Molecular Mechanisms of Gastric Cancer Initiation and Progression by *Helicobacter pylori*

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Abstract

Purpose of Review—Infection with the Gram-negative, microaerophilic pathogen *Helicobacter pylori* results in gastric cancer (GC) in a subset of infected individuals. As such, *H. pylori* is the only World Health Organization classified bacterial class I carcinogen. Numerous studies have identified mechanisms by which *H. pylori* alters host cell signaling pathways to cause disease. The purpose of this review is to highlight recent studies that explore mechanisms associated with induction of GC.

Recent Findings—Over the last year and a half, new mechanisms contributing to the etiology of *H. pylori* associated GC development have been discovered. In addition to utilizing the oncogenic CagA toxin to alter host cell signaling pathways, *H. pylori* also induces host DNA damage and alters DNA methylation to perturb downstream signaling. Furthermore, *H. pylori* activates numerous host cell pathways and proteins that result in epithelial-to-mesenchymal transition (EMT) and induction of cell survival and proliferation.

Summary—Mounting evidence suggests that *H. pylori* promotes gastric carcinogenesis using a multifactorial approach. Intriguingly, many of the targeted pathways and mechanism show commonality with diverse forms of cancer.

Keywords

Helicobacter pylori; gastric carcinogenesis; PI3K/Akt; DNA damage and methylation; epithelial-to-mesenchymal transition

INTRODUCTION

Given its association with gastric cancer (GC), the World Health Organization classified *Helicobacter pylori* as a class I carcinogen in 1994; to date, *H. pylori* remains the only bacterium with this distinction [1, 2]. This Gram-negative, microaerophilic bacterium persistently colonizes the gastric mucosa of the majority of the population. While, infections with *H. pylori* are frequently asymptomatic, they are also associated with gastritis, peptic

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ulcers, and in the most severe form, GC. Currently, GC is the fifth most common cause of cancer worldwide with a case fatality rate of 75%; these statistics make GC the third most common cause of cancer-related death [3]. The most recent reports from the International Agency for Research on Cancer (IARC) estimates that 78% of all gastric cancers are attributable to *H. pylori* infection [2]. Furthermore, seroprevalence studies estimate that 90% of GC patients have encountered *H. pylori* [4]. Given the striking association between *H. pylori* and GC, decades of research has sought to determine the molecular mechanisms underlying *H. pylori*-associated cancer development. Indeed, numerous molecular pathways have been identified as targets of this pathogen (reviewed in [5, 6]). Recent reports have revealed several *H. pylori*-targeted pathways that are also altered in various forms of cancer. Thus, a greater understanding of *H. pylori*-associated cancer development may shed insight into other cancer types. Herein, we highlight recent studies that explore *H. pylori*'s ability to induce DNA damage and aberrant methylation patterns that effect downstream host cell signaling. Additionally, we highlight recent discoveries that demonstrate how *H. pylori* initiates epithelial-to-mesenchymal transition (EMT) and potentiates pro-survival and proliferation signals that contribute to the cancer phenotype.

H. pylori induces DNA Damage and Aberrant DNA Methylation

Genome instability and mutation are emerging hallmarks of cancer development [7]. These genomic changes result from direct DNA damage, DNA repair failure, or via epigenetic mechanisms such as DNA methylation [7]. *H. pylori* targets each of these pathways: aberrant methylation [8], decreased expression of mismatch repair genes [9], increased expression of activation-induced cytidine deaminase (AID) [10], and induction of double strand DNA breaks (DSBs) [11**].

While the concept of *H. pylori*-induced DNA damage is not new, the mechanisms underlying these processes remain poorly defined [11**]. Two recent studies further characterize the molecular mechanisms behind *H. pylori* associated DNA damage [11**, 12*]. From a global perspective, distinct DNA damage patterns, which involve accumulation of DSBs in regions of active transcription and near telomeres, occur in response to *H. pylori* infection [12*]. Moreover, *H. pylori*-induced damage patterns are similar to structural variations found in gastric cancers [12*, 13]. Strains lacking the *cag*-pathogenicity island (*cag*-PAI) are impaired in their ability to induce DSBs [12*], suggesting a role for the type IV secretion system (T4SS) in this process. This suggestion is supported by additional work which shows that the DSBs occur in an NF-κB dependent manner [11**]. A molecular model has been proposed that suggests that binding of the T4SS pili to β_1 integrin triggers activation and translocation of NF-κB to the nucleus where it serves as a chromatin binding platform for RelA, the NER endonucleases XPG and XPF, and recruitment factor XPA [11**]. XPA and XPF then induce DSBs that result in enhanced expression of NF-κB-dependent anti-apoptotic genes that promote cell survival [11**]. Taken together, these studies suggest that the DSBs induced by *H. pylori* are unique and perhaps directed by this pathogen.

Another mechanism by which *H. pylori* contributes to genomic instability is via manipulation of host gene methylation [8]. In this regard, DNA methylation is increased in

the gastric antrum of *H. pylori* infected patients as compared to uninfected patients [14]. DNA methylation is also increased in the "normal tissue" surrounding the lesions [15**]. The role of aberrant DNA methylation in adverse disease outcome is supported by a 16-year cohort study that showed that changes in specific gene methylation can serve as predictors for gastric cancer progression [16]. Additionally, several studies have delineated the downstream consequences of altered gene methylation. For example, *H. pylori* has a profound effect on N-Myc downstream-regulated gene 2 (*NDRG2*) promoter methylation; methylation was seen in 54% of primary gastric cancer specimens [17*]. NDRG2 is a potential tumor suppressor that aids in dephosphorylation of the phosphatase and tensin homolog (PTEN) [18]; PTEN is frequently found inactivated in gastric cancers due to increased levels of C-terminal phosphorylation [19]. In the active, unphosphorylated form, PTEN dephosphorylates PIP3, which results in decreased PI3K/Akt activity [18, 20]. Thus, as methylation increases, expression of *NDRG2* decreases, PTEN C-terminal phosphorylation increases, and suppression of the PI3K/Akt pathway is relieved [17*, 20]. It is currently thought that the up-regulation of DNA methyltransferase 3b (DNMT3B), which is correlated with *H. pylori* infection, is responsible for the increase in *NDRG2* promoter methylation [17*].

DNA methylation also impacts micro-RNA (miRNA) regulation in chronic inflammatory diseases and in cancers [15**, 21]. In *H. pylori* positive patients with gastric disease, aberrant methylation of the CpG island in the miR-210 promoter occurs. This methylation results in a decrease in miR-210, which leads to an increase in expression of miR-210 pro-proliferation targets such as stathmin1 (STMN1) and demethyladensosine transferase 1 (DIMT1) [15**]. *H. pylori* also stimulates miRNA promoter hypermethylation via cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2) activation [21]. PGE2 promotes methylation in tumor cells, and in the case of *H. pylori* infection, miR-149 is a target of PGE2 induced DNA methylation. miR-149 targets and suppresses IL-6 and the PGE2 receptor genes. Thus, methylation of miR-149 results in a decrease in miR-149 and an increase in IL-6. Downstream consequences of IL-6 induction include EMT, stem-like traits, and invasive properties [21]. Interestingly, IL-6 regulates the cancer cell environment by activating the COX-2/PGE2 pathway and by regulating miRNAs [21]. *En masse* the data suggest that *H. pylori* regulates DNA damage and DNA methylation in a manner that promotes tumorigenesis via enhanced genome instability, and via the activation of cell signaling pathways that promote cellular proliferation, EMT, and stemness, [15**–17*].

Role of *H. pylori* in EMT

One process that has taken a prominent role in cancer etiology research is EMT. EMT is critical to the developmental process; however, EMT also plays a physiopathological role in epithelial tumorigenesis [22]. During EMT, epithelial cells loose properties required to maintain a healthy cellular barrier and instead acquire mesenchymal cell like properties: loss of cellular interactions, loss of polarization, and increased mobility [22]. Several recent studies link infection with *H. pylori* to EMT-like changes in gastric epithelial cells [23–26]. Indeed, molecular classification of GC tumors identified a cancer subtype associated with EMT [27]. This EMT subtype occurs at a younger age than the other subtypes, is associated with poor prognosis, and has a higher chance of recurrence [27]. Another clinical study

found decreased expression of the epithelial marker E-cadherin and increased expression of the mesenchymal markers SNAIL, TWIST, SLUG, and vimentin in tissue biopsies from *H. pylori* positive GC patients [24*]. Eradication of *H. pylori* results in a reversal of marker expression; E-cadherin increases while SNAIL, TWIST, SLUG, and vimentin decrease [24*]. Other studies also document EMT associated changes in gastric epithelial cells after *H. pylori* infection and identify associated key players in the process [23, 25*, 26]. For example, an association between CD44 and *H. pylori* induced EMT has been shown; increased CD44 is associated with a corresponding increase in mesenchymal markers [23, 24*]. While neither of these studies show a direct interaction between *H. pylori* and CD44, *H. pylori* may affect CD44 via suppression of miR-328, which is a known negative regulator of CD44 expression [28]. Interestingly, mir-328 expression is suppressed in response to H_2O_2 [28], suggesting that the gastric mucosal inflammation associated with *H. pylori* infection may increase host cell changes that further promote EMT.

The multipotent virulence factor, CagA, is also directly linked to *H. pylori*'s induction of EMT and cell proliferation via CD44 [29, 30]. Both *in vivo* and *in vitro* models demonstrate that CD44, c-Met, and CagA form a complex, which leads to increased cellular proliferation, an increase in mesenchymal markers (α-SMA, SNAIL2, TWIST1, N-cadherin, and ZEB1), and disruption of membrane associated E-cadherin [29]. The increase in mesenchymal markers, CD44, and matrix metalloproteinases occurs in a CagA phosphorylation-dependent manner [30]. Similarly, transfection with CagA leads to an increase in TWIST and vimentin with a corresponding decrease in E-cadherin [23]. This later decrease in E-cadherin appears to be tied to the downregulation of programmed cell death factor 4 (PDCD4), suggesting that CagA influences EMT through multiple pathways [23].

The factors that that are commonly used as mesenchymal or epithelial markers have diverse mechanistic roles during EMT. For instance, the aforementioned decrease and disruption of E-cadherin contributes to the weakening of cell-to-cell junctions, a hallmark of EMT. Interestingly, the histone demethylase RBP2 also plays a role in regulating E-cadherin; RBP2 forms a complex with p-SMAD3 on the E-cadherin promoter to suppress E-cadherin expression [26]. These types of junctional effects are a common theme of GC and *H. pylori* infection. The gap junction protein, connexin-43 (Cx43), is perturbed during *H. pylori* infection; *H. pylori* induces a GATA3 dependent decrease in Cx43, which leads to weakening of gastric epithelial integrity [31]. An additional study suggests that expression of Cx43 renders cells more susceptible to VacA induced cell death [32]. Combined, these data suggest a duel mechanism by which *H. pylori* generates a cell population with reduced levels of Cx43; *H. pylori* causes Cx43 down regulation, while VacA induces cell death in any cells that maintain Cx43. The end result is a defective gastric epithelial maintenance program and perhaps an increased propensity towards EMT.

Unlike the structural proteins, Cx43 and E-cadherin, SNAIL and TWIST function as transcription factors that control downstream effectors that contribute to EMT. For example, SNAIL acts as a transcriptional repressor of the gene encoding the junctional protein claudin-7 [25*]. Addition of *H. pylori* to AGS cells or a gastroid model leads to an increase in SNAIL with a corresponding decrease in claudin-7; this interaction is β-catenin dependent [25*]. Mechanistically, CagA binding to GSK-3 decreases GSK-3 activity, subsequently

increasing SNAIL stability and the active, cytoplasmic form of β-catenin [33]. *En masse*, current data suggest that *H. pylori* utilizes multiple mechanisms to disrupt epithelial adhesins as a means to contribute to EMT.

Role of cell proliferation in gastric carcinogenesis

As mentioned, the interaction between CagA and β-catenin plays a role in disruption of cell-to-cell junctions, which strongly suggests that β-catenin directly or indirectly contributes to EMT. Additional evidence supports CagA-dependent, nuclear accumulation of β-catenin, which results in increased pro-proliferation gene expression; several mechanisms have been implicated in orchestrating this outcome [34–36]. Recently, the multi-functional glycol-phosphoprotein, osteopontin (OPN), has emerged as another intermediary in the CagA-dependent activation of β-catenin. Intracellular OPN increases in a CagA and T4SS-dependent manner in response to *H. pylori* infection [37*]. Furthermore, siRNA knockdown of OPN abolishes the *H. pylori*-dependent nuclear accumulation of β-catenin [37*]. The downstream effects of the OPN increase and subsequent β-catenin nuclear localization include increases in c-Myc and cyclin D1 (*CCDN1*), both of which are known cellular proliferation contributors. These *in vitro* studies are supported by mouse models that show that the loss of OPN suppresses oncogenic changes such as inflammation, epithelial proliferation, and decreased apoptosis [38, 39].

Another major cell proliferation pathway activated during *H. pylori* infection is PI3K/Akt. The importance of this pathway is evidenced by the dual mechanisms of regulation by *H. pylori*; infection enhances cell proliferation through PI3K/Akt and suppresses proteins that inhibit PI3K/Akt activation. As mentioned above, PTEN is maintained in an inactive state following suppression of NDRG2 [17*, 18]. As an additional control mechanism, *H. pylori* infection also leads to an increase in miR-221/222 expression; both of these miRNAs target PTEN as well as the tumor suppressor, RECK [40]. A third component altered in the PI3K/Akt pathway is PDK-1. Early in infection, *H. pylori* dephosphorylates PDK-1, which results in dephosphorylation of Akt and induction of apoptosis; apoptosis is followed by an increase in cellular proliferation of the surviving cells [41]. Lastly, CagA induces activation of SP1, a downstream protein in the PI3K/Akt pathway, via Akt [42]. SP1 serves as a transcription factor for RBP2; RBP2 binds to the *CCDN1* promoter, which results in upregulation of cyclin D1 [42]. Thus, the role of RBP2 appears two-fold; RBP2 induces EMT and stemness, as well as cellular proliferation [26, 42].

H. pylori exploits numerous targets to foster an environment geared for enhanced cellular proliferation and survival. In addition to activating pathways that promote these features, *H. pylori* also suppresses mechanisms meant to keep these pathways in check. For instance, *H. pylori* modulates several miRNAs to affect cellular proliferation. Among these, miR-203 is downregulated in *H. pylori* positive tissue samples [43]. This decrease results in a corresponding increase in the PIK3CA and CASK targets, which in turn leads to enhanced cellular proliferation and invasion [43, 44]. Similarly, miR-101 is downregulated in *H. pylori* infected GC tissues [45]. Decreased levels of miR-101 results in the upregulation of SOCS2 and subsequent deregulation of the cell cycle, promoting tumorigenesis [45]. Other miRNAs downregulated during *H. pylori* infection, including miR-375 and miR101b, further

potentiate carcinogenic signals [46]. Thus, alteration of miRNA targets as a mechanism to promote cell survival and proliferation appears to be a broad theme employed by *H. pylori*.

Finally, *H. pylori* also has the ability to negatively influence numerous tumor suppressors. For example, gastrokine 1 has the ability to inhibit CagA-induced cell proliferation and EMT by directly binding to CagA; this interaction prevents CagA interaction with SHP2 and E-cadherin [47]. However, *H. pylori* overcomes this effect via downregulation of gastrokine 1 in GC tissues. This is a CagA-mediated event and the resulting decreased gastrokine 1 levels result in activation of NF-κB and PI3K/Akt [47]. An additional tumor suppressor example can be found with trefoil factor 1 (TFF1), which inhibits β-catenin and NF-κB-p65 nuclear translocation via negative regulation of Akt/GSK-3β. This in turn prevents an increase in *H. pylori*-induced inflammation and pro-proliferation signals [48–50]. While TFF1 can be protective, it is downregulated or lost in almost half of all adenocarcinomas [48]. Therefore, loss of TFF1 tumor suppressor activity allows for unchecked activation of β-catenin and cell proliferation through activation of c-Myc and cyclin D1 [49]. Clearly, *H. pylori* utilizes a multifaceted approach to induce host cell proliferation.

CONCLUSIONS

While *H. pylori* was identified as a carcinogen over two decades ago, researchers are still elucidating novel oncogenic mechanisms used by this fascinating pathogen. Indeed, increasing evidence indicates that *H. pylori* can influence numerous host pathways to promote cancer development. Though all of the mechanisms are unclear, evidence suggests that as *H. pylori* establishes infection, the subsequent host response creates an environment conducive to tumorigenesis. Remarkably, the host cell changes undergone in response to *H. pylori* infection (Figure 1) involve several hallmarks of traditional cancer progression. Included among these are DNA damage and methylation, activation of EMT pathways, activation of pro-proliferative/anti-apoptotic effectors, and suppression of tumor suppressors. *H. pylori* is a clearly a cunning pathogen that has the ability to directly target universal cancer pathways as a means to induce gastric carcinogenesis.

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KEY POINTS

- Known oncogenic pathways are targeted by *H. pylori* through the activation of upstream signaling components or through inhibition of known tumor suppressors.

- Recent work highlights the importance of activation of the PI3K/Akt axis in *H. pylori* induced cancer development.

- *H. pylori* has the ability to alter the human genome through DNA damage, methylation, and regulation of micro-RNAs.

- *H. pylori* initiates epithelial-to-mesenchymal transition leading to loss of cell-cell junctions and induction of cellular proliferation.
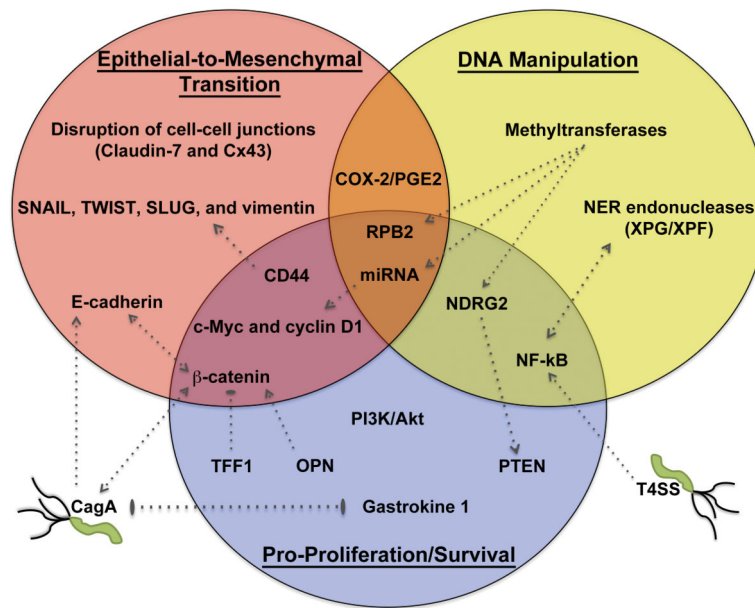
Figure 1. *H. pylori* activation of cancer pathways
Recent literature highlights *H. pylori's* ability to activate EMT (top left), manipulate host DNA (top right), and initiate cell pro-survival/proliferation signals (bottom center). Key components in each pathway are indicated within each circle. Stimulation of these pathways occurs primarily through interactions with proteins that serve overlapping functions in multiple pathways (overlap regions). Where appropriate, arrows indicate activation signals, blunt ended lines indicate inhibitory signals, and double-ended arrows or lines indicate reciprocating events between two proteins. Also indicated are direct interactions between host cell and bacterial factors.