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Review

CagA-mediated pathogenesis of *Helicobacter pylori*

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ABSTRACT

Helicobacter pylori has been described as the main etiologic agent of gastric cancer, causing a considerable rate of mortality and morbidity in human population across the world. Although the infection mainly begins asymptomatically, but simply develops to peptic ulcer, chronic gastritis, lymphoma of the gastric mucosa and eventually adenocarcinoma. The major pathological feature of *H. pylori* infection is due to the activity of the cytotoxin-associated gene A (CagA), a 125–140 kDa protein encoded by the cag pathogenicity island (cagPAI). CagA is also known as the first bacterial onco-protein, ranking the *H. pylori*-mediated adenocarcinoma as the second most deadly cancer type worldwide. Upon cytoplasmic translocation CagA undergoes interacting with numerous proteins in phosphorylation dependant and independent manners within the gastric epithelial cells. The profound effect of CagA on multiple intracellular pathways causes major consequences such as perturbation of intracellular actin trafficking, stimulation of inflammatory responses and disruption of cellular tight junctions. Such activities of CagA further participate in development of the *hummingbird* phenotype and gastric cancer. This review is sought to provide a structural and functional analysis of the CagA protein with focus on demonstrating the molecular basis of the mechanism of CagA intracellular translocation and its interaction with intracellular targets.

Contents

1. Introduction

Helicobacter pylori is a gram negative, microaerophilic and spiral shaped bacterium, which colonizes the epithelial layer of human stomach. Since its discovery in 1983 by Warren and Marshall [58], a strong relationship between infection with *H. pylori* and a group of gastric diseases such as peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, chronic gastritis, non-Hodgkin's lymphoma and gastric adenocarcinoma has been outlined. Although a small number of infection cases can lead to peptic ulceration or develop gastric cancer (adenocarcinoma) and a lesser amount of

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infections (approximately 0.1%) will develop to MALT [9,44,79], but it is verified that the infection with *H. pylori* increases the risk of gastric diseases and gastric adenocarcinoma. Recent studies have shown a strong association between coevolution of *H. pylori* and human host for almost 60000 years [54], which points to some possible advantages for the human as a host from the natural selection point of view. However, the coexistence of *H. pylori* within the gastric lumen stopped serving to the human host after converting into a serious health threat. *H. pylori* has remained as the most occurring bacterial infection in human population [9]. Although a great number of patients that are colonized with *H. pylori* might not show infection symptoms throughout their life, but almost all will develop gastric chronic inflammation to some extent [79]. Colonization of gastric epithelium with *H. pylori* normally occurs during the childhood and if not treated, can remain during adolescence and retains a lifelong persistence. Although it is still not clear whether early childhood colonization causes any specific symptoms, it has been shown that the onset of colonization in adults can trigger symptoms such as hypochlohydria, nausea or gastric pain [10,30,77]. It is clear that a continuous infection by *H. pylori* is required in order to develop the pathogenic profile of the infection. For instance, the occurrence of gastric ulcers requires several years of persistent *H. pylori* infection and normally takes place in the late adult-hood as well as adenocarcinoma of gastritis which occurs after a prolong period of gastric inflammation and injury of epithelial cells [10]. Despite the clear indication of such *H. pylori* infection-related disease symptoms, which are majorly stimulated by the host immune system, the complete eradication of the infection is not simply achieved. Having the ability to resist the acidic environment of the stomach has awarded *H. pylori* the ability to survive as the only bacterium, which can exist and grow in the mucosa of human gastric tissue [19]. Presence of various regulatory genes encoding acid tolerance proteins such as urease, (which breaks the secreted urea by gastric glands into ammonium and carbon dioxide), enables *H. pylori* to neutralize its surrounding niche and persist within the gastric mucosa of stomach [119,59]. *H. pylori* is able to adapt to the gastric mucosa of its host through transferring DNA materials during colonization. This will also induce a significant degree of immunologic and genetic diversity, and assists to effectively reduce the stimulation of immune response and therefore guarantees the persistence within its niche [19]. There are various factors which determine the severity of *H. pylori* infection and whether they can elaborate the risk of *H. pylori*-mediated inflammation and diseases, such as the strain type, the genetic background of the host which determines the state of immune response to the infection (in particular the proteins that regulate host inflammatory responses such as interleukins or tumour necrosis factor-α (TNF-α)) and environmental factors which stimulate the onset of *H. pylori*-mediated inflammation such as diet and smoking habit [10,37].

2. Cytotoxin associated gene A (CagA)

Virulent strains of *H. pylori* express an immunodominant, 120–145 kDa protein named cytotoxin associated gene A (CagA) [29,114]. One of the key characteristics of CagA is the ability to interact with the host cell kinases and undergo tyrosine phosphorylation modifications [28]. The tyrosine phosphorylation site of CagA includes a conserved array of amino acid motifs at the carboxyl terminal of CagA and consists Glu-Pro-Ile-Tyr-Ala (so called EPIYA) [11,100,41,7]. Based on the geographical distribution of CagA-bearing strains of *H. pylori* and the flanking sequence of amino acids, four distinctive types of EPIYA motifs have been identified, named EPIYA-A, B, C and EPIYA-D [38]. Two EPIYA motifs –A and –B are commonly carried by all CagA-positive strains of *H. pylori*, whereas EPIYA-C is mainly found in strains that isolated from regions such as USA, Europe and few Asian countries such as Malaysia and India (Western type CagA). The EPIYA-D is mainly isolated from the Eastern strains of *H. pylori* (Far East Asian regions such as China, Japan and Korea) (Eastern type CagA) [13] (Fig. 1). The repeat number of EPIYA motifs at the carboxyl end of CagA can vary as a result of repetitive amino acids surrounding these motifs. This determines the variability in the number of EPIYA motifs carried by different CagAs [6,49]; as well as difference in the pathogenicity of various CagA-positive strains of *H. pylori* [17,62,38,121,85,110]. For instance, the *in vitro* transfection of human gastric epithelium (AGS) with the ABCCC type-CagA (carries three consecutive repeats of EPIYA-C at the very C-terminus) can significantly trigger the transcription of numerous genes involved in gastric carcinogenesis as well as active stimulation of interleukin-8 (IL-8) production, manipulation of the Crk-related proteins and other proteins involved in the cellular apoptosis pathway (via its anti-apoptotic effect) in compare to the ABC-type CagA [116]. Moreover, the polymorphism of the EPIYA B of Western type CagA (at the pY+1 amino acid position), shown as EPIYA and EPIYT has different impacts on the development of adenocarcinoma. Accordingly, the EPIYT-B motif type is associated with the interaction of CagA with host P13-kinase and activation of the serine/threonine kinase AKT during the colonization phase of *H. pylori* and comparatively has a weaker effect on triggering the production of IL-8 or development of *hummingbird* phenotype than EPIYA-B type motif [124]. The estimated half-life of CagA within the gastric epithelial cells is around three hours [45], which enables CagA to undergo simultaneous and independent tyrosine-phosphorylation of two EPIYA motifs (EPIYA-C or EPIYA-D) [12]. Accordingly, a conserved motif sequence FPLxRxxxVxDLSKVG, plays as dimerization site of CagA within the gastric epithelium [84]. This consensus motif has been suggested to role as a membrane-targeting signal that assists localization of CagA to the surface of the plasma membrane prior to the entry into host cells [43].

3. Structure of cag pathogenicity island (cagPAI) and its relationship to development of gastric cancer

The gene encoding CagA is found as an insert into a locus encoding the glutamate racemase [24] and located within a 40 kb DNA segment, called cag pathogenicity island (cagPAI). It is believed that cagPAI has been originally inherited by *H. pylori* from unknown ancestors during horizontal gene transfer (i.e. conjugation) [2,24,39]. The rapid acquisition of foreign DNA by *H. pylori*, which is more significant than most of other bacteria, has resulted in a vast genetic diversity of *H. pylori* strains [102]. Such effect has subsequently succeeded the *H. pylori* to effectively adapt to the gastric mucosa of their individual hosts [46]. Some 20 genes carried by cagPAI (out of 27–30 genes) encode subunits of a type IV secretion system (T4SS), which functions as a molecular funnel to translocate CagA into the cytoplasm of gastric epithelial cells [39] (Fig. 2). However it is necessary to mention that the detailed description of the pathway, which illustrates the delivery of CagA into the cytoplasm of host cells by T4SS is yet to be known. Based on the presence or absence of cagPAI, strains of *H. pylori* are classified into three main groups; cag^+ strains, which carry an intact copy of cagPAI and cag^- strains, which do not possess the cagPAI and are hardly able to cause any disease. The third group, also called the intermediate strains, carry a partial or deleted form of cagPAI and yet cannot effectively express the genes located on cagPAI and function similarly to cag^- strains [70]. The cag^+ strains are associated with high ratio of peptic ulcer and gastric cancer, and are more globally distributed than the cag negative or intermediate strains [10,31,71,20,33]. Moreover, *in vivo* studies have presented a clear