Helicobacter pylori and the Development of Gastric Cancer

Abstract

Helicobacter pylori infects more than half of the world's population, and its CagA virulent strain induces 63% of the world's gastric cancer cases. Although this gram-negative stomach pathogen is easily eradicated using antibiotics, antibiotic resistance is becoming more common and poses a significant concern for the future of all antibiotic-treated diseases. Due to this rise in antibiotic resistance, it is important to understand the mechanisms in which CagA-positive *H. pylori* induces gastric cancer in order to effectively prevent or treat *Helicobacter pylori*-induced gastric cancer that does not rely on the use of antibiotics. This mini review presents an overview of the mechanisms in which *H. pylori* causes gastric cancer. Although some of this research has led to half-hearted public funding for the development of a vaccine, integration of CagA-positive *H. pylori* with more recent groundbreaking studies such as Nelson et al.'s molecular labeling of *Staphylococcus aureus* (2010), or Mullis et al.'s targeting of *Bacillus anthracis* using a present condition of the body's adaptive immune response (2013) may prove more successful in developing an effective gastric cancer treatment.

Introduction

Helicobacter pylori is a gram-negative spirochete or bacillus-shaped bacteria that colonizes the stomach's mucosal lining of more than half of the world's population (Lamb and Chen, 2013). While many who are infected remain asymptomatic, others develop inflammation leading to peptic ulcers, irritable bowel disease, Crohn's Disease, and gastric cancer. *H. pylori* has been classified as a Group I carcinogen by the International Agency for Research on Cancer since 1994 and remains a causative agent for 63% of the world's gastric cancer cases (Peek,

2005). Taken from a broader perspective, *H. pylori* is responsible for 5.5% of the world's overall cancer burden (Parkin, 2006).

Unlike most pathogens, *H. pylori* is unique in that it is a vector-less, free-floating bacterium found in the water column that has special characteristics for colonization, maturation, and reproduction in its human host. In fact, research has shown that *H. pylori* has likely been colonizing and coevolving in the human gut for over 100,000 years (Blaser, 2006). Discovered in 1982, *H. pylori* infection is greatest in developing countries with poor sanitation, leading to an infection rate of up to 80% in middle-aged adults. However, western or industrialized countries experience a much lower prevalence of *H. pylori* with a current infection rate of less than 10% in the US (Wang and Peura, 2011).

Helicobacter pylori specifically functions by colonizing the stomach of its host, where it lodges itself into the stomach's mucosal epithelial cells. From this position, the spirochete eludes the host's immune system, triggering inflammation and a series of complex mechanisms ensuring pathogen survival and proliferation, often leading to the development of gastric cancer. *H. pylori* virulence and the onset of gastric cancer has largely been attributed to the presence of cytotoxin-associated gene A (CagA), since the majority of individuals infected with *H. pylori* lacking this gene are asymptomatic (Wang et al., 2014).

Despite the high prevalence of infection and this bacteria's alarming role in the development of cancer, *H. pylori* can be removed from the body with antibiotics. However, antibiotic resistance is becoming more common, requiring multiple treatments at higher concentrations to eradicate the bacteria (Chuah et al., 2011). Given this rise in antibiotic resistance, there is a possibility that *H. pylori* may become untreatable in the near future. With

the current worldwide colonization of this pathogen, it is important to understand the cancer mechanisms of *Helicobacter pylori* as a means to develop new and effective cancer treatments. With this in mind, this mini review will analyze the different known mechanisms of *H. pylori*-induced gastric cancer with the goal to eventually target and prevent the spread of gastric cancer using a unique approach that does not result in the overuse of non-specific antibiotics. This mini review will specifically analyze CagA-positive *Helicobacter pylori's* ability to promote intestinal metaplasia, induce inflammation and oxidative stress, as well as its epigenetic strategies in causing gastric cancer.

Promotion of Intestinal Metaplasia

From an ecological standpoint the two most important objectives of *H. pylori* are to survive and reproduce inside its human host. Following ingestion, *H. pylori* migrates with its flagellum to the stomach's lining, where it uses its internal enzyme urease to break down urea into carbon dioxide and ammonia as a means to buffer itself against the stomach's low pH (Mobley et al., 2001). *H. pylori* then anchors itself into the stomach's mucosal epithelial cells, absorbing essential nutrients for survival. Once embedded in the stomach's epithelial cells, *H. pylori* is safe from an immune response as white blood cells are unable to penetrate the stomach's lining. It is at this point that *H. pylori* collects enough nutrients to reproduce and begins to degrade epithelial cells to make room for replication. Researchers have found that *H. pylori* induces transdifferentiation of epithelial cells leading to a precancerous stomach lesion known as intestinal metaplasia, or the reversion of a specialized cell into its original undifferentiated form (Murata-Kamiya et al., 2007). An increase in the number of transdifferentiated cells that no longer communicate with adjacent epithelial cells from the reproduction of *H. pylori* can lead to a negative phenotypic change in tissue known as dysplasia,

or the pre-metastatic phase of cancer. From this stage malignant or metastatic cancer is easily inducible, however, this transition from cellular transdifferentiation to metastatic cancer usually takes decades (Tsang et al., 2010). There are currently two known mechanisms in which *H. pylori* induces intestinal metaplasia. The first involves the degradation of the E-cadherin/β-Catenin complex by the virulence factor CagA, while the second mechanism involves a series of phosphorylation events.

E-Cadherin is an important transmembrane protein that binds adjacent cells to one another, fostering cell-to-cell communication (Takeichi, 1991). E-Cadherin specifically binds to the adhesion protein β-Catenin found on the surface of epithelial cells. Upon infection, *H. pylori* has been found to degrade this E-cadherin/β-Catenin complex, liberating free β-Catenin in the cytoplasm and nucleus. Accumulation of β-Catenin, a known carcinogen, then results in transdifferentiation and intestinal metaplasia (Murata-Kamiya et al., 2007).

Additionally, the CagA virulence factor protein of *Helicobacter pylori* has been found to target and alter a protein kinase known as SHP-2 tyrosine phosphatase, resulting in a phosphorylation cascade, eventually leading to cytoskeletal rearrangement and the transdifferentiation of gastric epithelial cells (Higashi et al., 2002). *H. pylori* also directly influences another type of phosphorylation cascade system by stimulating mitogen activated protein (MAP) kinases, controlling the expression of cyclin D-1 in the cell cycle. Specifically, *H. pylori* stimulates MAP kinases, inducing the overexpression of cyclin D-1, which shortens the G_1 phase of the cell cycle and increases cellular growth (Hirata et al., 2001).

Inflammation

One of the hallmarks of *H. pylori* infection, regardless of CagA pathogenicity, is innate and chronic inflammation. Since the 19th century, it has been known that inflammation is associated with cancer following Rudolf Virchow's discovery of the high frequency of cytokines or inflammatory cells surrounding cancerous tumors (Balkwill and Mantovani, 2001). Since then, the transcription factor NF-κB has been widely recognized as the "master regulator" of immune and inflammatory response (Lamb and Chen, 2013). In a study conducted by Lamb et al. in 2009, it was discovered that CagA-positive *H. pylori* stimulates the host protein transforming growth factor-β-activated kinase 1 (TAK1), triggering NF-κB activation and a resultant immune response. Further research has shown that there are many potential mechanisms in which *H. pylori* can trigger NF-κB as this spirochete can provoke a certain canonical inflammation pathway in gastric epithelial cells as well as distinct canonical and non-canonical pathways in immune cells, specifically in B lymphocytes (Lamb and Chen, 2013). Although this research into *H. pylori*-induced activation of NF-κB is ongoing, some trends in later research can be described.

Research has shown that besides CagA, the outer lipopolysaccharide (LPS) and peptidoglycan coatings of *H. pylori* play a role in NF-κB stimulation and cytokine release. In an experiment conducted by Mandell et al. in 2004, *H. pylori* LPS was shown to bind to innate immune receptor Toll-Like Receptor 2 in mice, eliciting a corresponding inflammatory response. However, LPS was selective in that it did not bind to other innate immune receptors such as Toll-Like Receptor 4, indicating that *H. pylori* LPS may serve as a low-grade, but persistent activator of cytokine secretion and inflammation (Lamb and Chen, 2013). Additionally, LPS may also be involved in early inflammatory activation of leukocytes over epithelial cells as Toll-Like Receptors tend to elicit immune responses more often from monocytes and macrophages

(Obonyo et al., 2007). Other research has shown that *H. pylori* peptidoglycan activates MAP kinases in the NF-κB pathway (Allison et al., 2009). Specific drug targeting or vaccine incorporation of *H. pylori's* CagA virulence factor, LPS, and peptidoglycan layers may therefore be a logical step towards cancer treatment and prevention.

Oxidative Stress

In addition to specific pathways involved in the promotion of intestinal metaplasia and inflammation, other mechanisms in which *H. pylori* stimulates the development of gastric cancer involve the induction of oxidative stress. *H. pylori* is known to generate reactive oxygen and nitrogen species, resulting in DNA damage and mutation in epithelial cells leading to cancer (Handa et al., 2011). Nitric oxide (NO) is an example of a harmful reactive nitrogen species that is synthesized and released into epithelial cells upon *H. pylori* infection. Specifically, *H. pylori* activates a transcription factor known as AP-1, inducing the inflammatory gene cylcooxygenase-2 and inducible nitric oxide synthase (iNOS) (Cho et al., 2010). This synthase then produces NO, resulting in epithelial DNA damage. Interestingly, it has been found that *H. pylori* LPS up-regulates the production of iNOS, synthesizing more harmful NO (Cavallo et al., 2011). Despite this increase in toxin production, it has been found that NO also harms *H. pylori*. However, this clever bacteria has developed a way of protecting itself by selectively converting NO into a harmful reactive oxygen species that has a greater affinity for epithelial DNA than its own. *H. pylori* stimulates host cell ornithine decarboxylase (ODC), generating spermine. Spermine then inhibits the production of iNOS, thereby inhibiting and preventing the synthesis of harmful NO. Instead, spermine becomes oxidized and converted into spermine oxidase, which catalyzes the oxidation reaction of water into toxic hydrogen peroxide (Chaturvedi et al., 2012). Since this reaction is taking place inside the epithelial cell, hydrogen peroxide is naturally closer and more

prone to harming epithelial DNA than *H. pylori* DNA. Additionally, *H. pylori* has its gram-negative defensive barrier comprising the LPS and peptidoglycan layers to protect its internal DNA, while the epithelial cell has no protective barrier or means of defense since hydrogen peroxide is already inside its cytoplasm. Besides resulting in eventual gastric cancer, these toxic reactive species *H. pylori* induces also more immediately cause noticeable symptoms such as peptic ulcers and irritable bowel movements in infected patients (Kwiecien et al., 2002).

Epigenetic Strategies

The last major category of mechanisms *H. pylori* employs to induce gastric cancer involves the use of epigenetic strategies or various gene expression targeting mechanisms. Compounding the negative effects of oxidative stress resulting in mutation, *H. pylori* actively inhibits certain DNA repair enzymes such as O6-methylguanine DNA methyltransferase (MGMT) that fixes single random base pair mutations through hypermethylation of this enzyme's promoter region (Sepulveda et al., 2010). DNA methylation, or the addition of hydrophobic methyl groups onto a specific DNA sequence, is known to protect DNA from harmful chemicals such as NO. However, hypermethylation results in loss of DNA transcription and protein expression (Herman and Baylin, 2003). Similarly, CagA-positive *H. pylori* is also known to induce hypermethylation of the promoter region of another tumor suppressor gene called RUNX3 (Kitajima, et al., 2008). However, unlike MGMT, CagA-positive *H. pylori* also targets RUNX3 for cell death through lysine labeling or ubiquitination and subsequent proteasome-mediated degradation, ensuring that this tumor suppressor gene does not function (Tsang et al., 2010). It is possible that *H. pylori* oversees the dysfunction and mediated cell death of RUNX3 through two distinct mechanisms because this tumor suppressor may be remarkably efficient in reverting *H. pylori's* induced oxidative stress mutations.

The CagA protein of *H. pylori* is also known to inhibit the expression of the widely studied tumor suppressor transcription factor p53 through a very unique manner. Transcription factor p53 regulates the gene expression of many tumor suppressor cells, making p53 a highly studied molecule in cancer research. Instead of hypermethylating the promoter region of p53 or labeling a segment of this transcription factor's DNA sequence for cell death, CagA interacts with the apoptosis regulating protein of p53 known as ASPP2. CagA physically binds to ASPP2 in leukocytes, subverting the normal activating function of ASPP2 to stimulate p53 to induce apoptosis of tumor cells, and instead causes ASPP2 to degrade p53 (Buti et al., 2011). In other words, CagA hijacks the activating function of ASPP2 to cause this regulating protein to destroy p53. The impact this mechanism has on the development of gastric cancer and of cancer more generally is significant because p53 is one of our body's main defense mechanisms against tumor cell development. Although there are many other ways in which CagA-positive *H. pylori* induces gastric cancer than through the suppression mechanism of p53, this pathway in particular may provide a model system for understanding and controlling a key component of many types of cancer.

Conclusion

CagA-positive *Helicobacter pylori* is one of the greatest etiological agents leading to the development of gastric cancer. Although *H. pylori* infection is currently treatable using antibiotics, this form of treatment is becoming more prone to antibiotic resistance and is typically not readily available for those most susceptible to infection. It is therefore important to understand *H. pylori's* host-pathogen interactions inside humans and to specifically research the different mechanisms of cancer onset in the aims of developing an effective cancer treatment that will not become prone to antibiotic resistance. Given the literature on current *H. pylori*-induced

gastric cancer mechanisms, there are several promising fields of study that may lead to the synthesis of an effective treatment for gastric cancer.

Taken as a whole, it is clear that targeting one specific mechanism will not produce an effective cancer treatment as there are many alternative ways in which *H. pylori* can induce gastric cancer. Instead, it would be better to identify and isolate a specific regulator of various cancer mechanisms such as *H. pylori's* virulence factor CagA. Development of a vaccine containing the DNA sequence of CagA would likely inhibit all of the cancer-promoting events associated with this protein. Although vaccines for CagA-positive *H. pylori* are not currently available, there has been progress towards vaccine development specifically targeting the CagA virulence factor (Zhang et al., 2011). Despite this progress, vaccine approval through FDA trials has been painstakingly incremental. This is largely attributed to lack of funding and public interest (Zhang et al., 2011). This lack of interest is likely due to the efficacy of current antibiotic treatment in eradicating *H. pylori* infection and the fact that few industrialized countries such as the US experience widespread infection. However, as mentioned before, antibiotic resistance to *H. pylori* treatment is increasing– emphasizing the need for an effective treatment. Given public sentiment, a more prompt treatment will likely be discovered outside of the field of vaccine development.

Besides vaccine development, there are two other studies that may be influential in creating an effective gastric cancer treatment. The first involves engineering the bacterial cell wall of gram-positive *Staphylococcus aureus*, utilizing a periplasmic enzyme called Sortase A to insert a labeling molecule (Nelson et al., 2010). This molecular labeling of *S. aureus* has many fascinating applications such as observing *S. aureus* activity within the body and for bacterial detection and destruction. Although *H. pylori* is a gram-negative bacteria and has a different cell

wall composition than *S. aureus*, it would be interesting to understand the ways in which *H. pylori* may be labeled and targeted for degradation. Additionally, this study has the potential of reducing or preventing antibiotic resistance by engineering molecular labels to target a variety of drugs that could kill *H. pylori*, since drug penetration through the bacterial cell wall may reduce antibiotic resistance. Considering that molecular labeling proves effective, this technique could also allow for physicians to prescribe a series of weaker antibiotics that may still be effective in killing off the bacteria. Patients could also frequently alter their drug regiments as well as their molecular labeling to prevent *H. pylori* from becoming resistant to any one drug.

The other study involves targeting and destroying unwanted bacteria such as *Bacillus anthracis* by chemically linking this undesired bacteria to an adaptive immune response to the alpha-gal epitope (Mullis et al., 2013; Carlson et al., 2007). By chemically synthesizing a DNA aptamer that both recognizes the CagA virulence factor of *H. pylori* and links this protein to the alpha-gal epitope, which is naturally targeted and degraded by the body, virulent *H. pylori* can be effectively targeted and destroyed in the body without resulting in bacterial resistance, as this treatment is immune-driven and would only require one dose to develop immunity. Taken collectively, these two studies provide a more realistic foundation for a potential *H. pylori*-induced gastric cancer treatment as both of these studies have multiple fascinating practical applications that would likely gain general attention and financial support. To conclude, this alternative to building off of new and groundbreaking research applicable to many fields may be more convincing than persuading an unconcerned public towards costly vaccine development.

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