REVIEW ARTICLE

Cancer Science WILEY

Helicobacter pylori-induced DNA double-stranded break in the development of gastric cancer

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Abstract

Infection with *cagA*-positive *Helicobacter pylori* strains plays an etiological role in the development of gastric cancer. The CagA protein is injected into gastric epithelial cells through a bacterial Type IV secretion system. Inside the host cells, CagA promiscuously associates with multiple host cell proteins including the prooncogenic phosphatase SHP2 that is required for full activation of the Ras–ERK pathway. CagA–SHP2 interaction aberrantly activates SHP2 and thereby deregulates Ras–ERK signaling. Cancer is regarded as a disease of the genome, indicating that *H. pylori*-mediated gastric carcinogenesis is also associated with genomic alterations in the host cell. Indeed, accumulating evidence has indicated that *H. pylori* infection provokes DNA double-stranded breaks (DSBs) by both CagA-dependent and CagA-independent mechanisms. DSBs are repaired by either error-free homologous recombination (HR) or error-prone non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ). Infection with *cagA*-positive *H. pylori* inhibits RAD51 expression while dampening cytoplasmic-to-nuclear translocalization of BRCA1, causing replication fork instability and HR defects (known as "BRCAness"), which collectively provoke genomic hypermutation via non-HR-mediated DSB repair. *H. pylori* also subverts multiple DNA damage responses including DNA repair systems. Infection with *H. pylori* additionally inhibits the function of the p53 tumor suppressor, thereby dampening DNA damage-induced apoptosis, while promoting proliferation of CagA-delivered cells. Therefore, *H. pylori cagA*-positive strains promote abnormal expansion of cells with BRCAness, which dramatically increases the chance of generating driver gene mutations in the host cells. Once such driver mutations are acquired, *H. pylori* CagA is no longer required for subsequent gastric carcinogenesis (Hit-and-Run carcinogenesis).

KEYWORDS
CagA, DNA damage response, DNA double-stranded break, genome instability, *Helicobacter pylori*

1 | INTRODUCTION

Cancer, a major cause of human death worldwide, has been considered to be primarily a disease evoked by malfunctioning of the host cell genome. Many cancers including gastric cancer are recognized as inflammation-related cancers, which are generally associated with chronic viral/bacterial/parasitic infection, where inflammation and direct genotoxicity by oncogenic pathogens may be critical events that promote carcinogenesis. A causal link between viruses and particular cancers has been well accepted because several viruses possess viral oncogenes. For example, chronic infection with an RNA virus HCV plays an etiologic role in the development of HCC. At least four HCV gene products (core, NS3, NS5A, and NS5B) have been shown to deregulate oncogenic signaling pathways in the infected host cells.[1] The human retrovirus human T-cell leukemia virus type 1 (HTLV-1), which caused ATL, possesses two viral oncogenes, *HBZ* and *tax*.[2] Similarly, oncogenic DNA viruses encode oncoproteins that neutralize the function of tumor suppressors, such as p53 and pRB, in infected host cells. For instance, HBV is an etiological agent of HCC. HBV not only uses viral DNA integration into the host genome but also inhibits p53 function by binding with viral oncoprotein HBx.[1] It is well understood that high-risk types of HPV are causative agents of cervical cancers. The HPV oncoproteins E6 and E7, which are viral oncoprotein that are responsible for initiation and progression of cervical cancer, inactivate p53 and pRB, respectively, by physical complex formation.[3] In contrast, bacteria have long been considered not to be etiologically involved in the development of human cancer. However, the results of recent epidemiological studies as well as those of molecular cell biological analyses have suggested that there is a causal link between several bacterial infections and carcinogenesis. Specifically, *Helicobacter pylori* has been recognized as a bacterium that is etiologically associated with the development of gastric cancer.[4,5] Genotoxic colibactin-producing *pks*-positive *Escherichia coli* has also been recognized as a causative agent of colorectal cancer.[6,7] *Salmonella typhi* has been associated with gallbladder cancer.[8] and *Chlamydia trachomatis* has been involved in the development of cervical cancer or ovarian cancer.[9,10] Although the mechanisms by which these bacteria promote neoplastic transformation of the host cells have not been fully understood, a common feature is that infection with these bacteria induces DNA DSBs in the host genome. DSBs are the most dangerous type of DNA damage, in which the phosphate backbones of the two complementary DNA strands are broken simultaneously. Unrepaired DSBs induce apoptosis or cellular senescence, whereas misprocessing of DSBs leads to various types of mutation including insertion, deletion, and translocation, thereby causing genome instability that promotes carcinogenesis.[11]

Helicobacter pylori is a micro-aerophilic Gram-negative bacterium, which was discovered by Barry Marshall and Robin Warren in 1984.[12] *H. pylori* colonizes the human stomach and is estimated to infect at least half of the world's human population.[5] Gastric adenocarcinoma is histopathologically divided into two major types: intestinal type and diffuse type. Long-term infection with *H. pylori* is intimately associated with intestinal-type gastric cancer, which proceeds through Correa's cascade with the following sequential stages: chronic gastritis, atrophy, intestinal metaplasia, and dysplasia culminating in gastric cancer.[13] The causative role of *H. pylori* in gastric carcinogenesis has been shown by clinical evidence that eradication of *H. pylori* significantly decreased the rate of gastric cancer in *H. pylori*-infected patients without precancerous lesions.[14] *H. pylori* is subdivided into *cagA*-positive and *cagA*-negative strains based on the presence or absence of the *cag* pathogenicity island (*cag*PAI). The *cag*PAI, an ~40-kb DNA segment, contains ~30 genes (open reading frames), which include *cagA* and several genes encoding components of a bacterial microsyringe, termed the Type IV secretion system (T4SS), that delivers CagA into the attached gastric epithelial cell (Figure 1).[15] Whereas almost all the *H. pylori* isolates circulating in East Asian countries, including Japan, China, and Korea, are *cagA*-positive strains, ~40% of the *H. pylori* isolates in other countries are *cagA*-negative.[16] Chronic infection with *cagA*-positive strains plays a critical role in the development of gastric cancer.[4,17] Consistently, the oncogenic potential of CagA has been directly demonstrated by its transgenic expression in *Drosophila*, zebrafish, and mouse.[18-20]

Inside the host cells, CagA undergoes tyrosine phosphorylation by Src family kinases and c-Abl kinase.[21,22] Tyrosine-phosphorylated CagA acquires the ability to bind to SHP2 and thereby stimulates the pro-oncogenic phosphatase activity, which potentiates RAS–ERK signaling.[23-25] CagA also interacts with the polarity-regulating serine/threonine kinase PAR1b (also known as MARK2) in a tyrosine phosphorylation-independent manner through the CM motif, resulting in junctional and polarity defects of gastric epithelial cells.[26,27] CagA also interacts with the c-MET HGF receptor through the CM motif, thereby activating the PI3K–AKT pathway.[28] These CagA activities have been thought to play a central role in gastric carcinogenesis (Figure 2).[5,29]

In addition to CagA, *H. pylori* produces several virulence factors that contribute to its pathogenesis (Figure 2). Vacuolating cytotoxin A (VacA), which is secreted from *H. pylori* and internalized into gastric epithelial cells by endocytosis, inducing vacuole formation and apoptosis.[30,31] Although almost all *H. pylori* strains possess the *vacA* gene, ~50% of *H. pylori* strains produce the toxigenic and pathogenic VacA due to gene sequence variations.[32] All *H. pylori* strains produce urease that catalyzes the hydrolysis of urea to yield ammonia, thereby neutralizing the acidic environment of the stomach. *In vitro* studies have shown that urease-mediated ammonia production disrupts tight junctions of the gastric epithelial cells.[33,34] Therefore, urease functions as both a colonization factor and a virulence factor. All *H. pylori* strains express γ-glutamyl transpeptidase (GGT) that catalyzes the consumption of glutamine and glutathione, thereby producing ammonia and reactive oxygen species (ROS), which play a role in the colonization of gastric mucosa.[35] Neutrophil-activating protein (NapA), produced by all the *H. pylori* strains, has also been identified as a virulence factor, which attracts and activates neutrophiles, thereby provoking gastric inflammation (Figure 2).[36,37]

In addition to CagA, metabolic precursors of *H. pylori* lipopolysaccharide (LPS), D-glycero-β-D-manno-heptose 1,7-bisphosphate (HBP) and ADP-β-D-manno-heptose (ADP-heptose), are delivered into host
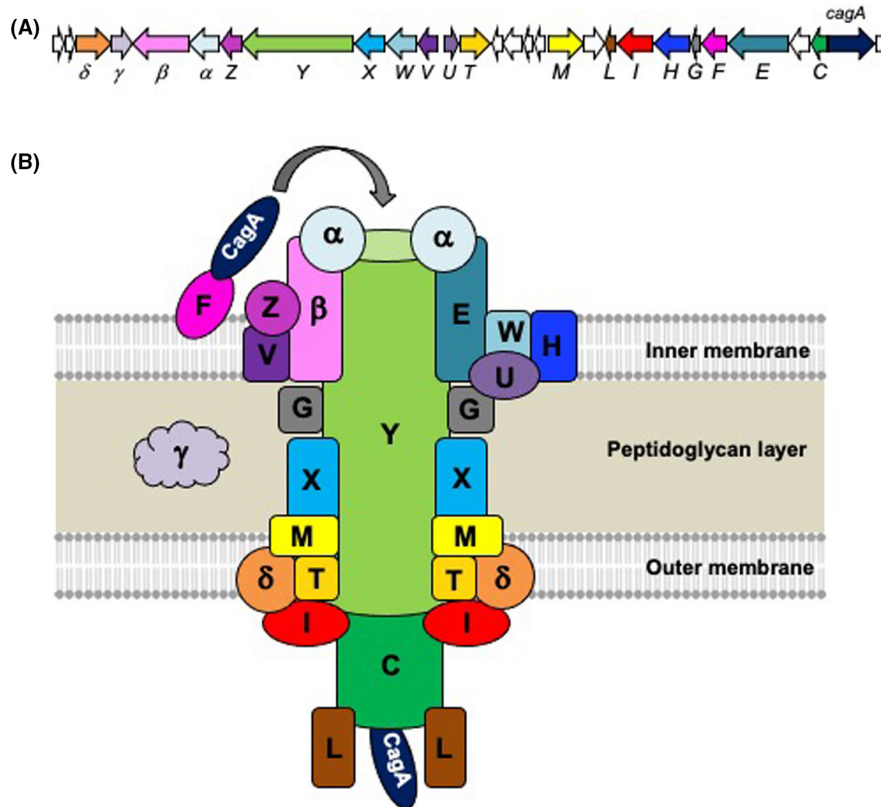
FIGURE 1 *Helicobacter pylori* Type IV secretion system (T4SS) and virulence factor CagA. (A) Schematic view of the *cag*PAI encoded by the *H. pylori* strain 26695. The *cag*PAI contains 29 open reading frames including the *cagA* gene that is located at the most downstream portion of *cag*PAI. Several genes encoding components of the T4SS are shown by colored arrows with a single letter corresponding to the last letter of each gene name, for example, *X* for *cagX*. White arrows indicate non-essential genes for CagA translocation through the T4SS. (B) A model for assembly of *H. pylori* T4SS. The non-essential components for CagA translocation are not shown here. The 19 components of T4SS are shown in a simple manner, in which they are not drawn to scale or with the exact number of subunits. *H. pylori* Cagγ contributes to the assembly of T4SS by digesting the peptidoglycan layer of the bacterial cell wall. CagA is delivered into gastric epithelial cells through bacterial T4SS

cells through the T4SS.[38,39] In gastric epithelial cells, HBP and ADP-heptose are recognized by alpha-kinase 1 (ALPK1) to activate the tumor necrosis factor receptor-associated factor (TRAF)-interacting protein with the forkhead-associated domain (TIFA)-mediated innate immune response to elicit NF-κB activation, which triggers the production of proinflammatory cytokines such as IL-8 (Figure 2).[39]

Helicobacter pylori has been thought to promote neoplastic transformation of gastric epithelial cells via CagA-dependent deregulation of cell proliferation, as well as cell motility in conjunction with CagA-independent chronic inflammation.[40] Intriguingly, while *H. pylori* infection appears to be mostly essential for gastric cancer initiation, the presence of this bacterium is no longer required for the later phase of gastric carcinogenesis. Therefore, previous studies that investigated pro-mitogenic activity of delivered CagA, as well as chronic inflammation induced by *H. pylori*-infected gastric mucosa, do not fully explain the mechanism of the later phase of gastric carcinogenesis. In this regard, recent studies have revealed that *H. pylori* infection induces DSBs in the host genome, which may elicit genome instability in the infected host cells. In this review, we focus on the mechanisms of DSBs induction by *H. pylori*, and discuss the possible

mechanisms leading to genome instability that underscores gastric carcinogenesis.

2 | INDUCTION OF DSBS BY *H. PYLORI* IN A CAGA-DEPENDENT MANNER

The main repair pathways used to resolve DSBs are HR, non-homologous end joining (NHEJ), and microhomology-mediated end joining (MMEJ). HR is an error-free pathway as it uses an homologous template for repair, whereas NHEJ and MMEJ are error-prone DSB repair pathways.[41] The tumor suppressor BRCA1 is indispensable for HR-mediated DSB repair. "BRCAness" is defined by a defect in HR-mediated DSB repair by mutational loss of *BRCA1*, *BRCA2*, or genes constituting components of the BRCA1/BRCA2 pathway.[42,43]

Infection with *cagA*-positive *H. pylori* induces DSBs in gastric epithelial cells, which is concomitantly associated with reduced expression of RAD51 that protects persistently stalled replication forks from Mre11-mediated nucleolytic degradation, while mediating HR as a recombination factor.[44] Consistently, *cagA*-positive *H. pylori* induces
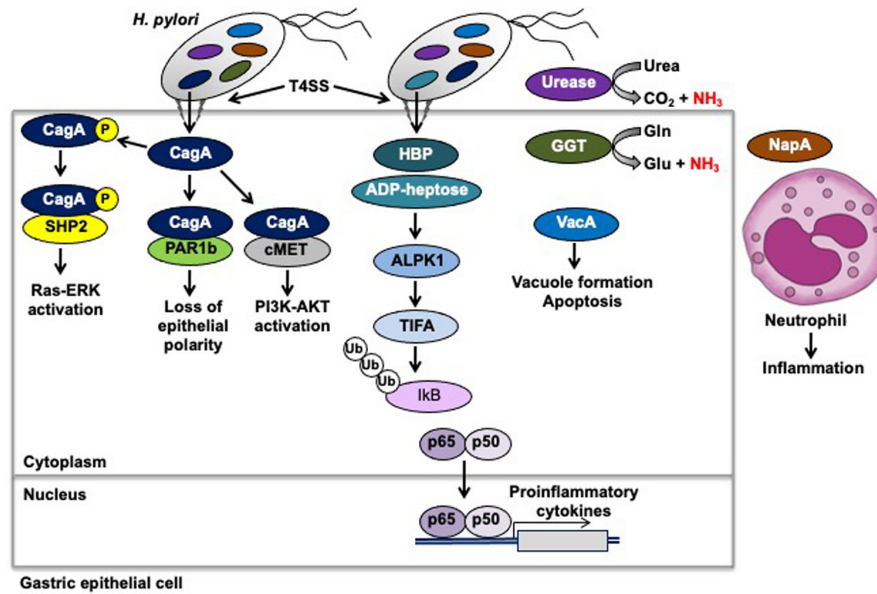
FIGURE 2 *Helicobacter pylori* virulence factors and host cell responses. CagA is injected into gastric epithelial cells through bacterial T4SS. CagA interacts with multiple host cell proteins and thereby deregulates prooncogenic signaling. D-Glycero-β-D-manno-heptose 1,7-bisphosphate (HBP) and ADP-β-D-manno-heptose (ADP-heptose) are injected into gastric epithelial cells through T4SS, which activates ALPK1/TIFA/NF-κB signaling. Urease and γ-glutamyl-transferase (GGT) produce ammonia and thereby neutralize the acidic environment. Vacuolating cytotoxin A (VacA) is secreted from *H. pylori* and internalized into host cells by endocytosis. VacA causes vacuole formation and apoptosis. Neutrophil-activating protein A (NapA) attracts and activates neutrophils and thereby promotes gastric inflammation
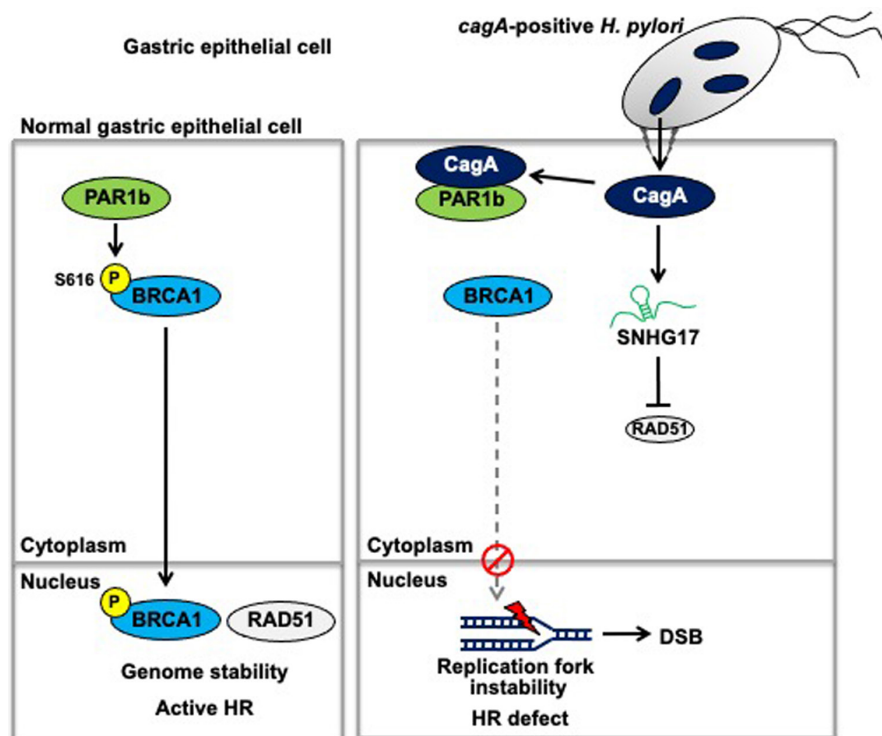

FIGURE 3 Induction of DSBs through CagA-mediated transient BRCAness. In normal cells, PAR1b phosphorylates BRCA1 on S616, which is responsible for the cytoplasmic-to-nuclear translocation of BRCA1. CagA prevents S616 phosphorylation of BRCA1 through PAR1b inhibition. CagA reduces RAD51 expression via the induction of long noncoding RNA SNHG17. These CagA activities cause replication fork instability leading to DSBs and HR defects

long noncoding RNA SNHG17 that functions as a decoy for miR-3909, thereby reducing the expression of RAD51 (Figure 3).[45] More recently, we found that CagA-mediated PAR1b kinase inhibition also gives rise to DSBs in *H. pylori*-infected human gastric epithelial cells.[46] Plasmid-mediated expression of CagA in gastric epithelial cells leads to DSB induction in a CM motif-dependent manner, indicating that CagA delivery on its own gives rise to DSBs.[46] BRCA1 shuttles between the cytoplasm and nucleus, where it functions as a key player in HR-mediated DSB repair, as well as in the protection of stalled forks from Mre11-mediated nucleolytic degradation.[47] BRCA1 has

two NLS, proximal NLS (residues 503–508) and distal NLS (residues 606–615). PAR1b phosphorylates BRCA1 on S616 that is located immediately downstream of the distal NLS, which is responsible for the cytoplasmic-to-nuclear translocation of BRCA1 (Figure 3).[46] CagA prevents phosphorylation of BRCA1 on S616 by inhibiting PAR1b kinase activity, resulting in a shortage of nuclear BRCA1. Consequently, CagA elicits replication fork instability that gives rise to DSBs and, at the same time, impairs HR-mediated DSB repair, which cooperatively provoke genome instability through error-prone end joining.[46] *In vivo* experiments have also shown that CagA induces dysfunction of BRCA1. Floxed *cagA*-transgenic mice that conditionally express the *cagA* transgene by tamoxifen treatment show reduced nuclear BRCA1 in the stomach mucosa upon CagA induction. Also, CagA induction in mice increases DSBs in the stomach mucosa.[46] Furthermore, loss of nuclear BRCA1 is observed in human stomach mucosa infected with chronic *cagA*-positive *H. pylori* but not in those without *H. pylori* infection.[46]

3 | INDUCTION OF DSBS BY *H. PYLORI* IN A CAGA-INDEPENDENT MANNER

Helicobacter pylori induces DSBs in infected gastric epithelial cells, and the DSB induction requires bacterial T4SS and host cell β1-integrin.[48,49] DSB induction is triggered by translocation of HBP and ADP-heptose into gastric epithelial cells through bacterial T4SS, which activates the ALPK1/TIFA/NF-κB signaling axis.[38,39] When NF-κB activation occurs during the S-phase of the cell cycle, active transcription results in R-loop formation, which is a three-stranded nucleic acid structure made up of an RNA/DNA hybrid. R-loops cause replication fork stalling, where the nucleotide excision repair endonucleases XPG (also known as ERCC5) and XPF (also known as ERCC4) cut the DNA strand in the RNA/DNA hybrid, producing a single-strand gap that is converted into a DSB by replication or additional strand breaks (Figure 4).[39,49] Whereas formation of *H. pylori*-induced DSBs depends on NF-κB signaling, inhibition of NF-κB signaling that completely suppressed the production of IL-8 can reduce the levels of DSBs by only half.[49] In addition, treatment of cells with the canonical NF-κB activator TNF-α does not induce DSBs.[39,49] These results indicated that NF-κB activation is not a major player for the induction of DSBs in the host cell genome.

Because CagA-mediated DSB induction is not influenced by inhibition of NF-κB signaling, CagA and ADP-heptose may independently give rise to DSB generation in gastric epithelial cells. Nevertheless, it should be important to emphasize that CagA exacerbates DSB formation triggered by both CagA-dependent and CagA-independent mechanisms by inducing the state of BRCAness.

4 | INDUCTION OF DSBS THROUGH OXIDATIVE STRESS INDUCED BY *H. PYLORI*

While ROS are produced during normal metabolisms, high levels of ROS elicit tissue damage and carcinogenesis. When ROS react with DNA,
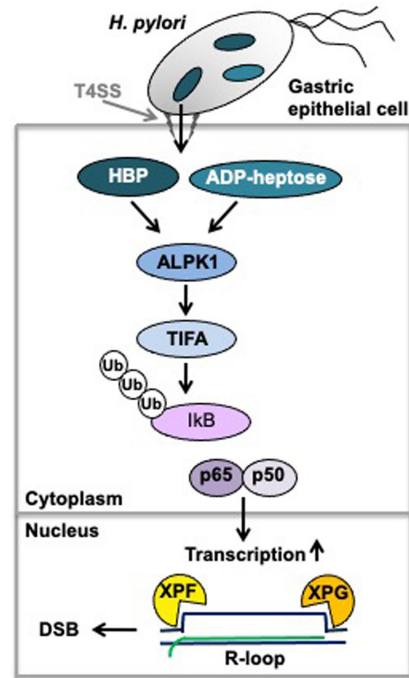

FIGURE 4 Induction of DSBs through NF-κB activation by *Helicobacter pylori*. *H.pylori*-derived HBP and ADP-heptose activates ALPK1/TIFA/NF-κB signaling. Active transcription causes R-loop formation in which DSBs are induced by nucleotide excision repair endonucleases XPG and XPF

various kinds of DNA damage causing mutational events are induced. Major oxidative DNA lesions are 2-hydroxydeoxyadenosine (2-OH-dA), 5-formyldeoxyuridine (5-CHO-dU), 5-hydroxydeoxycytidine (5-OH-dC), and 8-hydroxydeoxyguanosine (8-OH-dG).[50] Among them, 8-OH-dG has been widely used as a pivotal biomarker for oxidative damage of DNA, because it is relatively easy to detect using a high performance liquid chromatography-electrochemical detector (HPLC-ECD), a liquid chromatography-coupled tandem mass spectrometer (LC-MS/MS), or antibody labeling techniques such as ELISA and immunohistochemistry.[51,52] While unrepaired 8-OH-dG elicits base-pair substitutions, they are converted to abasic sites by 8-hydroxyguanine glycosylase (OGG1), resulting in single-stranded breaks (SSBs) that lead to DSBs.[53,54] ROS can be produced not only by immune cells but also by gastric epithelial cells infected with *H. pylori*.

In a study of human gastric biopsy samples, the presence of the *cagA* gene and high levels of 8-OH-dG were substantially correlated with severe gastric inflammation and gastric cancer.[55] It was also reported that *cagA*-positive *H. pylori* strains, but not *cagA*-negative strains, induced SMO, which catabolizes the polyamine spermine to produce spermidine and hydrogen peroxide.[56] In addition to the induction of ROS, *H. pylori* modulates the functions of antioxidant protein in gastric epithelial cells. Heme oxygenase (HO-1, also known as HMOX1) is a potent antioxidant enzyme that is responsible for the degradation of heme.[57] An *in vitro* study revealed that induction of HO-1 by nitric oxide (NO) is inhibited by *H. pylori* infection

in a CagA-dependent manner. Consistently, HO-1 is downregulated in gastric epithelial cells of patients infected with *cagA*-positive *H. pylori*, but not in those of patients infected with *cagA*-negative *H. pylori*.[58] Therefore, *cagA*-positive *H. pylori* can induce DSBs in gastric epithelial cells through production of ROS. In contrast, plasmid-mediated expression of CagA in gastric epithelial cells induces DSB without ROS production, indicating that CagA delivery on its own does not produce ROS.[46]

Another virulence factor VacA induces Ca^{2+} influx into the cytoplasm in *H. pylori*-infected cells, which is paralleled by Ca^{2+} flux into the mitochondria, resulting in increased hydrogen ion production in the respiratory chain.[59] Urease and NapA recruit neutrophils to the site of *H. pylori* attachment, which stimulate the production of ROS.[60,61] *H. pylori* also induces hydrogen peroxide production in epithelial cells in a GGT-dependent manner.[62] Taken together, ROS can be produced in gastric epithelial cells in response to multiple virulence factors of *H. pylori*, which variably induce DSBs in the host genome (Figure 5).

5 | IMPAIRMENT OF THE DNA DAMAGE RESPONSE BY *H. PYLORI*

As described above, *H. pylori* inhibits HR that is essential for error-free DSB repair by depleting nuclear BRCA1 in a CagA-dependent manner. DSBs that are associated with impairment of HR factors are also induced by *cagA*-positive *H. pylori*.[44,63,64] In addition, *H. pylori* has been reported to impair multiple DDRs including the DNA repair system. DNA mismatch repair (MMR) corrects DNA mismatches generated during DNA replication, thereby preventing mutations from being inherited in daughter cells.[65] *In vitro* studies showed that infection with *H. pylori* downregulates the expression of MMR components including MLH1, MSH2, MSH3, MSH6, PMS1, and PMS2 in a CagA-independent manner.[66,67] Consistent with these *in vitro* observations, the expression of MLH1 and MSH2 in patients with chronic *H. pylori* infection increases after *H. pylori* eradication, indicating that *H. pylori* downregulates the expression of MLH1 and MSH2.[68] *H. pylori* also impairs the DNA repair system of alkylative lesions. O^6-methylguanine methyltransferase (MGMT) repairs damaged guanine nucleotide by transferring the methyl group at the O^6 position of guanine to its cysteine residue, thereby avoiding genome mutation. The level of MGMT expression in individuals with chronic *H. pylori* infection is significantly lower than that in *H. pylori*-negative control subjects. The reduced level of MGMT is due to hypermethylation of the *MGMT* promoter, which is rescued after *H. pylori* eradication.[69] These findings indicate that DNA repair is impaired during *H. pylori* colonization, further increasing genome instability in *H. pylori*-infected gastric epithelial cells (Figure 6).

Under physiological conditions, the expression level of p53 is kept low through its ubiquitination by MDM2, an E3 ubiquitin ligase for p53, which is followed by proteasomal degradation. In response to DNA damage, however, p53 is rapidly activated via elevated protein levels and increased DNA-binding activity, causing transactivation of target genes that mediate cell cycle arrest, DNA repair, apoptosis, and cell senescence.[70]

The expression of AID is highly restricted to B cells in germinal centers, in which it plays an important role for somatic hypermutation and class-switch recombination in immunoglobulin genes. However, aberrant expression of AID in non-B cells can induce mutations that contribute to tumorigenesis.[71,72] Strikingly, the infection
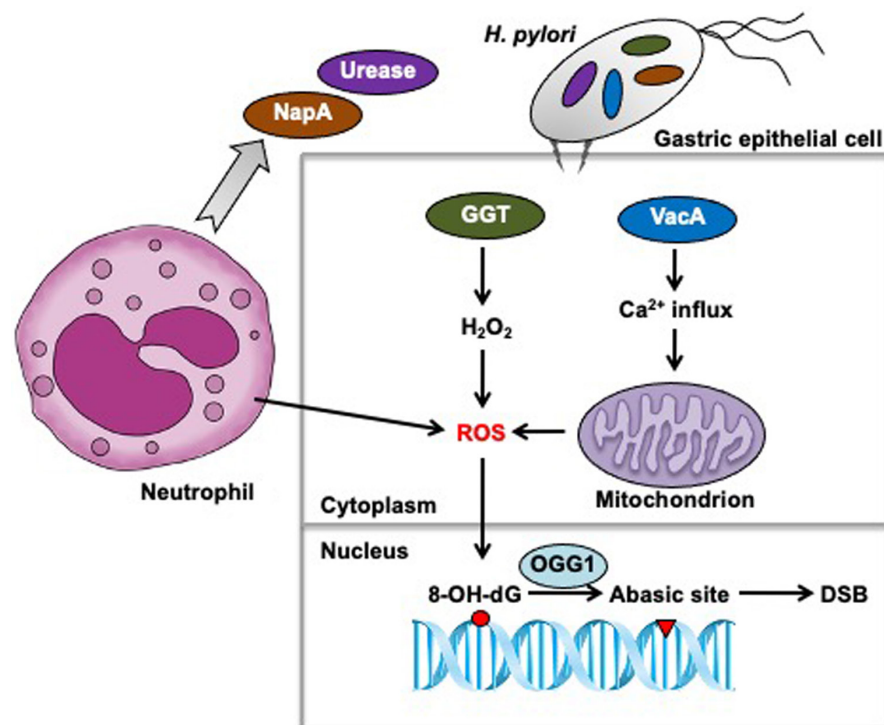

FIGURE 5 Induction of DSBs through oxidative stress induced by *Helicobacter pylori*. VacA, γ-glutamyl-transferase (GGT), urease, and NapA contribute to ROS production that causes DSBs

FIGURE 6 Impairment of the DNA damage response by *Helicobacter pylori*. The expression levels of MLH1, MSH2, and MGMT are downregulated in *H. pylori*-infected gastric epithelial cells. CagA induces *TP53* mutations through the aberrant expression of AID. CagA and VacA activate MDM2 through an AKT/ERK-dependent S166 phosphorylation. CagA/ASPP2 interaction promotes the proteasomal degradation of p53. *H. pylori* T4SS activates AP1, and thereby induces Δ133p53 and Δ160p53 that inhibit p53 and p73
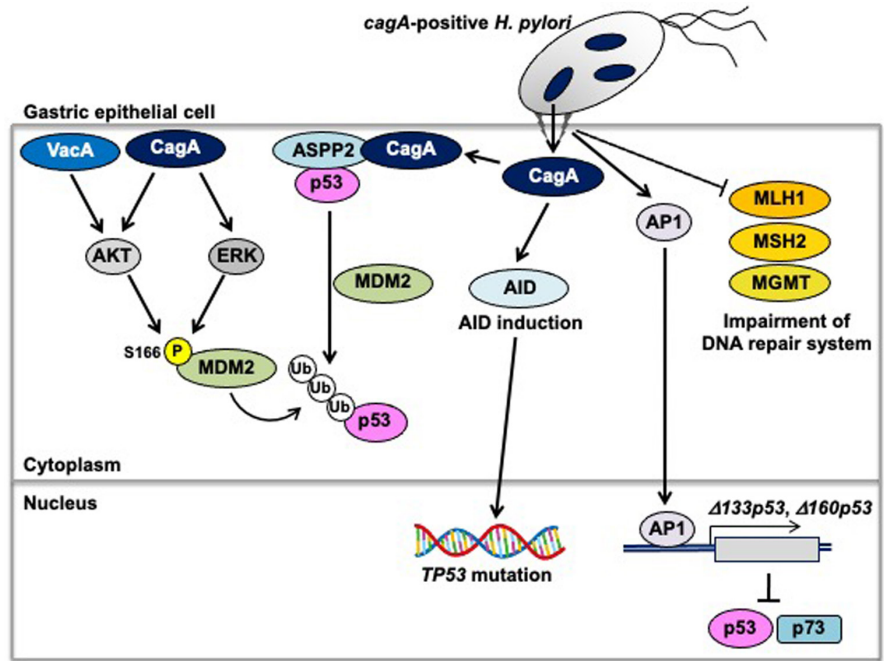
of gastric epithelial cells with *H. pylori* induces *TP53* mutations through ectopic expression of AID in an NF-κB-dependent manner.[73,74] Recent studies have further shown that *H. pylori* can induce p53 inactivation without its mutation. MDM2 activity is regulated by serine–threonine kinase ERK and AKT that phosphorylate MDM2 at Ser166.[75,76] *H. pylori* activates MDM2 through ERK/AKT activation in a CagA-dependent manner, which negatively regulates p53 by increasing ubiquitination and proteasomal degradation.[77,78] It was further reported that another virulence factor, VacA, also activates MDM2 through AKT activation.[79] In addition, CagA interacts with the human tumor suppressor apoptosis-stimulating protein of p53 (ASPP2) that functions as a proapoptotic protein by associating with and activating p53.[80] CagA/ASPP2 interaction leads to cytoplasmic retention of p53 and thereby promotes proteasomal degradation of p53.[81] Moreover, *H. pylori* activates the transcription factor AP1 and thereby inducing N-terminally truncated p53 isoforms, Δ133p53 and Δ160p53, that inhibit p53 and p73 activities and increase the survival of infected cells (Figure 6).[82]

The *TP53* gene is the most frequently mutated gene in human gastric cancer and, indeed, mutations are found in intestinal metaplasia (38%), dysplasia (58%), and intestinal-type gastric cancer (71%), indicating that the *TP53* mutation is intimately associated with this type of gastric cancer that typically arises after long-term *H. pylori* infection.[83]

6 | CONCLUDING REMARKS

Infection with *H. pylori* induces DSBs in the host genome, whereas CagA impairs HR-mediated DSB repair. *H. pylori* also inhibits p53 tumor suppressor functions, thereby preventing damage-induced apoptosis of infected cells. Conversely, *H. pylori* CagA promotes cell

proliferation through aberrant activation of SHP2.[23,25] Although CagA-transgenic mice develop gastrointestinal tumors, transgenic mice expressing a phosphorylation-resistant CagA mutant do not, indicating that CagA-mediated SHP2 activation is also important for malignant transformation of CagA-delivered cells displaying BRCAness, which greatly enhances the chance of acquiring driver gene mutation(s).[20] Therefore, both tyrosine phosphorylation-dependent and phosphorylation-independent CagA activities are critically involved in the development of gastric cancer.

Germline loss-of-function mutations in *BRCA1* or *BRCA2* cause an increased risk of breast, ovarian, and pancreatic cancers. BRCAness induces two unique mutational signatures, the COSMIC signature "SBS3," which is characterized by relatively equal distributions of single base substitutions, and a marked increase in the number of InDel signatures denoted "ID6," which is characterized by small deletions of >5 bp with extended stretches of overlapping microhomology at breakpoint junctions.[84,85] Markedly, mutational signatures revealed that intestinal-type gastric cancer exhibits the BRCAness signatures, SBS3 and ID6, despite the lack of *BRCA1/2* mutations.[85,86] Recently, we showed that sustained expression of CagA in gastric epithelial cells gives rise to SBS3 and ID6 corroborating that CagA provokes transient and reversible BRCAness in the delivered host cells, thereby causing genome instability.[46] While *H. pylori* infection can evoke genome instability in a CagA-independent manner, CagA-dependent BRCAness greatly increases the chance of driver gene mutations.

Although *cagA*-positive *H. pylori* plays a critical role in the early phase of gastric carcinogenesis, the bacterial pathogen is no longer necessary in the carcinogenic process once key driver mutations have been introduced into cancer precursor cells. It can be speculated that additional triggers and stimulants, both cell intrinsic and cell extrinsic, may also exist in the later phase of gastric carcinogenesis.

As a result, *H. pylori* CagA directs the neoplastic transformation of gastric epithelial cells through the Hit-and-Run mechanism.[87] In the CagA-mediated Hit-and-Run oncogenesis, *cagA*-positive *H. pylori* eradication is neither capable of inhibiting gastric cancer development, nor poly(ADP-ribose) polymerase (PARP) inhibitors, which are utilized for the treatment of cancer cells with BRCAness.[42,88] This does not work as the activity of BRCA1 is fully restored in the established gastric cancers in the absence of CagA delivery.

CONFLICTS OF INTEREST

The authors have no conflict interest. Masanori Hatakeyama is the Editor-in-Chief of Cancer Science.

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