Commentary

CagA protein of Helicobacter pylori: A hijacker of gastric epithelial cell signaling

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ABSTRACT

Epidemiological study has shown strong correlation between the Helicobacter pylori (H. pylori) infection and gastric carcinogenesis. However, the mechanism by which H. pylori induces gastric carcinogenesis is not known. In this review, we focused on the product of cytotoxin-associated gene A (CagA), one of the important virulence factors of H. pylori. H. pylori injects CagA protein into the host gastric epithelial cells through its needle-like structure, type IV secretion system. Injected CagA hijacks physiological signal transduction and causes pathological cellular response such as increased cell proliferation, motility, apoptosis and morphological change through different mechanisms. H. pylori has been shown to produce reactive oxygen species (ROS) in infected gastric mucosa. Although the main source of ROS production is possibly host neutrophil, we propose novel source of ROS production in this review; CagA itself can induce ROS production in gastric epithelial cell. Excessive ROS production in gastric epithelial cells can cause DNA damage and thus might involve in gastric carcinogenesis. Understanding the molecular mechanism by which H. pylori-induced carcinogenesis is important for developing new strategies against gastric cancer.

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1. Introduction

In 1994, Helicobacter pylori (H. pylori) was defined as a group 1 (a definite) carcinogen by International Agency for Research on Cancer (IARC), a part of World Health Organization (WHO). To date, extensive research has been done in many laboratories to clarify the mechanism by which H. pylori induces gastric carcinogenesis. One of the candidate proteins is cytotoxin-associated gene A (cagA) protein injected into host gastric epithelial cells by type IV secretion system (T4SS) of H. pylori. Injected-CagA protein, a H. pylori virulence factor, is thought to hijack the cellular signal transduction and involve in gastric carcinogenesis. In this review, we summarized reported CagA-induced pathological signal transduction pathways and focused on the reactive oxygen species (ROS) production, in H. pylori-infected gastric epithelial cells and possible mechanism by which H. pylori involve gastric carcinogenesis, since ROS have been shown to involve in tumor initiation and in particular enhance the expression of oncogenes and stimulate cell proliferation.

2. Helicobacter pylori (H. pylori)

H. pylori in the base of a gastric ulcer was first reported by Marshall and Warren in 1984, which revolutionized the
pathogenesis and the treatment of chronic gastritis, peptic ulcer, and gastric cancer. This small (3.5 μm × 0.5 μm) non-spor-forming and spiral-shaped Gram-negative rod bacteria transmitted from human-to-human possibly by the fecal–oral or oral–oral route [1] and induces infiltration of the gastric mucosa by neutrophils, macrophages, and T and B lymphocytes. However, our immune system and inflammatory response cannot clear the infection of this bacterium, and leaves the host prone to complications resulting from chronic inflammation or ulcer formation to gastric carcinogenesis.

Recent studies of the cagA gene, located in the most downstream portion of the cag pathogenicity island (cagPAI), have demonstrated that the CagA protein is associated with peptic ulcer disease, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma. In Western countries, persons carrying cagPAI have an enhanced risk for developing atrophic gastritis, non-cardia gastric adenocarcinoma, and peptic ulcerations, but in East-Asian countries, the relationship of cagPAI with disease is more difficult to establish [2].

3. CagA protein

H. pylori strains can be divided into two major sub-types based on their ability to produce a CagA protein (125–145 kDa), a cagA gene product of H. pylori, which is injected directly from the bacterium into the infected gastric epithelial cell via the type IV secretion system (T4SS) [3,4] (Fig. 1). The cagA gene is localized at the most downstream portion of the cag pathogenicity island (cagPAI) [5], a 40 kb DNA segment that was most likely incorporated into the H. pylori genome by a process of horizontal transfer in the same way as many other Gram-negative rods. Approximately, 60% of H. pylori strain in Western countries possess cagPAI [5], however, almost 90% of those in East Asian countries possess cagPAI [6]. The cagPAI segment contains 31 genes, including cagA–cagZ and cagA–cagZ, some of which code components required to form T4SS.

CagA protein can be sub-classified into two main types, East-Asian CagA and Western CagA, based on its polymorphisms in tyrosine phosphorylation sites [7] (Fig. 2). Tyrosine phosphorylation of CagA occurs at the EPIYA motif; a five amino acid sequence (Glu-Pro-Ile-Tyr-Alu) that is present in the carboxy-terminal variable region of the protein. The EPIYA motif is part of four distinct EPIYA sites; EPIYA-A, -B, -C, and -D. Each motif is defined by the amino acid sequence that surrounds the EPIYA sequence. In the Western countries, commonly circulate CagA possess EPIYA-A and EPIYA-B sites followed by one to three times repeated EPIYA-C sites (ABC, ABCB and ABCBC type) in which most prevalent type is ABC type [8]. In the East-Asian countries, on the contrary, most of CagA possesses EPIYA-A, -B and -D (ABD type) [9]. Among these EPIYA sites, EPIYA-C and -D is a major phosphorylation site in each type.

Azuma et al. reported that the prevalence of East-Asian CagA-positive strains appeared to be associated with the rate of gastric cancer mortality worldwide and endemic circulation of H. pylori populations with more virulent East-Asian CagA proteins may affect the prevalence of gastric cancer in East-Asian countries [10].

4. CagA as a hijacker of cellular signaling (Fig. 3)

As described above, CagA is injected directly from the bacterium into the infected gastric epithelial cells via the type IV secretion system [3,4]. Since most, but not all, CagA proteins contain tyrosine phosphorylation motifs, majority of injected CagA localizes to the plasma membrane and undergoes tyrosine phosphorylation by the host Src family protein

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Fig. 1 – cagPAI and cagA gene.

Fig. 2 – EPIYA. EPIYA motif consists of five amino acids: Glu-Pro-Ile-Tyr-Alu.

Fig. 3 – CagA as a hijacker of cellular signaling.
The complex formation between tyrosine phosphorylated CagA and SHP2 has been reported in infected cells in vitro as well as in transfected cells with CagA and in biopsy specimens of gastric mucosa (even in atrophic mucosa but not in mucosa with intestinal metaplasia or gastric carcinoma [19]) in CagA positive H. pylori-infected patients [20], indicating the importance of the complex formation in the pathogenesis of H. pylori. SHP2 has two tandem-repeated SH2 domain (N-SH2 and C-SH2) on its N-terminus and one protein tyrosine phosphatase (PTP) domain on its C-terminus [7,9,21]. Two SH2 domain of SHP2 specifically recognize two tyrosine phosphorylated EPIYA motifs of CagA. Upon binding, conformational change of SHP2 is occurred and results in its continuous protein tyrosine phosphatase (PTP) activation [13]. Recent studies have shown that gain-of-function mutations in PTPN11, the gene encoding SHP2, are associated with various human malignancies such as childhood leukemia and some solid tumors [22,23], indicating that SHP2 is an oncoprotein that is substantially involved in human malignancies.

A fraction of phosphorylated CagA interacts with the carboxy-terminal Src kinase (Csk) (~20%), although with lower affinity than SHP2 (~80%), and forms a complex, which in turn inhibits SFK activity by tyrosine phosphorylation [24]. CagA has also been reported to bind to Src and inhibit its kinase activity regardless of the CagA phosphorylation status [25]. By suppressing excessive CagA phosphorylation and subsequent recruitment of SHP2, H. pylori might down-regulate the excessive cytotoxicity of CagA.

Phosphorylation-independent hijackings of signal transduction of CagA has also been reported. CagA interacts with the scaffolding protein zonula occludens-1 (ZO-1) and tight junctional adhesion protein (JAM), by recruiting SHP2 and...
other signaling molecules, which causes ectopic assembly of a tight junction at the site of H. pylori attachment, ultimately resulting in monolayer leakage [26]. CagA can bind to growth factor receptor bound protein 2 (Grb2) forming a complex with son of sevenless (Sos), and to C-Met hepatocyte growth factor receptor and phospholipase C-γ (PLCγ) [17,27]. Although the complex of CagA with C-Met involves in morphological change of epithelial cells, the biological function of the complex formation of CagA with PLCγ remains to be elucidated. Interestingly, Grb2 binding to CagA occurred independently of CagA phosphorylation, but did require the presence of the CagA phosphorylation sequence. More recently, CagA was found to activate the nuclear factor of activated T cells (NFAT) in gastric epithelial cells in tyrosine phosphorylation-independent manner [28]. One of the NFAT-dependent genes activated by CagA in gastric epithelial cells is p21<sup>Cip1</sup> cyclin-dependent kinase inhibitor. Thus, although CagA activates a growth promotion signal via SHP2 deregulation, it simultaneously inhibits progression of the cell cycle through NFAT activation.

CagA could interact with several intracellular signaling molecules as described above in both tyrosine phosphorylation-dependent and -independent manners and thus might promote early stage of gastric carcinogenesis.

### 5. CagA-induced ROS production and gastric carcinogenesis

Reactive oxygen species (ROS) has been shown to play an important role in carcinogenesis by inducing DNA damage. In patients with H. pylori infection, production of ROS was found to be enhanced in endoscopic biopsied samples from the duodenum and stomach [29,30]. Davies et al. [29] found a positive association between ROS production and the infective load of H. pylori. ROS production was also found in gastric mucosa of H. pylori-infected patients with gastric ulcer [30] and chronic gastritis in the absence of peptic ulcer [31]. The main source of ROS production in H. pylori-infected gastric mucosa is probably host neutrophils, which are activated by soluble factors of H. pylori known as a neutrophil-activating protein (NAP). CagA positivity also affects ROS production in gastric epithelial cells. Suzuki et al. found that ROS production in gastric epithelial cells was significantly enhanced by the infection of cagA-positive H. pylori species with an extensive accumulation of neutrophils [30,32]. An in vitro study demonstrated that cagA-positive strains induce an increased oxidative burst in polymorphonuclear neutrophils (PMNs) with higher ROS production [33]. Besides neutrophils, injected CagA itself might induce ROS production in gastric mucosa. By transfecting cagA gene in gastric epithelial cells, we found that fraction of expressed CagA protein localizes to mitochondria and produce significant amount of ROS in the cells [34]. In addition, increased ROS production might involve in acceleration of cell cycle and subsequent cell proliferation [35]. The mechanism by which CagA induce ROS production in gastric epithelial cells are not known. However, CagA localize to mitochondria may deregulate the function of mitochondria electron transport chain and produce primarily super oxide (O₂<sup>•⁻</sup>). To clarify the importance of ROS production in CagA expressed gastric epithelial cells, an in vitro study using CagA transfected normal rat gastric epithelial cells (RGM1) are now underway in our laboratory.

One of the mechanisms by which H. pylori-induced carcinogenesis is now believed to be dependent on an accumulative oxidative DNA damage [36]. In gastric carcinoma patients, significantly higher levels of 8-hydroxydeoxyguanosine (8-OHdG), the main oxidative modifying product of DNA, was reported in their tumor-adjacent tissues and tumor tissues than in normal tissues [37]. Farinati et al. stated that H. pylori infection is the single most important factor in determining oxidative DNA damage, as assessed by 8-OHdG levels [38]. In addition, accumulated oxidative DNA damage such as 8-OHdG can be only partially repaired through enzyme pathways that may, in turn, cause further DNA damage [39] including DNA mutation and ultimately gastric carcinogenesis. Eradication therapy could not easily repair this DNA oxidative damage and is partially irreversible after eradication [40]. In addition to 8-OHdG production, an accumulation of intracellular ROS can induce point mutation in the DNA, thus disrupting the expression and function of several tumor suppressing genes such as p53, which might contribute to the pathogenesis of gastric cancer [41]. Therefore, in younger patients with cagA-positive H. pylori, DNA oxidative damage in the gastric mucosa is accumulated in earlier stage of their life, and may cause more extensive gastric mucosal derangement [40,42].

As described above, CagA induces ROS production in mitochondria. The mitochondrial DNA (mtDNA) is more susceptible to ROS damage than nuclear DNA because of its close proximity to the electron transport chain and its lack of protective histones or DNA-binding proteins [43]. In addition, mtDNA damage is not sufficiently repaired because of little amount of repair enzymes in some cells [44]. As a result, the respiratory enzymes containing the defective mtDNA-encoded protein sub-units may exhibit impaired electron transport function and thereby increase the electron leak and ROS production, which in turn elevate the oxidative stress and oxidative damage to mitochondria. Consequently, CagA might induces oxidative stress to the gastric mucosa, and may damage cellular components, including polyunsaturated fatty acids, proteins and mtDNA, which may enhance nuclear DNA damage, and possibly result in the pathogenesis of gastric carcinogenesis.

### 6. Concluding remarks

The precise mechanism of gastric carcinogenesis induced by H. pylori is still unclear. In this review, we propose a novel pathological signal transduction that CagA itself can induce ROS production in gastric epithelial cell. However, there are many factors involve in gastric carcinogenesis, such as another virulence factors of H. pylori, host factors and environmental factors. In addition, different mechanism was proposed by Houghton et al. in which they indicated that malignant cells immigrate to the injury site from the bone marrow [45]. If this is the case, increased turnover of gastric stem cells eventually lead to depletion of the resident stem cell pool and to subsequent recruitment and settlement of bone
marrow-derived cell (BMDC) into gastric mucosa that do not differentiate properly resulting ultimately in carcinogenesis.

We should be aware that the road to carcinogenesis is not through the single way. Moreover, recent reports have indicated that the ratio of eradication therapy-resistant case is increasing in H. pylori-infected patients and the effective eradication therapy has been anticipated. Therefore, to understand the signal transduction in H. pylori-infected gastric epithelial cells and the molecular mechanism by which H. pylori-induced carcinogenesis will be important for deciding strategies against eradication therapy-resistant H. pylori and for developing new strategies against gastric cancer.

REFERENCES


