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Outer membrane inflammatory protein A, a new virulence factor involved in the pathogenesis of *Helicobacter pylori*

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Abstract Outer membrane proteins (OMPs) represent an important class of proteins that are observed in gram-negative bacteria, mitochondria and chloroplasts. These proteins play diverse biological roles in protein translocation, cell–cell communication and signal transduction. A variety of OMPs have been identified in the gastrointestinal pathogen Helicobacter pylori (*H. pylori*) since it was first isolated in 1983. Among these proteins, outer membrane inflammatory protein A (OipA), which is encoded by *hopH* and unique to this pathogen, is a differentially expressed outer membrane protein that has been confirmed to be directly linked to *H. pylori* colonization, as well as to the pathogenesis of *H. pylori* and disease outcome. In this review, we will describe the progress of recent studies on OipA, particularly those on the functions and biological significance of this unique protein.

Keywords *Helicobacter pylori* · Outer membrane inflammatory protein A · Signal transduction · Biological significance

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Introduction

Helicobacter pylori (*H. pylori*), a spiral-shaped gram-negative rod bacterium that colonizes the epithelial cells of the stomach, is the cause of gastritis and peptic ulcers and is a risk factor for gastric cancer [1, 2]. This bacterium infects more than 50 % of the world population and typically causes chronic infection, persisting for the life of the host. The relationship between the pathogenic factors of *H. pylori* and gastrointestinal disease has been under extensive study in recent years, with a number of new pathogenic factors identified gradually, such as OipA [3], DupA [4], GGT [5], AlpA and AlpB [6]. Among these, OipA is an outer membrane protein that is crucial in clinical presentation, gastric inflammation and mucosal IL-8 production in *H. pylori*-related diseases. This review summarizes the recent progress in studies on OipA.

Gene polymorphisms of OipA

OipA was first identified in *H. pylori* isolates in 2000; it is encoded by the *hopH* gene (*hp0638*), with a *Mr* of approximately 34 kDa. As OipA was shown to be essential for IL-8 production by host epithelial cells, it was named outer inflammatory protein [7]. The expression of OipA by *H. pylori* is regulated by a slipped-strand repair mechanism, with a CT dinucleotide repeat motif located in the 5′ signal peptide-coding region of the gene. The number of CT repeats in this region determines whether the complete open reading frame is in frame [7]. For example, when the 5′ region has 6 or 9 CT repeats, the gene state is "on" and the OipA gene is functional. When the 5′ region has 4 or 5 CT repeats, the gene state is ''off'' and the OipA gene is non-functional. However, certain deletion or insertion

Table 1 Variations in Hp0638 signal peptide coding region in *H. pylori* isolates from 410 patients

Origin	No. of CT repeats	HP0638 CT repeats sequence	Frame states	Total
Asian countries	1 + 1+1 (3)	CTTT**CT**GT**CT**TT**CT**CGTT	In	4
	2 + 1 (3)	CTTT$(\mathbf{CT})_2$TTTT**CT**CGTT	In	1
	2 + 1 (3)	CTTTTA $(\mathbf{CT})_2$TT**CT**GTCT	In	1
	3	CTAA$(\mathbf{CT})_3$TTTTATCGTT	In	1
	1 + 1+2 (4)	CTAA**CT**TT**CT**TT$(\mathbf{CT})_2$CGTT	In	1
	1 + 3 (4)	CTTT**CT**GT$(\mathbf{CT})_3$CGTT	In	2
	3 + 1 (4)	CTAA$(\mathbf{CT})_3$TTTT**CT**CGTT	In	33
	3 + 2 (5)	CTAA$(\mathbf{CT})_3$TT$(\mathbf{CT})_2$CGTT	In	2
	2 + 3 (5)	CTAA$(\mathbf{CT})_2$ TT$(\mathbf{CT})_3$CGTT	In	1
	5	CTTTTACTAA$(\mathbf{CT})_5$TTCGTT[a]	In	4
	6	CTTACTAA$(\mathbf{CT})_6$CGTT	In	4
Western countries	4	CTTACTAA$(\mathbf{CT})_4$CGTT	Out	2
	1 + 4 (5)	CTTACTAA**CT**TT$(\mathbf{CT})_4$CGTT	In	1
	2 + 3 (5)	CTAA$(\mathbf{CT})_2$ TT$(\mathbf{CT})_3$CGTT	In	1
	5	CTTACTAA$(\mathbf{CT})_5$CGTT	Out	5
	5	CTTACTAACC$(\mathbf{CT})_5$CGTT[a]	In	2
	5	CTTACTAATT$(\mathbf{CT})_5$CGTT[a]	In	1
	4 + 2 (6)	CTTA$(\mathbf{CT})_4$TT$(\mathbf{CT})_2$CGTT	Out	1
	2 + 3+1	CTTA$(\mathbf{CT})_2$TT$(\mathbf{CT})_3$TT**CT**CGTT	In	1
	6	CTTA$(\mathbf{CT})_6$CGTT[b]	Out	3
	6	CTTACTAA$(\mathbf{CT})_6$CGTT	In	187
	3 + 4	CTTA$(\mathbf{CT})_3$TT$(\mathbf{CT})_4$CGTT	In	2
	5 + 2	CTTA$(\mathbf{CT})_5$TT$(\mathbf{CT})_2$CGTT	In	11
	7	CTTTTA$(\mathbf{CT})_7$CGTT	Out	3
	7	CTTACTAA$(\mathbf{CT})_7$CGTT	Out	7
	7	CTTA$(\mathbf{CT})_7$CGTT	Out	43
	8	CTTA $(\mathbf{CT})_8$CGTT[c]	In	41
	8	CTTACTAA$(\mathbf{CT})_8$CGTT	Out	8
	9	CTTA $(\mathbf{CT})_9$CGTT[b]	Out	7
	9	CTTACTAA$(\mathbf{CT})_9$CGTT	In	22
	10	CTTTTA $\mathbf{CT})_{10}$CGTT	Out	2
	10	CTTA $(\mathbf{CT})_{10}$CGTT	Out	1
	10	CTTACTAA$(\mathbf{CT})_{10}$CGTT	Out	2
	12	CTTA **(CT)12**CGTT[b]	Out	1
	12	CTTACTAA$(\mathbf{CT})_{12}$CGTT	In	2

[a] ORF with 5 CT repeats is in frame due to insertion of TT sequence 6 bp upstream of CT repeats or CC (TT) sequence immediately upstream of CT repeats

[b] ORF with 6,9 or 12 CT repeats is out of frame due to deletion of CTAA sequence immediately upstream of CT repeats

[c] ORF with 8 CT repeats is in frame due to deletion of CTAA sequence immediately upstream of CT repeats

mutations upstream of the CT repeats, such as the insertion of a TT sequence 6 bp upstream or the insertion of a CC sequence immediately upstream of the CT repeats and the deletion of CTAA sequence, can change the state of the gene.

Three reports have analyzed the differences in the signal peptide-coding region sequences of *hopH* from *H. pylori* isolates from 410 patients (Table 1), including 54 isolates from Asian countries (China, Japan and India) and 356 isolates from Western countries (American, Europe and Columbia) [3, 8, 9]. As shown in Table 1, the CT dinucleotide repeats present in the signal sequence-coding region of the *hopH* gene varies from 3 to 12, and the number of CT repeats was not greater than 6 in Eastern countries, while more than 96 % (344/356) of the isolates from Western countries harbors greater than 6 CT repeats [9]. Typically, the sequence immediately upstream of the CT repeats is dominated by the sequence CTTACTAA, and the downstream of the CT repeats is occupied by CTGG. The on and off states of the signal region and the number of CT repeats are related to the geographical area of distribution. The OipA signal region of 54 isolates of *H. pylori* from Asian countries are all in the open status, while only 76 % of the isolates from western countries are in the open status. The number of CT dinucleotide repeats was significantly different among strains in which OipA was in

frame, with a 6 CT repeat pattern dominant in Western countries (69 %), followed by 8 and 9 CT repeats (15 % and 8 %, respectively). In contrast, a pattern of 3 CT repeats with another CT after four T's (3 + 1-CT-repeat pattern) was dominant in East Asia (61 %).

Interestingly, the state of OipA strongly correlated with the CagA status. By using PCR amplification and DNA sequencing, Ando et al. [9] confirmed that approximately 96 % (89/93) of CagA-positive strains had *hp0638* in frame, which was 0 % (0/16) in CagA-negative strains. A relatively lower correlation was also observed between *hp0638* and VacA [8], as CagA and VacA are believed to be directly involved in *H. pylori*-related disease [10, 11]. OipA may have a synergistic activity with other virulence factors in the pathogenesis of *H. pylori*. Thus, the analysis of OipA CT polymorphisms may serve as a new typing system to discriminate *H. pylori* isolates for epidemiological purposes.

OipA and *H. pylori*-related disease

As virulence factors are usually involved in the development of disease, they may thus serve as a type of antigen marker for prediction of the pathogenesis of the pathogen and be used for the prediction of bacterial virulence and clinical outcome. In *H. pylori*, some virulence factors, such as CagA, VacA and BabA, are believed to correlate with the development and severity of gastritis, gastric ulcer or even gastric cancer. As a newly identified virulence factor, OipA, in particular its relationship to *H. pylori*-related disease, is also under extensive study.

Recent studies have suggested that OipA-positive isolates are associated with more severe mucosal cellular inflammation and an increased risk of clinical outcomes such as peptic ulcer disease and gastric cancer [3, 7, 12–14]. A meta-analysis also confirmed that a functional "on" status for OipA showed an association with increased risks for peptic ulcer disease and gastric cancer [15]. By analysis of the expression profile of OipA in 200 patients with gastroduodenal diseases using western blot, Yamaoka demonstrated that the OipA positivity rate in duodenal ulcer, gastritis and gastric cancer is 90, 64 and 76 %, respectively. More importantly, a functional OipA was significantly associated with high *H. pylori* density, severe neutrophil infiltration and high mucosal IL-8 levels [3, 16], suggesting that OipA is involved in bacterial colonization of the host gastric mucosa and the induction of the inflammation reaction. In 2004, in volunteers challenged with a Cag-PAI-negative, OipA-positive *H. pylori* isolate, Graham et al. [14] found that this strain could cause gastritis, further proving that OipA plays an important role in *H. pylori* pathogenesis. Other results also indicate that OipA has a close relationship with gastric cancer. Franco et al. [12] infected different groups of mice with wild-type and OipA-negative strains of *H. pylori*; no animals infected with the OipA mutant developed gastric cancer, whereas 27 % of those infected with the wild-type strain developed gastric cancer, with the mutant strain's gastric cancer lesions limited to the submucosa, while the wild-type strain's lesions are not. OipA expression was detected significantly more frequently from *H. pylori* strains isolated from human subjects with gastric cancer precursor lesions versus strains from persons with gastritis alone. In addition, OipA is also involved in the expression of certain cytokines and plays a key role in certain signaling pathways essential for carcinogenesis (see details below). These results indicate that OipA is involved in the progression of gastric cancer.

Because functional OipA genes are linked to bacterial virulence factors such as the VacA s1, VacA m1, BabA2 and CagA genotypes [4], Yamaoka et al. [17] proposed that it might be more relevant to hypothesize that these factors interact synergistically with each other to induce serious diseases, rather than to discuss which factor is the most virulent. For example, *hopH* is located on the *H. pylori* chromosome approximately 100 kb from the Cag PAI; this strong linkage with CagA further suggests that OipA may contribute to the pathogenesis of CagA-positive strains in vivo [4]. Thus, the functional status of OipA, combined with analysis of the genotypes of other virulence factors, may serve as an effective marker for prediction of the pathogenesis and severity of clinical isolates and the clinical outcome of *H. pylori*-related disease. We are now focused on studies to analyze the genotypes of these virulence factors using clinical *H. pylori* isolates form different geographical areas of China.

OipA-mediated cellular signaling and biological function

OipA plays a role in *H. pylori* colonization

A host-pathogen interaction is essential for infection and subsequent pathogenesis. OipA has now been shown to mediate the adherence of *H. pylori* to gastric epithelial cells in vitro [7, 13, 18]. Dossumbekova et al. [13] reported that the adherence of OipA mutant strains to gastric cells was significantly lower than that of wild-type strains, while complementation of the *hopH* gene completely restored the adherence properties of the mutants. The role of OipA in bacterial colonization was also confirmed by in vivo studies. Yamaoka et al. [19] confirmed that compared with mice infected with wild-type strain, the bacterial density was considerably decreased in the mice infected with the

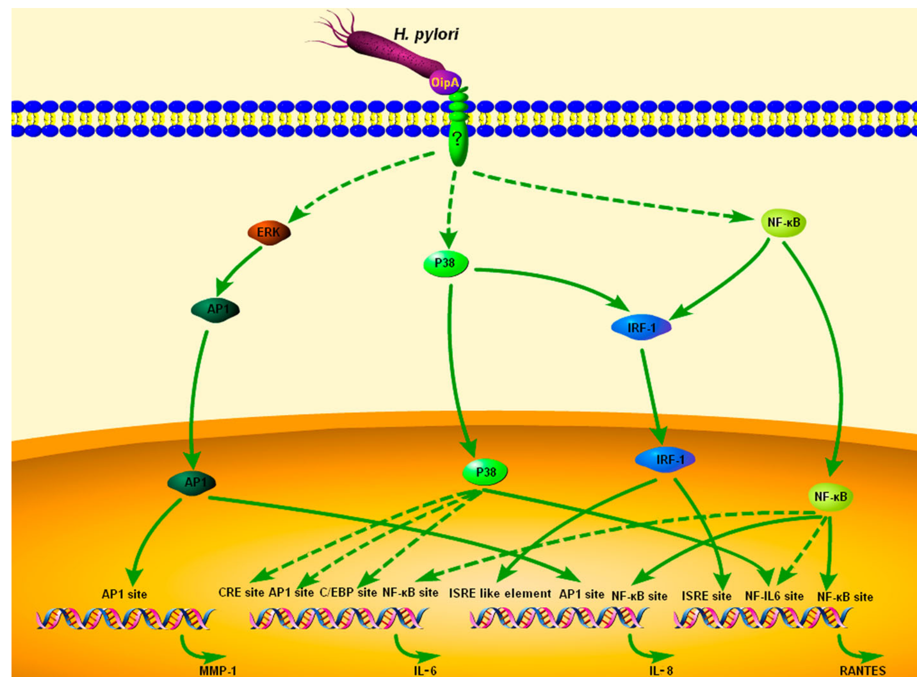

Fig. 1 The possible model for the induction of gene transcription of cytokines IL-8, IL-6, MMP-1and RANTES in gastric epithelial cells by OipA from *H. pylori*. The interaction of OipA with its receptor is involved in the activation of ERK, P38 and NF-κB pathway, the downstream signaling molecules of these pathways are involved in binding to the binding site in the promoter of cytokines IL-8, IL-6, MMP-1and RANTES, thus triggers the transcription and secretion of these cytokines

OipA mutant strain. This result was further confirmed by in vivo studies where bacterial colonization densities were lower in patients infected with OipA-off strains than in those infected with OipA-on strains [3]. In summary, OipA is involved in the colonization of *H. pylori* on host gastric epithelial cells.

OipA triggers secretion of cytokines

In addition to being an adhesion factor, OipA is also involved in the expression of certain proinflammatory cytokines such as IL-8, IL-6 and RANTES. The secretion of matrix metalloproteinase 1 (MMP-1), an interstitial collagenase involved in the pathogenesis of infectious disease, is also regulated by OipA [20]. The model by which OipA induce gene transcription of these cytokines is summarized in Fig. 1. Recent work using Mongolian gerbils infected with wild-type *H. pylori* and an isogenic OipA mutant strain further demonstrated a role for OipA in the expression of mucosal cytokines IL-1, IL-17, and TNF-α and in gastric mucosal inflammation [21]. However, due to the existence of functional and non-functional OipA states in different *H. pylori* isolates, the secretion of these cytokines was not observed in some studies [22, 23]. Interestingly, the biological function of OipA is similar to that of Cag-PAI, as the latter is also involved in the secretion of these cytokines [24–26], suggesting a close relationship between OipA and Cag-PAI, as these virulence factors may cooperate in the pathogenesis of *H. pylori*-related diseases. The following section summarizes the pathway and mechanism by which OipA influences the secretion of these cytokines.

OipA was initially named because its isogenic mutants reduced the induction of IL-8 in gastric epithelial cell lines [7, 27], and by far, the most studied function of OipA is still its influence on IL-8 secretion. A functional OipA status was independently and significantly associated with high mucosal IL-8 levels, as demonstrated by in vivo experiments using human gastric biopsy specimens [3], which provided solid evidence for the effect of OipA on IL-8 secretion. Studies indicated that OipA is involved in inducing interferon regulatory factor IRF-1 to bind and activate the ISRE-like element, which is required for inducing IL-8 gene transcription, thus dramatically increasing the levels of gastric mucosal IL-8 secretion [7, 18]. Studies also confirmed that binding sites for the transcription factors NF-κB and activator protein 1 (AP1) within the IL-8 promoter are involved in regulating IL-8 gene transcription in *H. pylori*-infected gastric epithelial cells in an OipA-dependent manner [28, 29]. These results indicate that OipA is directly involved in IL-8 secretion.

OipA is also required for full activation of the RANTES promoter, triggering the transcription and secretion of RANTES, a CC chemokine that functions in the inflammatory response in infectious disease [30]. Studies showed that RANTES mRNA levels in the antral mucosa were significantly higher in patients infected with OipA-positive *H. pylori* than in those infected with OipA-negative *H. pylori* or in uninfected patients. The signaling leading to the activation of the RANTES promoter is complicated, as

the binding sites for CRE, ISRE, NF-IL6, and NF-κB were all involved, for example, four pathways, including OipA → p38 → NF–IL6, OipA → NF-κB → NF-IL6, OipA → p38 → IRF-1 → ISRE pathway and OipA → NF-κB → IRF-1 → ISRE pathway, are involved in the activation of the NF–IL-6 site in the RANTES promoter [31].

IL-6 is a cytokine that is relevant to inflammatory disease and cancer. Studies indicate that OipA is also involved in *H. pylori* infection-induced IL-6 mRNA expression and IL-6 protein production. Four binding sites on the IL-6 promoter, including AP-1, CRE, C/EBP and NF-κB sites, were involved in *H. pylori*-induced IL-6 transcription activation. OipA participated in increasing IL-6 secretion through two pathways, including the OipA → p38 → AP-1 → CRE pathway and the OipA → RhoA → Rac1 → NF-κB pathway [32].

In addition, MMP-1 mRNA levels in the gastric mucosa and epithelial cells were observed in OipA-positive *H. pylori* infection [33]. MMP-1 is present in gastric epithelial cells of the human gastric mucosa [34], functioning to degrade the type I collagen that is mainly present in gastric mucosa [35]. An increased level of MMP-1 was observed in patients with gastric cancers [20]. *H. pylori* induce expression of MMP-1 mRNA through the Ras/Raf/RhoA → MAPK → c-Fos/c-Jun → PEA-3 pathway. It was confirmed that *H. pylori* induces MMP-1 expression by the promoter activation at AP-1 sites and by inhibition at the PEA-3 site and that the increase in MMP-1 expression is associated with two *H. pylori* virulence factors, cag PAI and OipA [33].

Cell phenotype and carcinogenesis

Host gastric epithelial cells undergo phenotypic changes during the process of *H. pylori* infection [36]. Focal adhesion kinase (FAK) is known to play key roles in regulating the organization of the actin cytoskeleton and cell motility through the FAK → Ser → Erk pathway [37]. FAK harbors six phosphorylation sites [38], and by using isogenic mutants of OipA from parental strains ATCC43504 and 26695, it was shown that infection with OipA mutants reduced phosphorylation of FAK at Y397, Y576, Y577, Y861 and Y925 but not at Y407, which is independent of Cag-PAI and other outer membrane proteins, the author propose that OipA-induced activation of FAK Y397 is an early event and possibly a prerequisite for complete activation of FAK and the subsequent intracellular signaling that results in actin stress fibre formation [39]. These data suggest a role for OipA in gastric carcinogenesis through its involvement with FAK.

Paxillin, a downstream effecter of FAK and Src, is thought to play an important role in tumor migration, invasion and metastasis [40, 41]. It has been reported that gastric cell infection with live *H. pylori* induced site-specific phosphorylation of Y31 and Y118 on paxillin in a time- and concentration-dependent manner, while isogenic OipA mutants significantly reduced paxillin phosphorylation at these two sites and also reduced actin stress fiber formation, thus abrogating FAK expression and inhibiting Src and PI3 K kinase activity [42]. These results suggest that OipA plays an essential role in *H. pylori*-mediated activation of paxillin, actin cytoskeletal reorganization and changes in cell phenotype, as the phosphorylation at Y31 and Y118 in paxillin promote the activation of RAC1 via CRK/CrKII, thereby promoting cancer migration and invasion [43]. Thus, OipA may play a crucial role in *H. pylori* induced carcinogenesis.

β-catenin is a factor involved in the transcriptional upregulation of genes implicated in carcinogenesis [44]. Franco et al. [12] reported that inactivation of OipA decreased nuclear localization of β-catenin and reduced the incidence of cancer in infected gerbils. However, the specific mechanism by which OipA regulates β-catenin signaling remains unclear.

OipA as vaccine candidates

Outer membrane proteins are often selected as candidates for vaccine development. Although the OipA protein is not expressed in all *H. pylori* isolates, it is strongly correlated with the severity of *H. pylori* related disease, so vaccination with OipA may benefit those who are infected with the bacterial strain that harbors a functional OipA gene. Chen et al. [45] reported that oral therapeutic immunization with an OipA-based DNA vaccine (poipA) significantly reduced *H. pylori* colonization in the stomach of C57BL/6 mice and that protection was related to a robust Th1/Th2 immune response. These authors also confirmed that the codon-optimized OipA gene improves protein expression by six-fold and consequently enhances the immunogenicity of the DNA vaccine. Interestingly, a strong Th2 immune response was observed when poipA was administered intradermally alone, whereas a Th1-biased immune response was achieved by co-delivery with either pIL-2 or pLTB adjuvants, with a strong Th1 immune response elicited by co-delivery with both pIL-2 and pLTB adjuvants [46]. As a Th1-biased immune response was thought to be essential for protection against *H. pylori* infection [47, 48], the use of pIL-2 or pLTB as adjuvants to shift the immune response from a Th2-to a Th1-biased response was crucial for OipA based vaccine development.

Despite the significant protective immune response induced by the DNA vaccine, the safety of DNA vaccines needs to be taken into consideration. Vaccination with a recombinant protein may be a better choice. By aligning

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HP_B8_OipA       1  MKKALLLTLSLSLSFWLHAERNGFYLGLNFAEGSYIQGQGSIGEKASAENALNEAINNAKNSLFPEQNTK
HP_F16_OipA      1  MKKALLLTLFF..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGEKASAENALNQAINNAKNSLFPEQNTK
HP_F32_OipA      1  MKKALLLTLFF..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGEKASAENALNEAINNAKNSVFPEQNTK
HP_F57_OipA      1  MKKTLLLFLSF..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGEKASAENALNQAINNAKNSLFPEQNTK
HP_F30_OipA      1  MKKTLLLFLSL..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGKKASAENALNQAINNAKNSLFPEQNTK
HP_OK310_OipA    1  MKKALLLFLFF..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGEKASAENALNQAINNAKNSLFPEQNTK
HP_OK113_OipA    1  MKKTLLLFLSL..SFWLHAERNGFYLGLNFAEGSYIKGQGSIGEKASAENALNQAINNAKNSLFPEQNTK

HP_B8_OipA      71  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFSYQLGGVGSVPGS
HP_F16_OipA     69  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS
HP_F32_OipA     69  AIRDAQNALNKVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS
HP_F57_OipA     69  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS
HP_F30_OipA     69  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS
HP_OK310_OipA   69  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS
HP_OK113_OipA   69  AIRDAQNALNEVKDSTKIANRFAGNGGSGGLFNELSFGYKYFLGKKRIIGFRHSLFFGYQLGGVGSVPGS

HP_B8_OipA     141  GLIVFLPYGFNTDLLINWTNDKRASQKYVERRVKGLSIFYKDMTGRTLDANTLKKASRHVFRKSSGLVIG
HP_F16_OipA    139  GLIVFLPYGFNTDLLINWTNDKRASQEYVERRVKGLSIFYKDMTGRTLDANTLKKVSRHIFRKSSGLVIG
HP_F32_OipA    139  GLIVFLPYGFNTDLLINWTNDKRASQEDVERRVKGLSIFYKDMTGRTLDANTLKKVSRHIFRKSSGLVIG
HP_F57_OipA    139  GLIVFLPYGFNTDLLINWTNDKRASQEYVERRVKGLSIFYKDMTGRTLDANTLKKVSRHVFRKSSGLVIG
HP_F30_OipA    139  GLIVFLPYGFNTDLLINWTNDKRASQEYVERRVKGLSIFYKDMTGRTLDANTLKKVSRHIFRKSSGLVIG
HP_OK310_OipA  139  GLIVFLPYGFNTDLLINWTNDKRASQEDVERRVKGLSIFYKDMTGRTLDANTLKKVSRHIFRKSSGLVIG
HP_OK113_OipA  139  GLIVFLPYGFNTDLLINWTNDKRASQEYVERRVKGLSIFYKDMTGRTLDANTLKKVSRHIFRKSSGLVIG

HP_B8_OipA     211  MELGGSTWFASNNLTPFNQAKSHTIFQLQGKFGVRWNNDEYDIDRYGDENYLGGSSVELGVKVPAFKVNY
HP_F16_OipA    209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGVRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKVNY
HP_F32_OipA    209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGVRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKVNY
HP_F57_OipA    209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGVRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKVNY
HP_F30_OipA    209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGVRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKVNY
HP_OK310_OipA  209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGVRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKANY
HP_OK113_OipA  209  MDIGASTWFASNNLTPFNQVKSHTIFQLQGKFGIRYNSDEYDIDRYGDEIYLGGSSVELGVKVPAFKVNY

HP_B8_OipA     281  YSDNYGDKLDYKRVVSVYLNYTYNFKNKH
HP_F16_OipA    279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
HP_F32_OipA    279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
HP_F57_OipA    279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
HP_F30_OipA    279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
HP_OK310_OipA  279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
HP_OK113_OipA  279  YSDNYGDKLDYKRVVSVYLNYTYNFK...
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Fig. 2 Sequence alignment of OipA from 7 *H. pylori* strains by ClusterX. Colored outlines indicate identical and similar amino acid residues, respectively. More than 90 % residues are identical among these sequences as shown in the figure, indicating that OipA is highly conserved among these strains

the sequences of OipA from eight *H. pylori* strains, we show that OipA was highly conserved among these strains (Fig. 2). In addition, this protein was unique to *H. pylori,* as no homologous proteins have been identified in other species. Thus, in our lab, we are focused on developing a recombinant OipA protein-based vaccine and evaluating its effect on animal models.

The possible receptor of OipA

As a bacterial outer membrane protein involved in the attachment of the organism to the host cells, the biological function of OipA must be mediated by proteins on the surface of the host cells, some of which must serve as the receptor of OipA. However, the host receptor for OipA remains unknown. Based on published information, we propose that two types of molecules on gastric epithelial cells may serve as the host receptor for OipA.

The first and most likely category for OipA receptor is EGFR, an important target for the treatment of several malignancies [49]. First, EGFR is involved in *H. pylori*-induced phosphorylation of FAK and Erk1/2, whereas OipA is responsible for most of the FAK phosphorylation. Inhibition of EGFR significantly reduced *H. pylori*-induced phosphorylation of EGFR Y845 and phosphorylation of FAK and Erk1/2, suggesting that OipA might associate either directly or indirectly with EGFR to modulate FAK and downstream signals [39]. Second, OipA is involved in *H. pylori*-mediated activation of paxillin, either by the silencing of EGFR or by inhibiting the kinase activity of EGFR, which notably reduced paxillin phosphorylation [42].

The second category for possible OipA receptor are integrins, which are cell adhesion receptors that mediate cell–cell, cell–extracellular matrix and cell-pathogen interactions [50]. It has been reported that CagL, a component of the Cag-PAI, binds to and activates integrin α5β1 receptor on gastric epithelial cells through an RGD motif, and then triggers CagA delivery into target cells as well as activation of FAK and Src [26]. As OipA is strongly associated with Cag-PAI and is also involved in the activation of FAK and Src, it may target host cell integrins for downstream cell signaling as well.

Concluding remarks

OipA is an outer membrane inflammatory protein that closely correlates with *H. pylori*-related diseases, and may be used for clinical diagnosis and prediction of disease outcome. OipA interacts with several cellular signaling pathways and regulates the expression of certain proinflammatory cytokines that may function in the pathogenesis of *H. pylori*. Due to the conservation of the protein, it could be used as an antigen candidate for vaccine development. Finally, OipA may target host cell EGFR or integrins for downstream cellular signaling.

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