an MOI of 100:1 than in the cells infected at an MOI of 50:1.

### *Cell growth in AGS cells was inhibited after* H. pylori *infection*

To further investigate cell growth after DNA damage, we used an ACEA RT-CES microelectronic cell sensor system to measure the cell index after infection with *H. pylori*. After 24 h of infection, cells were seeded in 96-well plates. As shown in Figure 5, the growth of the AGS cells was inhibited after *H. pylori* infection in a dose-dependent manner. Approximately 43 h after plating, the growth of the negative control cells reached a plateau. The growth rate was reduced in the cells previously infected by *H. pylori* at an MOI of 50:1 or 100:1 for 24 h. At ~60 h after plating, the growth of the cells previously infected at an MOI of 50:1 reached a plateau. Approximately 70 h after plating, the growth of the cells previously infected at an MOI of 100:1 reached a plateau. The results of the cell index measurements indicated that the cells with DNA damage induced by *H. pylori* infection might have a delay in a cell cycle checkpoint, and this leads to mitotic exit and genomic instability.

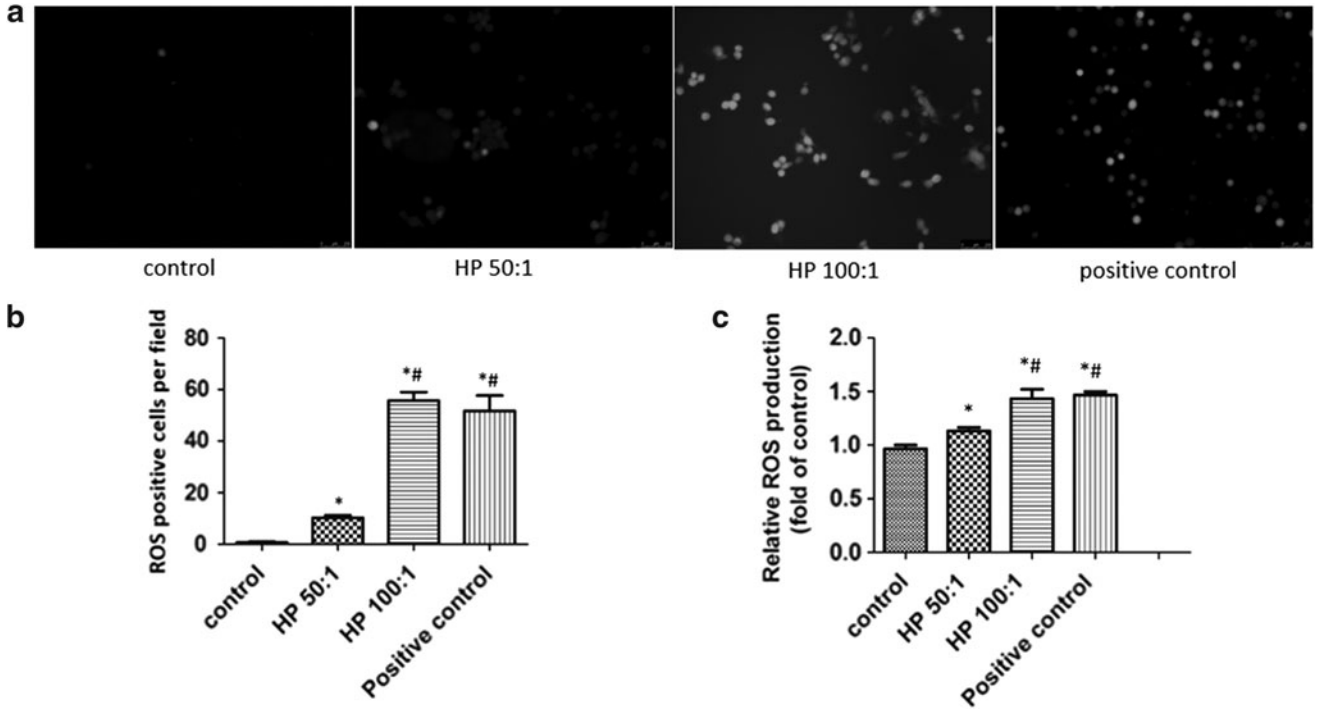


**FIG. 1.** *Helicobacter pylori* induced intracellular ROS in AGS cells. **(a)** After cells were cocultured with *H. pylori* at an MOI of 50:1 or 100:1, the intracellular ROS levels in the gastric cancer AGS cells were measured using DCF-DA molecular probe treatment followed by fluorescence microscopy. **(b)** Statistical analysis of ROS positive cells per field in three groups, including the control group without *H. pylori* infection, *H. pylori*-infected group at an MOI of 50:1, and *H. pylori*-infected group at an MOI of 100:1. **(c)** ROS production by measuring the level of fluorescent DCF was presented as fold of control. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$ was considered statistically significant versus control. $^{\#}p < 0.05$ was considered statistically significant versus the group with *H. pylori* infection at MOI of 50:1. DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; MOI, multiplicity of infection; ROS, reactive oxygen species; SD, standard deviation.

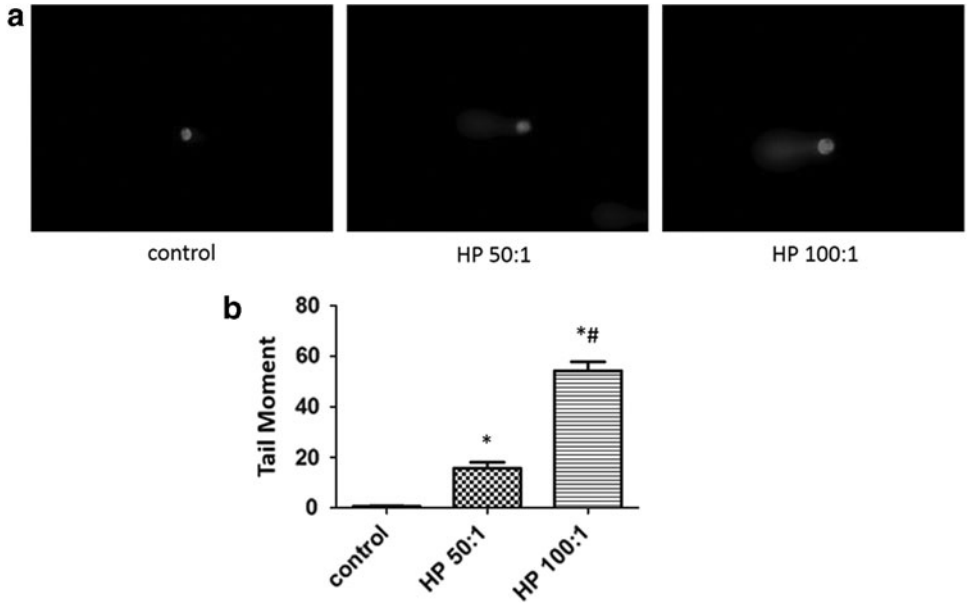


**FIG. 2.** *Helicobacter pylori* induced DNA damages in AGS cells. **(a)** The neutral comet assay was performed to examine DNA damage after cells were cocultured with *H. pylori* at an MOI of 50:1 or 100:1. **(b)** Statistical analysis of the tail moment in three groups, namely, the control group without *H. pylori* infection, the *H. pylori*-infected group at an MOI of 50:1, and the *H. pylori*-infected group at MOI of 100:1. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$ was considered statistically significant versus control. $^{\#}p < 0.05$ was considered statistically significant versus the group with *H. pylori* infection at MOI of 50:1.

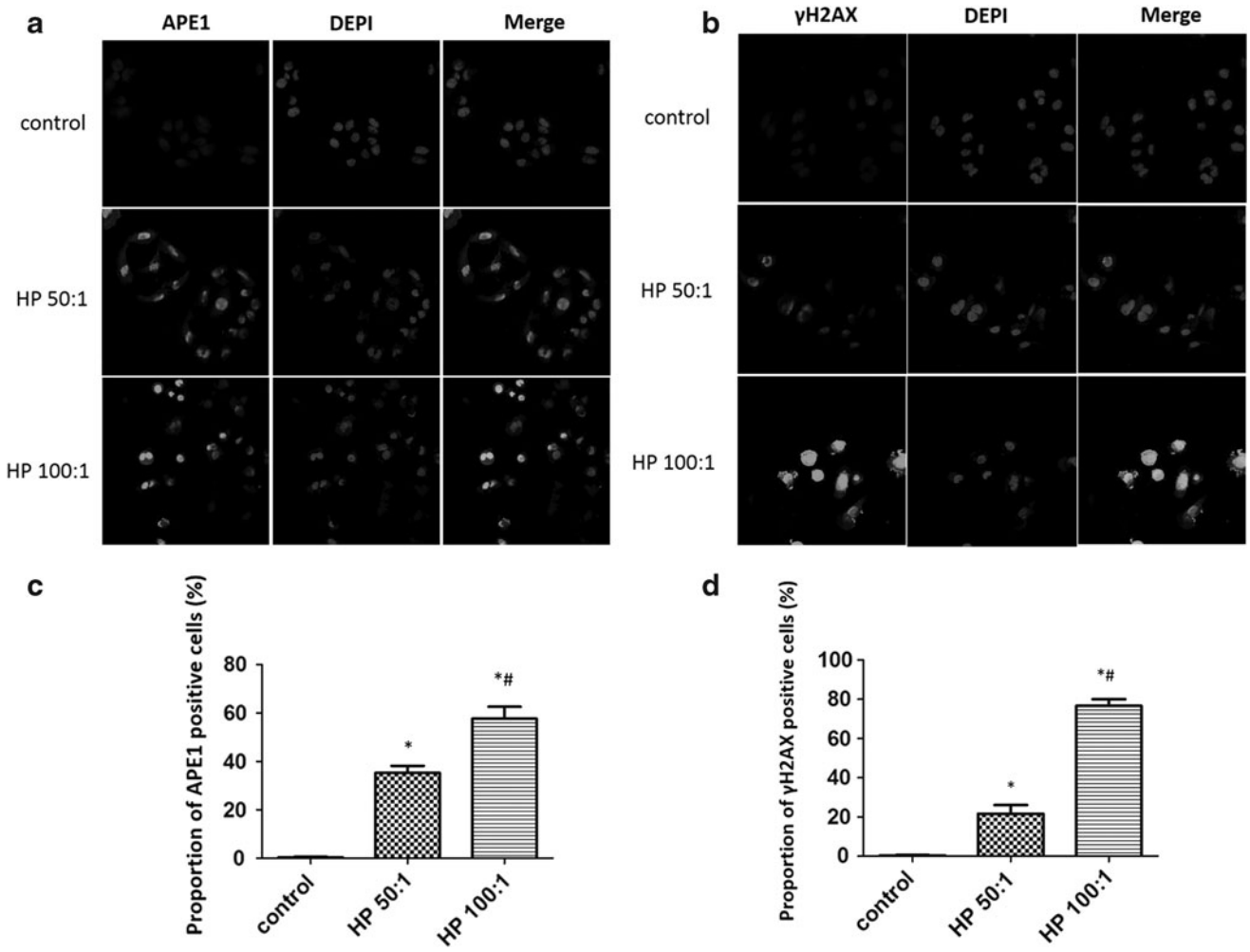


**FIG. 3.** *Helicobacter pylori* upregulated APE1 and γH2AX expression in AGS cells. **(a, b)** APE1 and γH2AX expression was measured using immunofluorescence microscopy after cells were cocultured with *H. pylori* at an MOI of 50:1 or 100:1. **(c, d)** Statistical analysis of fractions of APE1 and γH2AX in three groups, namely, the control group without *H. pylori* infection, the *H. pylori*-infected group at an MOI of 50:1, and the *H. pylori*-infected group at an MOI of 100:1. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$ was considered statistically significant versus control. $^{\#}p < 0.05$ was considered statistically significant versus the group with *H. pylori* infection at MOI of 50:1. APE1, apurinic/apyrimidinic endonuclease 1; γH2AX, phosphorylated H2AX.

## Discussion

Almost 1 million cases of gastric cancer are diagnosed each year, and ~700,000 people succumb each year to gastric adenocarcinoma, thus establishing gastric cancer as the fourth most common cancer and the second cause of cancer-related deaths in the world (Wroblewski *et al.*, 2010). Gastric cancer is a multistep disease that can be preceded by a cascade of precancerous lesions. Multifactorial pathway has been reported to lead to gastric carcinoma, including host, bacterial, and environmental factors. The mechanisms of the development of gastric cancer are complex (Ajani *et al.*, 2017). Further investigations of the pathogenesis can help to understand the mechanisms and might provide evidence in support of early intervention and targeted therapy in gastric cancer. *H. pylori* has been classified as a group I carcinogen that causes gastric cancer in humans, which accounts for at least 75% of gastric cancers worldwide (Plummer *et al.*, 2015). After decades of *H. pylori* infection, 1–3% of patients develop gastric adenocarcinoma (Malfertheiner *et al.*, 2012). Cohort studies from various countries have shown that *H. pylori* infection is associated with an increased risk of gastric cancer (Uemura *et al.*, 2001; Kim *et al.*, 2008). *H. pylori* eradication is beneficial for the prevention of gastric cancer development (Wong *et al.*, 2004; Takenaka *et al.*, 2007; Lee *et al.*, 2016; Choi *et al.*, 2018). *H. pylori* infection is one of the most important factors of etiology of gastric cancer. However, the mechanisms of *H. pylori* infection in gastric mucosal damage have not been clarified.

Studies have shown that *H. pylori* infection can trigger apoptosis of gastric epithelial cells (Correa and Piazuelo, 2008; Shi *et al.*, 2013). Surviving cells undergo genomic events that lead to malignant transformation (David and Meltzer, 2010). Our previous studies showed that after *H. pylori* infection, numerous *H. pylori* strains adhere to the cell wall. The cell surface was damaged, the mitochondria were swollen, the mitochondrial cristae were disrupted, and

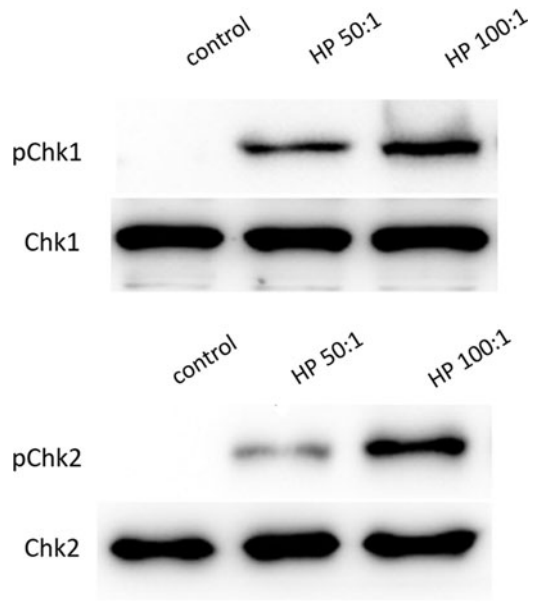


**FIG. 4.** *Helicobacter pylori* activated cell cycle checkpoint in AGS cells. Phosphorylation status of Chk1 and Chk2 in AGS cells was tested by western blotting. Total Chk1 and Chk2 were used for the loading controls.

the nuclei shrank (Shi *et al.*, 2018), indicating that DNA damage might have occurred in the *H. pylori*-infected cells. Several effects of *H. pylori* on genomic integrity have been described. Gastric carcinogenesis requires genomic instabilities; however, the mechanisms of *H. pylori* induction of genomic instabilities remains poorly understood (Hanada *et al.*, 2014; Koeppel *et al.*, 2015). In this study, we extend these observations by showing that *H. pylori* induced an increase in the intracellular ROS levels, DNA DSBs, and DNA SSBs in the gastric cancer cell line AGS, which has been routinely used in cell culture system infected by *H. pylori in vitro* (Wang *et al.*, 2016; Datta *et al.*, 2018). To comprehensively explore the occurrence of DNA damage in the *H. pylori*-infected cells, we measured ROS levels, DNA damage, cell cycle checkpoint activation, and subsequent cell growth. In our experimental setting, cell growth was assayed using a real-time cell proliferation assay that measured the cell index in real time.

Using DCF-DA molecular probe, we found that the intracellular ROS levels increased after *H. pylori* infection in a dose-dependent manner. ROS are a group of molecules produced during metabolism. Low concentrations of ROS are the fundamental signaling molecules (Gwangwa *et al.*, 2018). Exogenous stimulation can induce excessive ROS production, which is beneficial for tumorigenesis. The resulting increase in ROS leads to depolarization of the mitochondrial membrane potential and induction of apoptosis (Dhar *et al.*, 2018). ROS can produce multiple types of oxidative DNA damage, including SSBs. Closely spaced SSBs can form DNA DSBs (Hegde *et al.*, 2012). The DNA damage is induced by upregulated ROS levels in tumorigenic cells; this is a double-edged sword since tumorigenesis and genomic instability are promoted by DNA mutations or can lead to cell death (Nogueira and Hay, 2013; Gwangwa *et al.*, 2018).

In this study, we analyzed DNA damage induced in the *H. pylori*-infected cells using the comet assay. We found significantly longer comet tails in the *H. pylori*-infected cells than in the negative control cells indicating that DNA damage occurred after *H. pylori* infection. Furthermore, we examined whether *H. pylori* infection can induce APE1 and γH2AX accumulation in the gastric cancer cells. APE1 is a multifunctional enzyme. It is an important regulator of the cellular response to ROS and is known best for its DNA backbone cleavage activity during base excision repair.

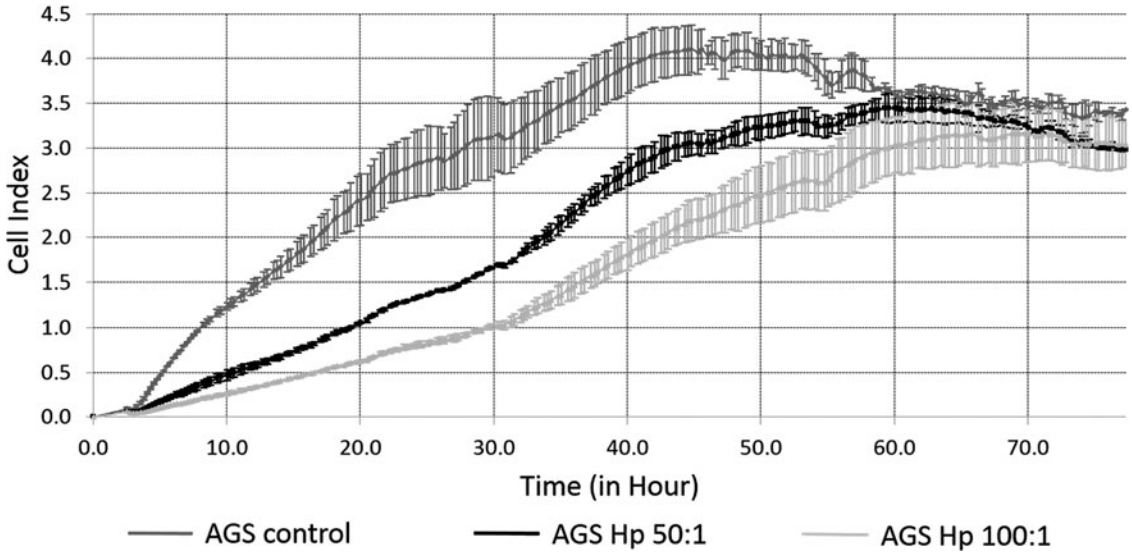


**FIG. 5.** *Helicobacter pylori* inhibited cell growth in AGS cells at the early stage after infection. Real-time cell proliferation assay using electric impedance as a measure of gastric cancer cell growth. Electrical impedance was normalized according to the background measurement at time point 0. The results showed a significantly lower growth rate of *H. pylori*-infected AGS cells 50 h after *H. pylori* infection ($p < 0.05$).

APE1 utilizes the intrahelical DNA space to remove mismatches and DNA damage (Ilina *et al.*, 2018). APE1 is involved in the DNA SSB repair pathway, and it can recognize DNA SSBs (Hegde *et al.*, 2012). H2AX plays a critical role in spreading of the DNA damage signal. γH2AX is required for the stabilization of various DNA damage response factors at the sites of DNA lesions. Therefore, γH2AX is usually used as a surrogate marker of DSB presence (Li and Yu, 2013). Our results showed that APE1 and γH2AX accumulation was induced after *H. pylori* infection in a dose-dependent manner. *H. pylori* infection activates the DNA damage response pathway after oxidative DNA damage in the gastric cancer cells.

It is well known that Chk1 and Chk2 phosphorylation reflects activation of Chk1 and Chk2 that can stop the cell cycle and may result in the mitotic exit and genomic instability (Yang *et al.*, 2010). To further investigate the cell cycle arrest, we measured the levels of active Chk1 and Chk2 in the *H. pylori*-infected cells using western blotting. The results showed that Chk1 and Chk2 were activated by *H. pylori* in a dose-dependent manner indicating that a cell cycle checkpoint was overactivated due to the DNA damage. The cell growth was confirmed to be inhibited using a real-time cell proliferation assay that can provide continuous measurements of the cell index. This study *in vitro* showed cell death after DNA damage, especially in the following 60 h after *H. pylori* infection. It indicated that after DNA damage induced by *H. pylori* infection, some cells underwent cell cycle arrested or cell death pathway, and other cells survived. At 70 h, cells in three groups all reached a plateau, and the plateau was almost at the same level. Combined with literature reports, it indicated that after *H. pylori* infection, surviving cells might undergo genomic events that would lead to malignant transformation (David and Meltzer, 2010). Future studies, including molecular mechanism investigation and chronic *H. pylori*-infected animal models, should be performed to certify this hypothesis.

When DNA damage was induced and the cell cycle checkpoint was activated in the gastric cancer cells, DNA damage repair pathway was clearly activated resulting in mitotic exit, genomic instability, or cell death. These changes in the cells are beneficial for tumorigenesis. After DNA damage, cell death, apoptosis, or necrosis pathway is induced. Parthanatos, similar to apoptosis, is thought to be a protective mechanism that counteracts carcinogenesis. Meanwhile, DNA repair and damage tolerance mechanisms will be triggered, which are key cell survival pathways (Ashour *et al.*, 2015; Stingele *et al.*, 2015). The DNA damage response activates both prosurvival and prodeath signaling. This has been reported to be regulated complexly by many downstream proteins and it is not well understood how the cell switches between these pathways. In survival cells, mismatch repairs, genomic instability, and tumorigenesis would be promoted (Roos *et al.*, 2016). In this study, the downstream molecular mechanisms after DNA damage were scarcely included. More studies should be carried out in the future to investigate DNA repair, and the mechanisms regulating the balance between cell survival and death in cancer biology.

In summary, this study comprehensively demonstrates that *H. pylori* infection can increase the ROS levels and induce both DNA SSBs and DNA DSBs in the gastric cancer cells; these may play an important role in gastric carcinogenesis. The results suggest that *H. pylori*-induced DNA strand breaks is a potential driver for human gastric cancer. Further studies will be performed to investigate the mechanisms of ROS and DNA damage in *H. pylori*-induced gastric carcinogenesis. The antioxidants should be chosen as a potential treatment option for *H. pylori* infection.

In this study, AGS cells were used, which have been commonly used in cell culture system infected by *H. pylori*. Normal gastric cells would be more reasonable than gastric cancer cells to explain gastric carcinogenesis induced by *H. pylori*. But there has been no mature cell culture system representing normal gastric cells at present. This problem has been paid attention to and some scientists have begun to investigate it specially and technically (Saberi *et al.*, 2018). It is expectable in the future, a simple, consistent, and mature cell culture system representing normal gastric cells can be used to better understand *H. pylori*-induced gastric carcinogenesis.

## Acknowledgments

This study was funded by the National Natural Science Foundation of China (Grant No. 81700496), the interdisciplinary medicine Seed Fund of Peking University (BMU2017MX015), and Key laboratory for *Helicobacter pylori* infection and upper gastrointestinal diseases, Beijing Key Laboratory (No. BZ0371).

## Disclosure Statement

No competing financial interests exist.

## References

(1994). Schistosomes, liver flukes and *Helicobacter pylori*. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum **61,** 1–241.

Ajani, J.A., Lee, J., Sano, T., Janjigian, Y.Y., Fan, D., and Song, S. (2017). Gastric adenocarcinoma. Nat Rev Dis Primers **3,** 17036.

Ashour, M.E., Atteya, R., and El-Khamisy, S.F. (2015). Topoisomerase-mediated chromosomal break repair: an emerging player in many games. Nat Rev Cancer **15,** 137–151.

Chaturvedi, R., de Sablet, T., Asim, M., Piazuelo, M.B., Barry, D.P., Verriere, T.G., *et al.* (2015). Increased *Helicobacter pylori*-associated gastric cancer risk in the Andean region of Colombia is mediated by spermine oxidase. Oncogene **34,** 3429–3440.

Choi, I.J., Kook, M.C., Kim, Y.I., Cho, S.J., Lee, J.Y., Kim, C.G., *et al.* (2018). *Helicobacter pylori* therapy for the prevention of metachronous gastric cancer. N Engl J Med **378,** 1085–1095.

Correa, P., and Piazuelo, M.B. (2008). Natural history of *Helicobacter pylori* infection. Dig Liver Dis **40,** 490–496.

Datta, C., Subuddhi, A., Kumar, M., Lepcha, T.T., Chakraborty, S., Jana, K., *et al.* (2018). Genome-wide mRNA-miRNA profiling uncovers a role of the microRNA miR-29b-1-5p/PHLPP1 signalling pathway in *Helicobacter pylori*-driven matrix metalloproteinase production in gastric epithelial cells. Cell Microbiol **20,** e12859.

Davalli, P., Marverti, G., Lauriola, A., and D'Arca, D. (2018). Targeting oxidatively induced DNA damage response in cancer: opportunities for novel cancer therapies. Oxid Med Cell Longev **2018,** 2389523.

David, S., and Meltzer, S.J. (2010). Stomach—genetic and epigenetic alterations of preneoplastic and neoplastic lesions. Cancer Biomark **9,** 493–507.

Dhar, S.K., Bakthavatchalu, V., Dhar, B., Chen, J., Tadahide, I., Zhu, H., *et al.* (2018). DNA polymerase gamma (Polgamma) deficiency triggers a selective mTORC2 prosurvival autophagy response via mitochondria-mediated ROS signaling. Oncogene **37,** 6225–6242.

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., *et al.* (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature **434,** 907–913.

Graham, D.Y., and Fischbach, L. (2010). *Helicobacter pylori* infection. N Engl J Med **363,** 595–596; author reply 596.

Gwangwa, M.V., Joubert, A.M., and Visagie, M.H. (2018). Crosstalk between the Warburg effect, redox regulation and autophagy induction in tumourigenesis. Cell Mol Biol Lett **23,** 20.

Halazonetis, T.D., Gorgoulis, V.G., and Bartek, J. (2008). An oncogene-induced DNA damage model for cancer development. Science **319,** 1352–1355.

Hanada, K., Uchida, T., Tsukamoto, Y., Watada, M., Yamaguchi, N., Yamamoto, K., *et al.* (2014). *Helicobacter pylori* infection introduces DNA double-strand breaks in host cells. Infect Immun **82,** 4182–4189.

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. J Gerontol **11,** 298–300.

Hegde, M.L., Izumi, T., and Mitra, S. (2012). Oxidized base damage and single-strand break repair in mammalian genomes: role of disordered regions and posttranslational modifications in early enzymes. Prog Mol Biol Transl Sci **110,** 123–153.

Ilina, E.S., Khodyreva, S.N., and Lavrik, O.I. (2018). Unusual interaction of human apurinic/apyrimidinic endonuclease 1 (APE1) with abasic sites via the Schiff-base-dependent mechanism. Biochimie **150,** 88–99.

Jin, Y., Xu, X., Wang, X., Kuang, H., Osterman, M., Feng, S., *et al.* (2016). Increasing sensitivity to DNA damage is a potential driver for human ovarian cancer. Oncotarget **7,** 49710–49721.

Kim, N., Park, R.Y., Cho, S.I., Lim, S.H., Lee, K.H., Lee, W., *et al.* (2008). *Helicobacter pylori* infection and development of gastric cancer in Korea: long-term follow-up. J Clin Gastroenterol **42,** 448–454.

Kim, S.S., Ruiz, V.E., Carroll, J.D., and Moss, S.F. (2011). *Helicobacter pylori* in the pathogenesis of gastric cancer and gastric lymphoma. Cancer Lett **305,** 228–238.

Koeppel, M., Garcia-Alcalde, F., Glowinski, F., Schlaermann, P., and Meyer, T.F. (2015). *Helicobacter pylori* infection causes characteristic DNA damage patterns in human cells. Cell Rep **11,** 1703–1713.

Kruk, J., and Aboul-Enein, H.Y. (2017). Reactive oxygen and nitrogen species in carcinogenesis: implications of oxidative stress on the progression and development of several cancer types. Mini Rev Med Chem **17,** 904–919.

Lee, Y.C., Chiang, T.H., Chou, C.K., Tu, Y.K., Liao, W.C., Wu, M.S., *et al.* (2016). Association between *Helicobacter pylori* eradication and gastric cancer incidence: a systematic review and meta-analysis. Gastroenterology **150,** 1113–1124.e5.

Li, M., and Yu, X. (2013). Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. Cancer Cell **23,** 693–704.

Liu, L.N., Ding, S.G., Shi, Y.Y., Zhang, H.J., Zhang, J., and Zhang, C. (2015). *Helicobacter pylori* with high thioredoxin-1 expression promotes stomach carcinogenesis in Mongolian gerbils. Clin Res Hepatol Gastroenterol **40,** 480–486.

Ma, T., Chen, Y., Zhang, F., Yang, C.Y., Wang, S., and Yu, X. (2013). RNF111-dependent neddylation activates DNA damage-induced ubiquitination. Mol Cell **49,** 897–907.

Malfertheiner, P., Megraud, F., O'Morain, C.A., Atherton, J., Axon, A.T., Bazzoli, F., *et al.* (2012). Management of *Helicobacter pylori* infection—the Maastricht IV/Florence Consensus Report. Gut **61,** 646–664.

Mera, R.M., Bravo, L.E., Camargo, M.C., Bravo, J.C., Delgado, A.G., Romero-Gallo, J., *et al.* (2018). Dynamics of *Helicobacter pylori* infection as a determinant of progression of gastric precancerous lesions: 16-year follow-up of an eradication trial. Gut **67,** 1239–1246.

Nogueira, V., and Hay, N. (2013). Molecular pathways: reactive oxygen species homeostasis in cancer cells and implications for cancer therapy. Clin Cancer Res **19,** 4309–4314.

Plottel, C.S., and Blaser, M.J. (2011). Microbiome and malignancy. Cell Host Microbe **10,** 324–335.

Plummer, M., de Martel, C., Vignat, J., Ferlay, J., Bray, F., and Franceschi, S. (2016). Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health **4,** e609–e616.

Plummer, M., Franceschi, S., Vignat, J., Forman, D., and de Martel, C. (2015). Global burden of gastric cancer attributable to *Helicobacter pylori*. Int J Cancer **136,** 487–490.

Roos, W.P., Thomas, A.D., and Kaina, B. (2016). DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer **16,** 20–33.

Rugge, M., Genta, R.M., Graham, D.Y., Di Mario, F., Vaz Coelho, L.G., Kim, N., *et al.* (2016). Chronicles of a cancer foretold: 35 years of gastric cancer risk assessment. Gut **65,** 721–725.

Saberi, S., Pournasr, B., Farzaneh, Z., Esmaeili, M., Hosseini, M.E., Baharvand, H., *et al.* (2018). A simple and cost-efficient adherent culture platform for human gastric primary cells, as an in vitro model for *Helicobacter pylori* infection. Helicobacter **23,** e12489.

Shi, Y., Guo, Y., Zhang, T., and Ding, S. (2018). Hydrotalcite can prevent the damaging effects of *Helicobacter pylori* on gastric epithelial cells. Microsc Microanal **24,** 277–283.

Shi, Y., Liu, L., Zhang, T., Shen, L., Liu, L., Zhang, J., *et al.* (2013). The involvement of *Helicobacter pylori* thioredoxin-1 in gastric carcinogenesis. J Med Microbiol **62(Pt 8),** 1226–1234.

Steer, H.W. (1985). The gastro-duodenal epithelium in peptic ulceration. J Pathol **146,** 355–362.

Stingele, J., Habermann, B., and Jentsch, S. (2015). DNA-protein crosslink repair: proteases as DNA repair enzymes. Trends Biochem Sci **40,** 67–71.

Takenaka, R., Okada, H., Kato, J., Makidono, C., Hori, S., Kawahara, Y., *et al.* (2007). *Helicobacter pylori* eradication reduced the incidence of gastric cancer, especially of the intestinal type. Aliment Pharmacol Ther **25,** 805–812.

Tan, P., and Yeoh, K.G. (2015). Genetics and molecular pathogenesis of gastric adenocarcinoma. Gastroenterology **149,** 1153–1162.e3.

Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet-Tieulent, J., and Jemal, A. (2015). Global cancer statistics, 2012. CA Cancer J Clin **65,** 87–108.

Torre, L.A., Siegel, R.L., Ward, E.M., and Jemal, A. (2016). Global cancer incidence and mortality rates and trends—an update. Cancer Epidemiol Biomarkers Prev **25,** 16–27.

Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., *et al.* (2001). *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med **345,** 784–789.

Vineis, P., and Wild, C.P. (2014). Global cancer patterns: causes and prevention. Lancet **383,** 549–557.

Wang, G., Romero-Gallo, J., Benoit, S.L., Piazuelo, M.B., Dominguez, R.L., Morgan, D.R., *et al.* (2016). Hydrogen metabolism in *Helicobacter pylori* plays a role in gastric carcinogenesis through facilitating CagA translocation. MBio **7,** pii: e01022-16.

Wong, B.C., Lam, S.K., Wong, W.M., Chen, J.S., Zheng, T.T., Feng, R.E., *et al.* (2004). *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA **291,** 187–194.

Wroblewski, L.E., and Peek, R.M. Jr. (2007). Orchestration of dysregulated epithelial turnover by a manipulative pathogen. Cell Host Microbe **2,** 209–211.

Wroblewski, L.E., Peek, R.M., Jr. and Wilson, K.T. (2010). *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev **23,** 713–739.

Yang, L., Besschetnova, T.Y., Brooks, C.R., Shah, J.V., and Bonventre, J.V. (2010). Epithelial cell cycle arrest in G2/M mediates kidney fibrosis after injury. Nat Med **16,** 535–543, 531p following 143.

Zaid, T.M., Yeung, T.L., Thompson, M.S., Leung, C.S., Harding, T., Co, N.N., *et al.* (2013). Identification of FGFR4 as a potential therapeutic target for advanced-stage, high-grade serous ovarian cancer. Clin Cancer Res **19,** 809–820.

Address correspondence to:
*Yuan Li, MD*
*Department of Gastroenterology*
*Peking University Third Hospital*
*No. 49, Garden North Road*
*Beijing 100191*
*P.R. China*

*E-mail:* leeeyuan@aliyun.com

*Shigang Ding, MD*
*Department of Gastroenterology*
*Peking University Third Hospital*
*No. 49, Garden North Road*
*Beijing 100191*
*P.R. China*

*E-mail:* dingshigang222@163.com

Received for publication October 13, 2018; received in revised form December 2, 2018; accepted December 12, 2018.