Mouse Model for Tissue-Specific Oxidative Stress

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Abstract: Infections contribute to carcinogenesis through inflammation-related mechanisms. *H. pylori* infection is a significant risk factor for gastric carcinogenesis. However, the molecular mechanism by which *H. pylori* infection contributes to carcinogenesis has not been fully elucidated. *H. pylori*-associated chronic inflammation is linked to genomic instability via reactive oxygen and nitrogen species (RONS). In this article, we summarize the current knowledge of *H. pylori*-induced double strand breaks (DSBs). Furthermore, we provide mechanistic insight into how processing of oxidative DNA damage via base excision repair (BER) leads to DSBs. We review recent studies on how *H. pylori* infection triggers NF-κB/inducible NO synthase (iNOS) versus NF-κB/nucleotide excision repair (NER) axis-mediated DSBs to drive genomic instability. This review discusses current research findings that are related to mechanisms of DSBs and repair during *H. pylori* infection.

Keywords: *H. pylori*; RONS; BER; DSBs; NF-κB; NER

1. Introduction

Infection contributes to 20% of cancer worldwide [1]. *H. pylori* infection is one of the most common risk factors for gastric carcinogenesis [2]. More than 50% of the human population is infected with *H. pylori*, but few develop gastric cancer [3]. Several studies have shown that *H. pylori* infection causes chronic inflammation with different degrees of pathological severity, including chronic gastritis, peptic ulcers that eventually cause gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4–6]. Chronic gastritis that is associated with *H. pylori* infection is the first and early stage of inflammation. When accompanied by gastric epithelial cell injury, it may contribute to gastric cancer development [7–9]. *H. pylori* virulence factors that contribute to host-pathogen interaction have been characterized, which increase the risk of gastric cancer pathogenesis [4,10]. These virulence factors enhance the severity of the mucosal inflammatory response, which may largely be responsible for the virulence factor-associated increased risk of gastric cancer [10].

*H. pylori* causes chronic gastritis and contributes to genotoxic activity [11,12]. However, the molecular mechanisms by which *H. pylori* promotes genotoxic activity and the host response to genotoxic factors to drive gastric carcinogenesis require more study. Based on the current knowledge, *H. pylori* infection induces a genotoxic effect via two potential mechanisms. First, *H. pylori* infection enhances the infiltration of immune cells, including neutrophils and macrophages, to produce reactive oxygen species and nitrogen species (RONS) [13]. RONS can cause DNA base damage that leads to single strand breaks (SSBs) and the enhanced expression of oncogenes [14–16]. Alternatively, RONS activate the oxidant-sensitive transcription factor NF-κB, which induces the expression of oncogenes and cell-cycle regulators [17,18]. Activated NF-κB is translocated to the nucleus and it forms a protein complex with NER proteins (XPG and XPF) to cleave the promoter regions of the genes and cause double strand breaks (DSBs) that impact gene expression [11].
2. *H. pylori* Induces Inflammation-Dependent DNA Damage

Chronic inflammation is estimated to contribute to approximately 25% of human cancers [19]. Gastric inflammation in *H. pylori* infection may be induced via two different mechanisms. The first mechanism is initiated via physical contact between the pathogen and the host epithelial cells, producing direct cell damage or enhancing the ability of epithelial cells to release pro-inflammatory mediators (Figure 1). The second mechanism is likely promoted by *H. pylori* virulence factors (e.g., CagA, VacA) that may target the potential cell signaling pathways to stimulate immune responses. Interestingly, the *H. pylori* CagA positive strain enhances chemokine activation, such as IL-8, a potent neutrophil-activating chemotactic cytokine or chemokine [20,21]. Furthermore, chemokines that are released from infected gastric epithelial cells can stimulate neutrophil infiltration and T lymphocytes to enhance RONS-mediated gastritis [22,23]. Overall, *H. pylori*-mediated gastric inflammation is associated with humoral and cell-mediated immune cells.

![Figure 1](image-url)

**Figure 1.** Molecular mechanisms of *H. pylori*-induced double strand breaks (DSBs). Schematic representation of how *H. pylori* induces DSBs. *H. pylori* infection causes DNA damage in gastric epithelial cells [24]. *H. pylori*-host cell interaction is a prerequisite for DSBs [25] (top panel). Persistence of the host-bacterium interaction leads to chronic inflammation and the release of inflammatory cytokines and chemokines, which contribute to oxidative DNA damage that is processed via base excision repair (BER) pathways (bottom panel). Processing oxidative DNA damage by DNA glycosylase (e.g., OGG1, NEIL1, etc.) contributes to accumulation of apurinic/apyrimidinic (AP) sites that are eventually converted to DSBs [26]. In addition, some cytokines (e.g., TNF-α) inhibit BER proteins to exacerbate genomic instability. The second pathway associated with *H. pylori*-mediated NF-κB activation leads to formation of a protein complex with nucleotide excision repair proteins (XPF and XPG), cleaves the promoter regions, and alters gene expression [11] including HR DNA repair proteins (Rad51). Alternatively, NF-κB/iNOS-mediated NO production leads to DNA damage and/or inhibits DNA repair proteins (AAG) that likely impact BER and cause DSBs. Note that solid arrow and dot arrow shows activation and alternative avenue for the down stream events respectively; T bar shows inhibition or suppression of protein activity or gene expression.
3. H. pylori Induces Base Excision Repair (BER) Intermediate-Dependent Double Strand Breaks (DSBs)

Chronic inflammatory conditions induce immune and epithelial cells to release RONS, which are capable of causing DNA damage and persistent cellular proliferation [27]. In addition, RONS accumulation may result in proto-oncogene activation, chromosomal aberrations, and DNA mutations [28,29]. There is considerable evidence that H. pylori itself induces genomic instability and epigenetic alteration in the host genome. However, there is little experimental evidence to provide mechanistic insight into how oxidative DNA damage leads to DSBs and how oxidative-damaged DNA processed via BER (Figure 1), which is thought to be the primary repair pathway against oxidative DNA damage [30]. The mechanism of H. pylori-induced host genomic instability remains poorly understood.

BER is crucial for maintaining genomic stability to prevent carcinogenesis [31–34]. BER is a major DNA repair pathway that removes the majority of oxidative and alkylating DNA damage without affecting the double helix DNA structure [30,35,36]. BER is the primary repair pathway of RONS-induced DNA damage during inflammation that occurs during H. pylori infection [37] (Table 1). Tight coordination of the different steps in BER is necessary to avoid genomic instability [38]. BER is initiated by the recognition and excision of the damaged base by specific DNA glycosylases. For example, the best characterized 8oxoG DNA lesions paired with cytosine are recognized and excised by bifunctional OGG1 glycosylase [39–42]. Subsequently, OGG1 remains bound to its abasic site (AP) and its turnover is stimulated by apurinic/apyrimidinic endonuclease1 (APE1) [43,44]. After AP site processing and end-remodeling, the single-nucleotide gap is filled by Pol β, and the nick is sealed by DNA ligase I to complete repair [45,46]. H. pylori can alter DNA repair gene expression and/or interfere with DNA repair activity [26,47,48]. Ding et al. reported live H. pylori upregulated APE1 expression in cultured gastric adenocarcinoma cell lines (AGS) and gastric epithelial cells that were isolated from uninfected human subjects [49]. Overexpressed APE1 likely interacts with other redox proteins to suppress ROS production [50]. In addition, Taller et al. show that coculture of H. pylori with gastric cancer cell lines induces DSBs in a contact-dependent manner [12]. DSBs in those cell lines lead to the activation of the ATM-dependent DNA damage response. H. pylori-induced DSBs likely cause chromosomal aberrations, such as deletions, insertions, and translocations, which are a major cause of the loss of heterozygosity.

Repair of oxidative DNA damage is critical for suppression of inflammation-associated carcinogenesis. However, host BER insufficiency caused by genetic polymorphism or loss of repair capacity likely exacerbates RONS-mediated DNA damage and cancer development [51–53] (Table 1). In addition, altered function of BER proteins causes aberrant function, including the processing of H. pylori-induced oxidative DNA damage that leads to SSBs [54] and mutation [47,48]. In vivo studies have shown that H. pylori infection in an OGG1 knockout mouse model enhances accumulation of 8oxoG DNA lesions and promotes resistance to inflammation [55–57]. In addition, loss of DNA glycosylase, such as MYH and alkyladenine DNA glycosylase (AAG), causes the accumulation of oxidative DNA damage lesions and promotes inflammation-associated tumor development [37,58,59]. H. pylori infection activates other BER proteins, such as PARP1 and enhances the inflammatory response, suggesting that the bacterium modulates the host PARP1 status to drive inflammation-associated gastric cancer [60]. However, cell culture experiments have shown that OGG1 downregulation in gastric epithelial cells decreases the formation of AP sites and suppress DSBs formation [26]. However, silencing of APE1 as part of the BER machinery failed to cause a significant level of H. pylori-induced DSBs [11].
Table 1. Interplay between *H. pylori* and relevant DNA repair gene products.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role of Gene Products</th>
<th>Interplay between <em>H. pylori</em> &amp; Gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER</td>
<td>OGG1 removes 8oxoG and FapyG DNA lesions</td>
<td>absence causes increased mutation frequency, fewer DSBs and decreased inflammation</td>
<td>[26,55,61]</td>
</tr>
<tr>
<td></td>
<td>NEIL1 removes 8oxoG and Tg lesions</td>
<td>decreases mRNA in tumor; unknown role during infection</td>
<td>[34]</td>
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<td></td>
<td>APE1 acts as a negative regulator of ROS and enhances chemokine release</td>
<td>enhances the expression of mRNA and protein</td>
<td>[49,62,63]</td>
</tr>
<tr>
<td></td>
<td>POLB removes 5'-dRP group and adds a single nucleotide base</td>
<td>infection does not affect gene expression and protein level</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>XRCC1 scaffold protein enhance ligation</td>
<td>downregulated via promoter hypermethylation</td>
<td>[64,65]</td>
</tr>
<tr>
<td>NER</td>
<td>XPG cuts the 3’ of the DNA damage site; forms complex with NF-κB and promotes target gene expression</td>
<td>moderates change in gene expression</td>
<td>[11,66,67]</td>
</tr>
<tr>
<td></td>
<td>XPF forms complex with NF-κB &amp; promotes targeted gene expression</td>
<td>moderates change in gene expression</td>
<td>[11,66]</td>
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<tr>
<td></td>
<td>XPA recognition bulk DNA adduct</td>
<td>increases IL-8 cytokine expression</td>
<td>[11,66]</td>
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<td>NHEJ</td>
<td>DNA-PK increases cellular proliferation &amp; facilitates NHEJ (nonhomologous DNA end-joining) repair</td>
<td>enhances activity and expression</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>Ku70/80 protects DNA DSB ends and prevents cell death</td>
<td>decreases protein level</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td>DNA ligase IV completes NHEJ repair by sealing DNA DSB regions</td>
<td>knock-down enhances DSBs</td>
<td>[11]</td>
</tr>
<tr>
<td></td>
<td>XRCC4 scaffold to hold DNA DSBs ends to enhance ligation</td>
<td>knock-down promotes DNA DSBs</td>
<td>[11]</td>
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<tr>
<td>HR</td>
<td>NBS1 DNA DSB end processing/DDR</td>
<td>decreases expression and may impair DNA end processing and DDR</td>
<td>[66]</td>
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<td></td>
<td>Rad51 strand exchange and enhances DSB repair</td>
<td>decreases gene expression; however, infection does not increase DSBs</td>
<td>[25]</td>
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<td></td>
<td>RPA1 ssDNA binding and DDR</td>
<td>downregulates mRNA</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>Mre11 DSB end processing and DDR</td>
<td>decreases expression and impairs end processing and DDR</td>
<td>[66]</td>
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</table>

DSBs are the principle cytotoxic lesions generated by *H. pylori* infection. DSBs can be caused by the accumulation of unrepaired BER intermediates in DNA replication independently and/or arise when DNA replication forks encounter BER intermediates including DNA SSBs [70,71]. Few studies have shown that accumulation of AP sites in *H. pylori*-infected human gastric epithelial cells leads to DSBs [26]. Toller et al. [12] reported that a direct bacterium-host interaction is a prerequisite to DSBs, rather than the release of DNA-damaging components. Overall, these results suggest that DSB formation is mediated by BER intermediates that are generated from a direct response of the host-bacterium interaction (Figure 1).
4. NF-κB-iNOS Axis-Dependent DSB Formation

*H. pylori* infection induces DNA damage in gastric epithelial cells [24]. Contact-dependent interactions between *H. pylori* bacteria and gastric epithelial cells activate intracellular signaling events that have further downstream effects via activation of the transcription factor NF-κB [72]. NF-κB activation is effected through a series of phosphorylation and transactivation events, triggering a downstream signaling pathway that contributes to gastric inflammation in *H. pylori*-infected individuals [73,74]. *H. pylori*-mediated NF-κB activation leads to the upregulated expression of a variety of inflammatory mediators, including IL-8 [75], and regulates genes that govern the innate and adaptive immune response [76,77]. However, aberrant NF-κB activation has been reported to function as a tumor promoter in inflammation-associated cancer [78,79].

Moreover, the host response to *H. pylori* infection enhances NF-κB activation in immune and epithelial cells, resulting in inducible nitric oxide synthase (iNOS) [80–84]. iNOS is an inflammatory mediator that causes the production of nitric oxide (NO) by immune cells, such as macrophages, linking chronic inflammation and tumorigenesis [85–87]. iNOS is expressed in response to bacterial endotoxins and cytokines and leads to NO production that enhances carcinogenesis [88]. iNOS-mediated NO induces oxidized DNA and leads to mutations associated with the infection [89,90] and DSBs [91]. Furthermore, NO has a biphasic effect on NF-κB to exert both pro- and anti-inflammatory actions. Although the ability of NO to directly damage DNA has been studied to a limited degree [92], its role in promoting potentially mutagenic changes in DNA has received far less attention. NO prevents NF-κB transactivation via the stabilization of IκBα [93] and nitrosate, a specific cysteine residue on the p50 subunit of NF-κB that reduces its DNA-binding capacity [94,95]. Few studies have shown that NF-κB plays a significant role in inhibition of pathogen-induced apoptosis in immune cells [96], suggesting that NF-κB may play a pro-inflammatory role to induce persistent macrophage activation. Other studies have shown that DNA repair proteins that are involved in BER and SSBs (PARP1) interact with NF-κB to facilitate the interactions with DNA to promote the expression of pro-inflammatory cytokines and enhance the activity of iNOS [97–99].

iNOS-mediated NO enhances inactivation of DNA repair enzymes that eventually contribute to genomic instability, leading to cancer development [86]. NO can nitrosylate thiol and tyrosine residues of the DNA repair proteins, causing loss of their function [100,101]. Thus, determining the effect of iNOS-generated NO on DNA repair proteins is scientifically important to uncover the impact of *H. pylori*-triggered iNOS-mediated DNA repair defects (Figure 1). Few studies have shown that DNA repair proteins are vulnerable to oxidative damage from NO because of their active sites, such as sulphydryl, tyrosine, and phenol side chains [102]. DNA repair enzymes, such as MGMT, FpyG, and PARP may be inactivated by NO-mediated nitrosylation of the cysteine-rich residues of the active site [103,104]. The integrity of the genome may be challenged during exposure to high concentrations of NO by direct oxidative damage to DNA and by inhibiting the DNA repair capacity of the enzyme (Table 1).

5. NF-κB-Nucleotide Excision Repair (NER) Axis-Dependent DSB Formation

*H. pylori* infection increases NF-κB activation to promote the inflammatory immune response [105]. NF-κB modulates many DNA repair genes to facilitate repair and generate DNA DSBs [106,107]. Endonucleases XPF and XPG are critical components of NER that are responsible for excising the damaged DNA strand to remove the DNA lesion. The endonuclease XPG cuts the DNA strand approximately 5–6 nucleotides downstream of 3' of the DNA damage site. In addition, the ERCC1-XPF protein complex performs an incision of the DNA strand 20–22 nucleotides upstream of the 5' end of the DNA [108,109]. Although these two endonucleases are recruited and form complexes with NF-κB to make preincision complexes, proper assembly of all the factors seems to be required for dual incision at the promoter region of a given gene. XPF/XPG-mediated DSBs amplify NF-κB target inflammatory gene expression and promote host cell survival [11]. The NF-κB complex in XFG and XPF in the formation of the active DSBs at the chromatin region of the genome likely promotes a hub that controls
gene expression (Figure 1). Furthermore, the expression level of XPG is significantly associated with an *H. pylori*-positive sample [110] (Table 1). However, silencing XPG strongly reduced the DSBs upon *H. pylori* infection, suggesting that NER-dependent DSBs contribute to genomic instability during infection. *H. pylori* infection modulates NER and enhances the interaction with NF-κB, likely providing a molecular basis for insights into how *H. pylori* infection induces transcription-associated DSBs (Figure 1).

6. *H. pylori* Impairs DSBs Repair

DSBs can be repaired via two major repair pathways [111,112]. Homologous recombination (HR) requires sequence homology of extensive DNA regions from an undamaged sister chromatid or homologous chromosomes. In contrast, nonhomologous DNA end-joining (NHEJ) occurs throughout the cell cycles and is processed without any sequence homology or few end homology sequences. *H. pylori*-induced DSBs are likely recognized by the MRE11-RAD50-NBS1 (MRN) complex [113,114], which is recognized and processed the DNA ends, resulting in the activation of ataxia telangiectasia mutated kinase (ATM) [115,116]. ATM is a major DNA damage response sensor of DSBs. It directly binds to the damaged DNA and phosphorylates target proteins, including H2AX protein at serine 139 of the histone (γH2AX) to mark DSBs sites [116–119]. Alternatively, ATM is involved in mediating the NF-κB response to DSBs [120].

In the NHEJ pathway, DNA-dependent protein kinase (DNA-PK) and Ku proteins play key roles in mediation of incompatible DNA ends. DNA-PK may function as a DNA damage sensor or scaffolding to assemble repair proteins including Ku proteins to bind the two ends of the break together. Then, ligase IV/XRCC4/XLF carries out the ligation reaction [121] to complete NHEJ repair. However, *H. pylori* causes an increase in Ku70/80, which may indicate that NHEJ-mediated repair contributes to genomic instability [69]. Recent evidence has shown that altered DNA-PK and Ku70/80 are associated with pathological processes in different types of cancer [122]. Moreover, Ku70 and DNA-PK are expressed in *H. pylori*-associated gastritis, intestinal metaplasia and gastric adenoma tissues [68]. Furthermore, Lim et al. [123] showed that activated NF-κB-Cox2 axis plays a significant role in enhancing the expression of KU70/80. In contrast, the loss of Ku proteins leads to an accumulation of DNA damage that eventually causes cell death in gastric epithelial cells [124].

Our previous study shows that DSBs significantly increase in the G1 stage of the cell cycle after *H. pylori* infection [26], suggesting that NHEJ repair might be involved in promotion of error-prone repair. When DSBs are generated during the S phase at DNA replication forks or after replication in the G2 phase of the cell cycle, HR may contribute to genome integrity (Figure 1). Few studies have shown that NF-κB interacts with HR proteins (e.g., CtIP-BRCA1 complexes) to stabilize BRCA1 and stimulate HR-mediated repair [125] Activation of multiple molecular targets by NF-κB enhances rapid activation of HR and may permit the accelerated proliferation of cells. However, *H. pylori* infection-mediated activation of the NF-κB/NER axis causes defects in HR that reduce the ability of DSB repair [11]. *H. pylori* infection modulates DSB repair efficiency to exacerbate genomic instability and facilitate gastric carcinogenesis.

7. Summary

*H. pylori* infection is a contributing factor for gastric cancer. This review highlights how *H. pylori*-associated DNA base damage in infected host cells is likely processed via BER to generate DSBs. In addition, this review provides a comprehensive overview of how *H. pylori*-associated DSBs are induced via the NF-κB/NER axis and NF-κB/iNOS axis, influencing DNA repair gene expression and enhancing genomic instability and carcinogenesis (Figure 1). Several studies have shown that *H. pylori* induces DSB and promotes gastric carcinogenesis. Host BER, NER, and NHEJ genetic variants could modify the process of carcinogenesis in *H. pylori* infected hosts. Alteration of the activity of enzymes that function in DNA repair as a result of genetic mutation could significantly impact gastric cancer risk. Other studies are needed to uncover the associations of the BER, NER and NHEJ genetic
variants with increased susceptibility to gastric cancer in *H. pylori*-infected hosts. In addition, many questions remain regarding the mechanism of DSB formation and how breaks are processed via the NHEJ or HR pathways. Does *H. pylori* infection decrease tumor latency for carriers of BER variant genotypes? Future studies will likely explore how *H. pylori* manipulates host DNA repair genetics and how NHEJ processes DSBs. Therefore, the relevant DNA repair of the host genetics and *H. pylori* infection status of the host should be considered in studies of gastric cancer susceptibility in the future.

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