Helicobacter pylori-induced AID expression and carcinogenesis.

Hiroyuki Marusawa* and Tsutomu Chiba.

Address

Department of Gastroenterology and Hepatology,

Graduate School of Medicine, Kyoto University

54 Kawara-cho, Shogoin, Sakyo-ku,

Kyoto 606-8507, Japan.

*Corresponding Author: Hiroyuki Marusawa

E-mail: maru@kuhp.kyoto-u.ac.jp

Summary of recent advances

Tumorigenesis is a multistep process in which the accumulation of genetic alterations drives the transformation of normal cells into malignant derivatives. Activation-induced cytidine deaminase (AID) contributes to immune system diversity by inducing somatic hypermutations and class-switch recombinations of human immunoglobulin genes. The mutagenic activity of AID, however, can also induce genetic changes in various genes and may lead to the development of cancer. *Helicobacter pylori*, a class 1 carcinogen for human gastric cancer, affects AID expression by two different mechanisms, introduction of bacterial virulence factors into host cells and induction of inflammatory responses, thereby contributing to the accumulation of mutations in tumor-related genes. Aberrant AID activity may therefore be a novel link between infection and carcinogenesis.

Introduction

Helicobacter pylori (*H.pylori*) is a gram-negative, spiral-shaped bacterium colonized in human populations for more than 58,000 years [1]. *H.pylori* infection is involved in the development of several human diseases, including gastro-duodenal ulcers, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach. *H.pylori* strains exhibit a high level of genetic diversity, and a striking difference among strains is the presence or absence of a 40-kb DNA segment, termed the cag pathogenicity island (PAI). The risk for developing *H.pylori* infection-mediated gastric disorders is closely associated with the strain [2]. The risk of developing gastric cancer is higher in patients infected with cagPAI-positive *H.pylori* compared with cagPAI-negative *H.pylori* [3,4], but how *H.pylori* infection contributes to gastric carcinogenesis remained unknown. Genetic changes in tumor-related genes are essential in the malignant transformation that leads to cancer cell development. How the intra-gastric residential bacteria induce the genetic changes required for tumorigenesis in host gastric epithelial cells is unclear, since the extracellular habitant *H.pylori* cannot directly access host genomic DNA located in the nucleus of gastric epithelial cells. Recent studies, however, revealed that cagPAI-positive *H.pylori* manipulates the host nucleotide editing enzymes to induce mutagenesis in human DNA sequences of the gastric epithelium [5].

Novel mechanism of active mutagenesis achieved by nucleotide editing enzymes.

Genetic changes in tumor-related genes are essential for malignant transformation in cancer cell development. Mechanisms that account for genetic changes required for tumorigenesis are unknown, except for defects in the DNA repair system that are observed in certain human cancers. Several enzymes that induce nucleotide alterations were recently identified, providing a new avenue for understanding the mutagenesis mechanism. The apolipoprotein B mRNA editing

enzyme catalytic polypeptide (APOBEC) family comprises nucleotide editing enzymes that insert nucleotide alterations in target DNA or RNA through cytidine deamination [6]. The human APOBEC family consists of APOBEC1, -2, -3A, -3B, -3C, -3DE, -3F, -3G, -3H, -4, and activation-induced cytidine deaminase (AID), and contributes to producing various favorable physiologic outcomes by modifying target gene sequences. For example, APOBEC1 participates in lipid metabolism through deaminating a specific cytidine to uridine in the Apo-B mRNA, resulting in the formation of a termination codon, which leads to the production of a half-length genomically-encoded Apo-B100. APOBEC3G is an anti-viral molecule that induces hypermutation in viral DNA sequences and acts as a host defense factor against viruses such as HIV-1. Although the majority of APOBEC family members exhibit mutagenic activity against human RNA or exogenous viral genomes, only AID has the ability to induce nucleotide alterations and double-strand DNA breaks in human genomic sequences. Under physiologic conditions, AID is expressed in germinal center B-cells and induces somatic hypermutation and class-switch recombination of immunoglobulin genes, thereby amplifying immune system diversity[7]. In sharp contrast to the favorable role of AID in the immune system, excessive AID activity might affect non-immunoglobulin genes, including tumor-related genes in non-lymphoid cells [8].

CagPAI-positive *H.pylori* induces aberrant AID expression in gastric epithelial cells.

AID can alter host genomic information, but there are safeguard mechanisms that restrict its potential tumorigenic activity, including post-transcriptional regulation by microRNA [9-11], post-translational modification by protein phosphorylation or ubiquitination [12-14], and regulation of subcellular localization [15-18]. Restriction of AID expression is also an important regulatory system that minimizes the aberrant mutagenic activity of AID. AID gene

transcription is restricted mainly to activated germinal center B lymphocytes where editing of the immunoglobulin gene is required[19,20], while AID expression is not detected in normal epithelial cells under physiologic conditions. How then is AID expressed in epithelial cells under pathologic conditions, especially where the tumorigenic risk is unusually high? Strikingly, endogenous AID is expressed in the epithelial cells of *H.pylori* -infected stomach. Gastric epithelial cells and some infiltrating lymphocytes are immunoreactive for AID protein expression in the majority of chronic gastritis tissues infected with cagPAI-positive *H.pylori* [21]. Moreover, eradication of *H.pylori* infection by antibiotics substantially decreases AID protein expression in gastric mucosa. These findings suggest that cagPAI-positive *H.pylori* somehow upregulates AID protein in the gastric epithelium of the infected host.

CagPAI contain approximately 30 putative genes encoding various bacterial proteins such as cytotoxin-associated gene A (cagA) [22].   CagPAI-positive *H.pylori* introduces several bacterial virulence factors into gastric epithelial cells through a type-IV secretion apparatus, and cagPAI-positive *H.pylori* -derived peptidoglycans introduced into the host cells has been shown to be responsible for activating the transcription factor NF-κB [23]. The AID promoter region also includes sites for several transcription factors, such as NF-κB, STAT6, HoxC4, Sp1, Sp3, and Pax5 [24-27],   and AID expression in B lymphocytes is induced in response to NF-κB activation through CD40 ligand signaling [28]. Together, these findings suggest that cagPAI-positive *H.pylori* induces AID expression via NF-κB activation by introducing bacterial virulence factors, and that the proinflammatory response caused by *H.pylori* infection also triggers AID expression via the activation of NF-κB in gastric epithelium, because proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1β can induce NF-κB activation in various types of cells. These hypotheses are supported by *in vitro* analyses showing

that AID expression is induced in response to cagPAI-positive *H.pylori* infection or stimulation with the proinflammatory cytokine TNF-α via NF-κB signaling in cultured human gastric epithelial cells [21]. Based on the clinical course of *H.pylori*-infected individuals, both bacterial factors that are introduced into epithelial cells and the inflammatory response against *H.pylori* infection would be responsible for aberrant AID expression in gastric epithelium (Figure 1), and the direct action of the bacterial virulence factors contributes to activate AID in the early stage of *H.pylori* infection when the number of bacteria is high. In the late phase of chronic gastritis, when gastric atrophy has progressed and the number of *H.pylori* is decreased, the proinflammatory cytokine plays a central role in causing the constitutive expression of AID in gastric epithelial cells.

AID induces DNA mutations in tumor-related genes in gastric epithelial cells.

The target of AID-mediated genotoxic effects is not restricted to immunoglobulin genes and several non-immunoglobulin genes are also targeted by AID in lymphocytes. Approximately 25% of expressed non-immunoglobulin genes analyzed, including Bcl6 and Cd83, accumulated AID-mediated mutations in germinal B cells [29]. Moreover, AID produces double-strand DNA breaks throughout the genome, including *c-myc* in B cells [30,31]. The impact of AID expression in non-lymphoid gastrointestinal epithelial cells was clarified using mouse model with constitutive and ubiquitous AID expression. AID transgenic mice accumulated somatic mutations in various tumor-related genes and developed tumors in both lymphoid and non-lymphoid tissues [32,33]. The tumors developed in the epithelial organs of AID transgenic mice included lung, liver, and gastric cancers.

The findings that AID mutagenic activity results in stomach cancer led us to speculate that

H.pylori infection in association with aberrant AID expression contributes to human carcinogenesis via the accumulation of genetic alterations in gastric epithelial cells[21]. In *in vitro*-cultured gastric epithelial cells, cagPAI-positive *H.pylori* infection led to somatic mutations in the tumor-suppressor *TP53* gene. The number of nucleotide alterations observed in *H.pylori*-infected cells was significantly reduced by knockdown of endogenous AID, indicating that the somatic mutations in the *TP53* gene in cells infected with cagPAI-positive *H.pylori* were due to the induction of endogenous AID expression in gastric cells. In wild-type mice, oral infection with cagPAI-positive *H.pylori* upregulated AID protein expression. Moreover, nucleotide alterations emerge in the *TP53* gene in stomach tissues after oral *H.pylori* infection in wild-type mice. These findings strongly suggest that *H.pylori* infection causes accumulation of somatic mutations in tumor-related genes such as *TP53* through aberrant upregulation of AID in gastric epithelial cells.

H.pylori-associated lymphoid tumorigenesis and AID expression

Low-grade lymphomas originating from MALT develop in the stomach, salivary and thyroid glands, bronchi, and small intestine, and are classified as MALT lymphoma [34,35]. The acquisition of MALT is induced prior to the development of lymphoma as a response to a persistent antigenic stimulation [36]. The development of gastric MALT lymphoma, a representative gastric lymphoma, is strongly associated with *H.pylori* infection [37]. The seroprevalence of *H.pylori* is higher in patients with gastric MALT lymphomas than in control patients without MALT lymphoma [38], and eradication of *H.pylori* leads to complete regression of the lymphoma in nearly 80% of patients with early-stage disease [39,40]. On the other hand, several studies have aimed to clarify the role of AID in the development of MALT lymphoma,

because AID is required for the development of germinal center-derived non-Hodgkin's lymphomas [41,42]. AID mRNA was, however, expressed in only some extranodal marginal zone B-cell MALT lymphomas [41]. More recent studies demonstrate that neoplastic marginal zone B cells did not express detectable AID, whereas AID expression was confined to reactive areas within MALT lymphomas [43,44]. In addition to the low frequency of AID upregulation in MALT lymphoma tissues, it remains unknown whether *H.pylori* infection enhances the aberrant mutagenic activity of AID in gastric B-cells. Further analyses are required to determine the role of AID in the development of *H.pylori*-associated MALT lymphomas.

Conclusions

The discovery of AID was a seminal finding that greatly advanced our understanding of the molecular mechanisms involved in immunoglobulin diversification [45]. Now, AID is central to our understanding of how inflammation and infection underlie the genetic alterations required for carcinogenesis in epithelial cells [46]. Indeed, proinflammatory cytokine induction of AID expression via the NF-κB pathway is not limited to gastric epithelial cells. AID expression is mediated by the inflammatory response in a variety of epithelial cells, including human hepatocytes [47,48], and biliary[49] and colonic epithelial cells[50]. Aberrant AID expression in these gastrointestinal organs results in somatic mutations in various tumor-related genes. Thus, AID may have a central role in genetic susceptibility to mutagenesis, which leads to cancers in these gastrointestinal tissues upon exposure to certain inflammation or infection (Figure 2).

A characteristic of *H.pylori*-associated gastric cancer is multicentric tumor development. Patients with a history of *H.pylori*-related gastric cancer are at high risk for subsequent development of gastric cancers[51], suggesting that each epithelial cell of the *H.pylori*-infected

stomach possesses sufficient genetic damage for malignant transformation. Efficient strategies to restrict aberrant AID activity might help to prevent carcinogenesis in gastric epithelial cells inflamed by *H.pylori* infection.

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