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SURVEILLANCE

Potential application of *Helicobacter pylori* against cancer: carcinogenic pathogen or therapeutic agent?

NEDA Soleimani[1], RAZIEH Ebrahimi Askari[2*], FOAD Rommasi[3*]

Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran. **foad.rommasi@gmail.com**

ABSTRACT
The repeated exposure of normal cells to carcinogenic agents may lead to mutations in their genetic material, changing them into cancerous cells. In this case, the structure and function of these cells would alter, and they would not behave like a normal cell. Treatment of cancer by bacteria is a promising and new strategy. Recently, scientists demonstrated that bacteria could induce apoptosis in cancerous cells. Cell death was identified by cellular cytotoxicity assays when bacterial structures were utilized, and these observations proved bacterial capability for apoptosis induction. *Helicobacter pylori* (or *H. pylori*) is known as a pathogenic and carcinogenic bacterium that can cause various problems. Recently, practical therapeutic applications of this bacterium has drawn attention. Here, we analyzed the advantages and merits of bacterial compounds of *H. pylori* as active remedial agents for cancer treatment. Besides describing *H. pylori* virulent factors and their role in cancer incidence, we also discuss how their potentials can significantly be strengthened and used for cancer treatment. This review results suggest that some factors of *H. pylori* can be exploited as therapeutic agents for cancer therapy in future *(Tab. 1, Fig. 3, Ref. 77)*. Text in PDF *www.elis.sk*
KEY WORDS: apoptosis induction, bacteriotherapy, cancer treatment, *Helicobacter pylori.*

Introduction

Cancer is the uncontrolled division of cells caused by environmental factors (such as carcinogenic compounds) and genetic mutations (1, 2). Gastrointestinal cancer includes about 20 percent of all cancer cases across the world (3, 4). Inflammations and infections are associated with 15 to 20 percent of malignancies globally and are among the remarkable factors predisposing to gastrointestinal cancers (5, 6). *Helicobacter pylori* is one instance of successful pathogens that can infect epithelial cells of the stomach and is classified by the World Health Organization (WHO) as a potential carcinogen. In developed and industrial countries, *H. pylori* infection involves less than 50 % of the adult population. In contrast, in developing countries, the infection rate is higher and involves about 80 % of the mature population (5–7). This gram-negative bacillus bacterium can colonize the bottom layers of human gastric mucosa and survive in the gastric mucosa for a considerable period due to its high capability for adaptation to diverse and challenging conditions. *H. pylori* virulence factors without considering detailed molecular studies are classified into general groups. Exotoxins, lipopolysaccharides, flagella, excreted proteins and enzymes and secretory systems are among them. The general virulent factors of *H. pylori* are shown in Figure 1.

As aforesaid, *Helicobacter pylori* has several pathogenetic factors such as urease enzyme, flagella, HpaA, CagA, VacA, BabA, SabA, AlpA/AlpB, IceA, DupA, LPS, OipA, and HP-NAP (7–14). This pathogen binds to the gastric epithelial cells by several receptor molecules present on its surface and exerts its pathogenicity by utilizing other virulent factors. In Table 1, by using different sources, the relationship between virulence factors of *H. pylori*, including CagA, VacA, BabA and OipA and diseases that lead to cancer is obviously demonstrated (15).

Survey method

In this study, a descriptive, analytical and interventional review of articles that were indexed in various databases (SID, DOJA, Scopus, Science Direct, Google Scholar and PubMed) was performed. Primary selection or removal of articles was based on their title, keywords and abstract content. A total of 93 Persian and English papers were selected and studied. Ultimately a summary from the content of 76 articles was extracted and used for writing the manuscript of this article. In order to examine more recent and novel studies, 43 % of used papers were published in the period between 2010–2017.

[1]Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran, [2]Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran, and [3]Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

Address for correspondence: N. Soleimani, PhD, Ass Prof, Department of Microbiology and Microbial Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

[*]These authors contributed equally to this article.

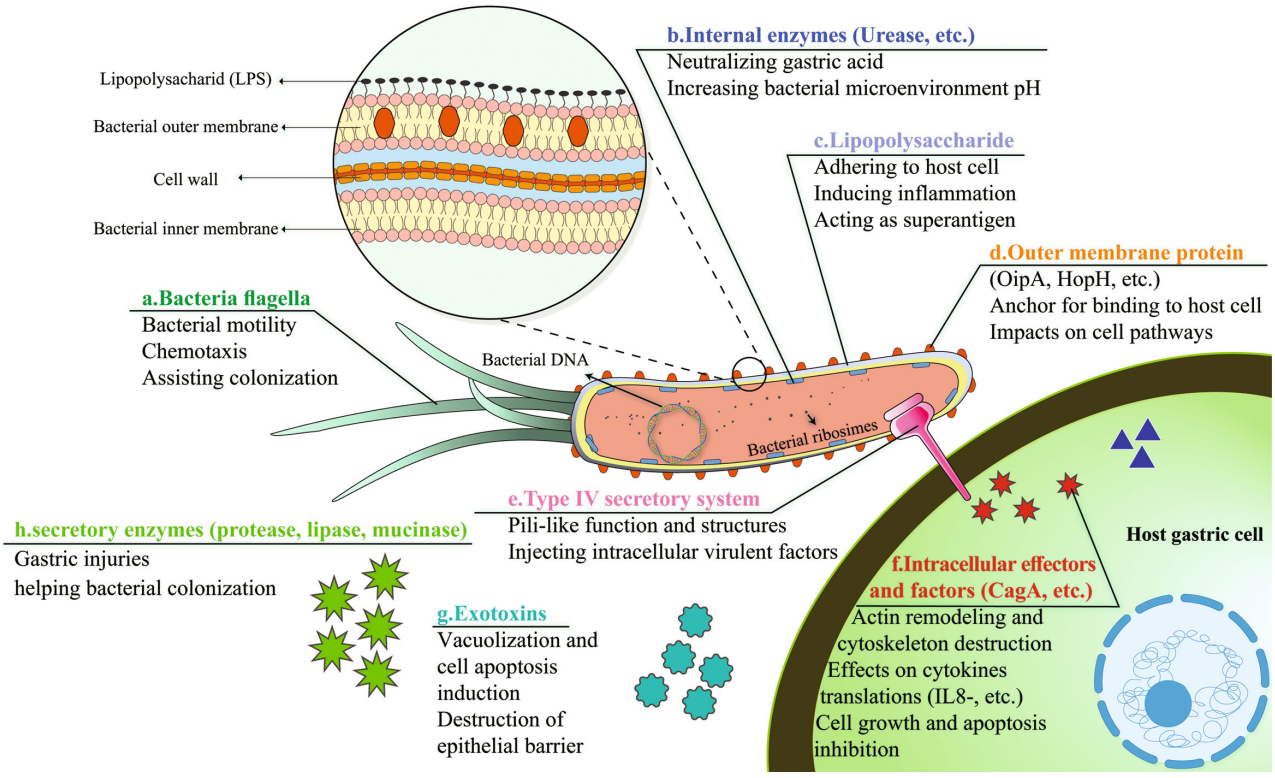

Fig. 1. General categorization of H. pylori virulence factors.

Main text

H. pylori can cause different diseases such as duodenal ulcer, stomach wound, gastrointestinal cancer and lymphoma cancer in mucosa-associated lymphoid tissue (MALT) of the stomach (5–8). Among the diseases caused by this bacterium, cancer is crucial and challenging to treat. Some pathogenic bacteria or some of their virulence factors can be used for treating cancer (32). Various studies have shown that *Enterococcus faecalis (33)*, clostridial spores (34) and *H. pylori* have a high potential for being applied for cancer treatment. If every single virulence factor of *H. pylori* gets examined in a lab, we can understand their action mechanisms and achieve applied approaches in cancer therapy, vaccine production, and defense mechanisms by using them. However, this bacterium can be investigated from two aspects. The pathogenicity process of bacteria as a carcinogenic factor in the stomach is one of these

Tab. 1. Relationship between virulence factors of *H. pylori*.

Factors	Role	Disease	References
CagA	Enter the cell via type IV secretion system, SHP-2 phosphorylation and inhibition of cytokine production (IL-8)	Increased risk of peptic ulcer or gastric cancer in 50 to70 percent, dependent on strain	(15, 16)
VacA	Apoptosis induction, inhibition of T-lymphocyte cells activation	Elevated risk of gastrointestinal cancer or peptic ulcer in 50 percent of strains in the Eastern American population	(17–19)
LPS	Endosomes and lysosomes disconnection	Induction of apoptosis and cell death	(20, 21)
BabA (HopS)	Binding to GECs receptor	More likely occurrence of peptic ulcer, increased risk of gastric cancer and metaplasia	(22–24)
SabA & B (HopP)	Binding to GECs receptor and neutrophil activation	–	(23–25)
HspA & B	Binding to GECs receptor	Increased risk of lymphoma and MALT cancer	(26)
AlpA & B	Binding to GECs receptor and toxin production	–	(23, 26, 27)
OipA (HopH)	Binding to GECs receptor and toxin production (IL-8)	Increased risk of gastric cancer and duodenal ulcer	(23, 28, 29)
IceA	Code limited endonucleases	Increased risk of peptic ulcer	(19, 30)
DupA	Increased synthesis of cytokines (IL-8)	Reduced risk of gastric cancer and increased risk of duodenal ulcer	(27)
NapA	Neutrophil activation and ROS, TNF-α, INF-γ and IL-12 production	–	(19, 31)

aspects. The second one is exploiting them in the medical and pharmaceutical fields and applying them as clinical treatments. So, *H. pylori* has various potentials and can be helpful or dangerous from different points of view. On the one hand, it is a pathogen bacterium and can cause disorders in the body. On the other, this pathogen can be utilized as a tool for cancer treatment by using Genetic Engineering and Biotechnology strategies for modifying its structure. This paper discusses the practical approaches and the pathogenesis of *Helicobacter pylori*.

The complexity of the pathogenesis of *Helicobacter pylori* is due to the fact that this bacterium has some factors that induce apoptosis. In contrast, other virulent factors of this bacterium can induce cell proliferation. In these conditions, virulent factors that induce apoptosis can be administered to destroy cancer cells directly. The factors that stimulate the immune system can be used to activate the immune responses to attack or kill tumors and cancer cells indirectly by the cell-mediated activities.

Helicobacter pylori and cancer incidence

For a pathogen to cause and develop the disease, the most critical initial event is binding to the host cell's surface receptors. The interactions between bacteria and host cells trigger a series of cascade pathways and signaling in intracellular space, leading to some cell function changes, ultimately resulting in damages to cells and tissues. *Helicobacter pylori* is initially connected to type-four collagen protein, and this attachment causes the establishment and invasion of bacteria into the lamina propria layer (35). Another important protein which this bacterium can connect to is laminin. The attachment of *H. pylori* to host cells and stimulated responses by this attachment are illustrated in Figure 2.

Laminin is the main protein in the composition of the basement membrane of various tissues. *H. pylori*, after damaging the host cells, may be exposed to the basement membrane and bind to Laminin using its receptors such as LPS and some proteins, which have 25 and 67 kDa molecular weight. This attachment causes better establishment of bacteria in the affected lesions and ulcers (36). After conjugation of the bacterium to host cells and its deployment on the cell surface, the other virulence factors get activated. Type-four secretory system is very beneficial for *H. pylori* to advance its pathogenicity (37). By utilizing this secretory system, *H. pylori* directly injects CagA (one significant virulent protein which causes damage) into the cell in the course of its infection. After the entry of this molecule to the cell, it is phosphorylated, and this phenomenon causes elevated cellular proliferation and the degradation of tight junctions between adjacent cells (38). The presence of CagA increases the danger of gastric ulcer or gastrointestinal cancer development by 50 to 70 percent (16). In 2013, Mobarez et al, in a critical study, examined the relationship between CagA-positive strains with MALT lymphoma. Results of this evaluation indicate that these strains are associated with an enhanced risk of lymphoma in infected people (39).

VacA is the other virulence factor of *H. pylori*. It is a toxin that forms pores and holes in cell membranes, resulting in the formation of vacuoles in the cell. By exploiting these toxins, *Helicobacter pylori* can damage host cells. All strains of *H. pylori* contain VacA genes, but only 50–60 percent of them show cytotoxic activity in *vivo* (40). VacA is a protein with 95 kDa molecular weight and comprises two identified domains with 37 and 58 kDa molecular weight, respectively. Out of the bacterial cell, the subunits of this protein form flower collections, which are composed of 6 or 7 petals (40, 41). After a brief exposure to an acidic envi-

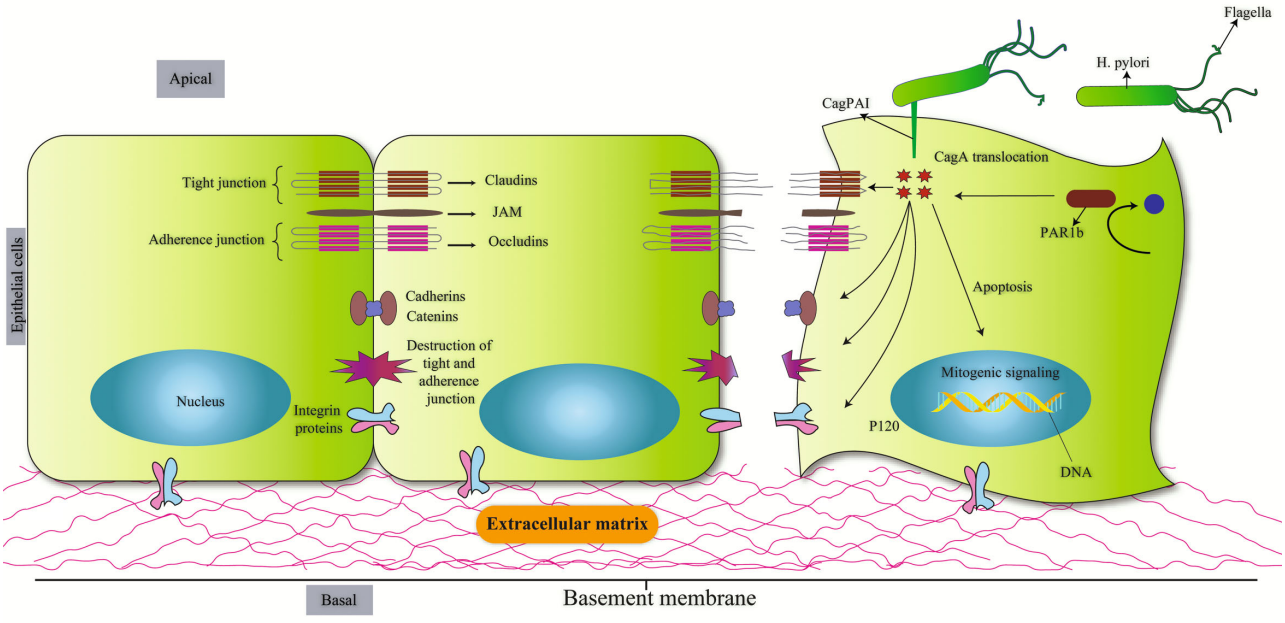

Fig. 2. The effect of CagA on cells, their proliferation and destruction of intercellular connections.

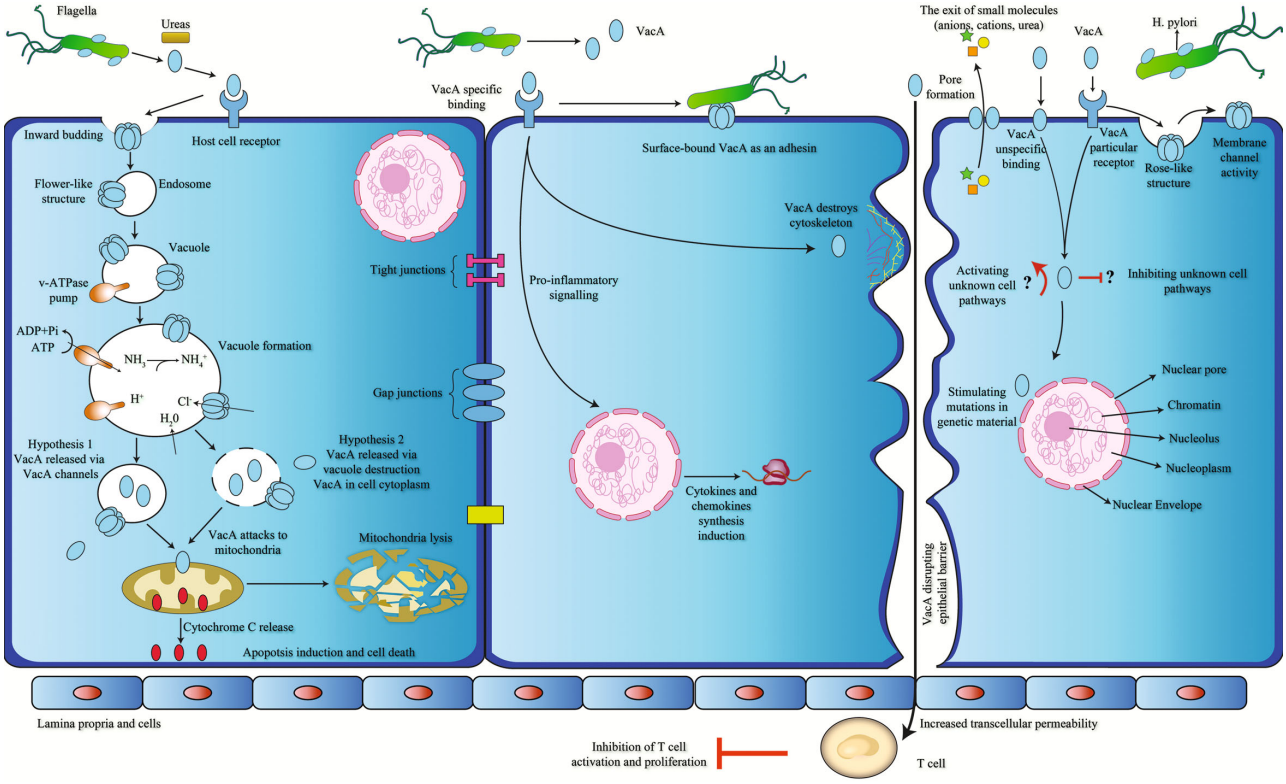

Fig. 3. Various mechanisms of disruption of cell function by VacA: specific and non-specific binding of VacA to host cell, vacuole formation, apoptosis induction, cell cytoskeleton destruction and T cells inhibition are illustrated.

ronment, structural modifications in VacA result in activation of this toxin. As aforesaid, VacA disrupts intracellular membranes and causes vacuoles' formation within the cell and inflammation (40). After connecting to the host cell, it is internalized via the endocytosis process and forms the anion-selective channels in the endosomal membrane. This factor stimulates the production of vacuoles and ultimately leads to cell death by apoptosis. It also increases the permeability of the epithelial cells, and this process provides more nutrients for bacterial growth. In other words, this toxin can cause the erosion of epithelial cells (41). In this virulent protein, 34 kDa domain is able to exert harmful activity, but 58 kDa domain is involved in connection to the target cell. Gene sequence of 58 kDa domain is preserved among the various subspecies, but allelic diversity is observed in the middle area of 58 kDa domain. It causes the secretion of acid hydrolytic enzymes in the extracellular environment. VacA enables *H. pylori* to escape from lysosomal degradation processes in target cells (42). This toxin is vital for intracellular survival but not crucial for the initial invasion.

VacA genome has two different gene placements. These two areas are Signal sequence (S) and median area (M) (which is exceptionally heterogeneous and is varying in different strains and diverse regions of the world) (31). These two genes have mosaic structure, and both of them have two alleles; the S area contains S1 and S2 alleles, while the M area alleles include M1 and M2. Strains that genotypically are S1/M1 express high toxicity against cells, while strains S2/M2 do not synthesize an active toxin. Iranian strains often have S1/M2 genotype, which is not as dangerous as S1/M1 strains. VacA cytotoxicity causes the vacuolization in the epithelial cells in vitro, and finally but not quickly leads to cell death through releasing cytochrome C from mitochondria (43). Evidence suggests the presence of VacA enhances the risk of peptic ulcer and gastric disorders development (17). Figure 3 demonstrates the hypothesized action mechanism of Vac A protein, and its carcinogenic activity.

Outer inflammatory protein A (OipA)

About 4 % of the *H. pylori* genome (significantly more than any other bacterium) has genes encoding outer membrane proteins (which mainly contains outer inflammatory protein A genes). *H. pylori* outer membrane proteins are essential for compliance with the host (44). This bacterium has 32 outer membrane proteins, and these proteins are involved in bacterial pathogenicity. There is a strong relationship between OMP and density of *H. pylori* with gastric mucosal damage, high levels of IL-8 and exudation of neutrophils at the site of inflammation. OipA gene

codes one of the outer membrane proteins and is associated with inflammation occurrence in host cells. It is located at a distance of 100 kb from CagA and PAI on the *H. pylori* chromosome (45). The function of this protein is strongly associated with VacA and CagA molecules. Since OipA probably acts as a binding agent, OipA-including strains have a stronger connection to gastric mucosa (46). Yamaavka et al separated the protein (which has a molecular weight between 33 to 35 kDa) from 97.5 % of patients with gastric ulcer and 70 % of patients with chronic gastritis. It is proved that the HPO638 gene encodes OipA (47). OipA is part of the outer membrane proteins and Hop, one of the first-class family of *Helicobacter pylori* outer membrane proteins. This protein is associated with duodenal ulcer, gastric cancer, and accumulation of neutrophils in patients' pathological lesions (47). This protein expression is under the control of the slipped-strand mispairing mechanism. There is a signal sequence in the N-terminal of this protein that has a controlling role. Gene expression is affected by the addition of C-T bases to the N-terminal of protein. *Helicobacter pylori* usually inactivates its OipA after several pathways in vitro using this mechanism (48). The protein expression is related to the CagA factor, and most of the ON-strains in terms of OipA expression are CagA positive while most of the OFF-strains are CagA negative (49). In a study conducted by Dosambkowa et al, the binding-to-cells role for OipA was also reported (50). The outer membrane proteins form numerous pores in the membrane of the target cell. These pores are necessary for the colonization and survival of *H. pylori* and play a crucial role in inflammation occurrence. The created pores cause malfunctions in the performance of cell membranes.

OipA has a synergistic effect with CagE in the production of IL-8 (50). The mode of OipA operation induced secretion of IL-8, which the presence of *Helicobacter pylori* will induce. Secretion of cytokines depends not only on CagPAI because most strains lacking this genetic locus also induce the expression of IL-8 in low-level quantities. *H. pylori* subspecies with CagPAI also have OipA expression. They are significantly associated with ulcers in the duodenum, stomach cancer, and mainly induced secretion of IL-8. OipA gene is located outside of pathogenicity islands. CagA-negative strains are almost babA2 negative and off-OipA with vacA S2/M2 alleles (46, 47). The mechanism of intracellular signaling production of IL-8 by protein OipA has been investigated in recent year. This protein has a considerable influence on the activation and binding of interferon regulatory factor 1 (IRF-1) to the Interferon-stimulated response element (ISRE), which is in the promoter area of the IL-8. It also takes part in the phosphorylation of STAT-1, which is located upstream of IRF-1. The proposed route for the activation of intracellular signals is so that OipA activates STAT-1, and this factor activates IRF-1 and eventually, ISRE factor is activated, respectively. Recent studies have proved that OipA is also involved in activating several transcription factors, including NF-KB, AP-1, ISRE, and CRE. The desired route in the activation of the CRE site in the RANTES promoter is OipA → p38 → ATF-2 → CRE. Also, OipA and cag PAI plays a role in activating the NF – IL-6 site in the RANTES promoter (49–51).

Moreover, OipA and P38 are involved in the induction of IL-18 synthesis in gastric mucosa infected with *Helicobacter pylori* (51). OipA is one of the outer membrane proteins which importance in the induction of inflammation and increased production of IL-8 has been shown; however, there are many unclear aspects of the interaction of this factor with the host (47, 49, 51).

Endotoxin

As other gram-negative bacteria contain endotoxin or lipopolysaccharide, *Helicobacter pylori* outer membrane is essential to maintain bacterium and bacterial interactions with the environment. In comparison to another lipopolysaccharide of gram-negative bacteria, the immunological activity of *Helicobacter pylori* LPS is lower (39, 52–55). *H. pylori* has unusual LPS, which is related to the fatty acid composition that forms the hydrophobic lipid A in the presence of 3-Hydroxy-Octadecanoic acid. LPS of this bacterium is 500–10000 times less toxic than *Escherichia coli* and *Salmonella*. Differences in the structure of lipid A are responsible for the low toxicity of this bacteria. The bacterial LPS is critical in decreasing gastric mucin layer thickness. *H. pylori* LPS stops the gastric mucosa glycosylation and converts structures with high molecular weights to structures with low molecular weights (which makes them more sensitive to stomach acid). It stimulates the secretion of pepsinogen, which is unique for the bacterium activity and inhibits parietal cells immunological responses and reduces the gastric acid secretion (53). The bacterial LPS stimulates the release of IL-8, IL-6, IL-10, and IL-12 and also induces the production of TNF-α and PGE2 (48–51). Mobarez et al. in 2014 indicated the relationship between the hopQ II gene and gastric duodenal disease in strains separated from patients (53). By investigating the presence of HomB in *H. pylori*, Talebi et al in 2011 proved that the presence of this gene without the presence of CagA gene could also be associated with stomach cancer (55). By studying people who suffer from *H. pylori* infection and investigating the presence of the iceA gene, Mobarez et al research in 2010 testified that this gene could be associated with ulcer patients (56). *H. pylori* has several virulence factors but their role in the pathogenesis compared to the listed factors is lower. Synergism of all virulent factors, including BAbA (Blood group antigen binding adhesion), Sialic acid-binding adhesin A and HOPP (all as adhesions factors), Adherence lipoprotein (a lipoprotein with 53 kDa weight), accelerating factor of duodenum, (dupA), heat shock proteins such as Gro-EI 58-2-kDa (HspB) Gro-Es B-kDa (HspA), disulfide reductase enzyme, purines, mucin, alcohol dehydrogenase, polyphosphatase kinase (PPK), RO53, FLdA, Locus Jhpo47-Jhpo949, gamma-glutamyl peptidase is very effective in carcinogenesis of *H. pylori* (54, 57–61). These factors may have a more diminutive role in cancer development or may be an unimportant treatment option; however, the simultaneous presence of these factors is efficient in bacterium pathogenesis (62).

***Helicobacter pylori* and cancer treatment**

Although various treatment methods are available for cancer therapy, surgery is still the first cancer treatment, especially breast

cancer (12). Common treatments of cancers may reduce tumor size but are transient and usually do not positively affect patient survival, and there is a risk of disease recurrence (63). Standard treatment methods are based on the hypothesis that a tumor is a population of homogeneous cells. Conventional treatment strategies are designed to target cells with rapid and differentiated proliferation (64). Nevertheless, since more than a century ago, cancer has been morphologically recognized as a heterogeneous population of different cells (65). Functional differences have also been considered for various cells in the tumors (64). In fact, cancer tissue includes small subpopulations of cells with particular properties (66), and these cells are responsible for the tumorigenesis, metastasis, recurrence of disease and resistance to current treatments (67). They also have the remarkable potential to induce cancer and tumors in mice with defective immune systems. Such diversifications make a finding of a general therapeutic strategy for definitive treatment hard. Due to the aggressive nature of cancer and complex mechanisms involved in its progression, traditional treatments such as surgery, chemotherapy, and radiotherapy are ineffective in many cases (65, 68). High side effects, low specificity and likely recurrence of disease are limitations of the mentioned methods. Therefore, the demand for more effective and specific alternative treatments with fewer side effects has been dramatically highlighted. Hence, the use of bacterial products, including bacterial proteins and toxins and so on in cancer treatment, has been considered (67).

The studies on several virulence factors of *Helicobacter pylori* proved their direct cytotoxic effects on tumors and their immunomodulatory impacts on immunity cells. Some of these factors that result in the immune system's activation are followed by a cascade of intracellular pathways and cytokine production that ultimately causes the activation of immune system cells. So, these virulence factors can indirectly cause the death of cancer cells. These microbial agents can include several pathogenic factors such as urease enzyme, flagella structure, and HP-NAP (69). These peptides or proteins with immunomodulatory properties can increase the activity of the endoplasmic reticulum (ER) system in various cells and cause the activation of macrophages and dendritic cells. The activated macrophages and dendritic cells engulf pathogenic structures and are able to form a more efficient response against cancerous or bacterial cells. In addition to increasing the immune system's response, these proteins can also guide the immune system to create specific responses. This strategy promotes the desired treatment efficiency in the induction of immune responses against tumors, which is vital in advancing medical applications and cancer treatment (70).

The role of *Helicobacter* OipA protein in cancer treatment

VacA and OipA, and even LPS have a direct cytotoxic effect on cancer cells. LPS of this bacterium can bind to Laminin, which causes acute gastritis and induction apoptosis in gastric epithelial cells. In this type of apoptosis, caspase-8 and mitochondria play an essential role (71). OipA is one of the relevant candidate factors for cancer treatment. The *H. pylori* outer membrane protein was discovered in 2000. In a study, Mavka et al found that less IL-8 is produced by *Helicobacter pylori* which has some mutations in the gene that encodes this protein. Hence, this protein was called OipA or outer inflammatory protein (28). Diverse mechanisms have been considered for *H. pylori* to interfere with the cell cycle and create apoptosis or stimulate proliferation. Factors in epithelial cells that changed under the influence of this bacterium and disrupted the cell cycle's natural process include mutations in the P53 protein, some modifications in Bcl-2 family proteins, increased telomerase activity, increased expression of receptor Fas, and increased NF-κB activity (72). Many proteins in the Bcl-2 family have a significant role in cell survival or apoptosis. However, two major proteins, including Bax and Bcl-2, have a fundamental role in activating or preventing apoptosis, respectively (73). In some studies, the effect of *H. pylori* infection on the Bcl-2 family has been investigated (72, 73). This family of proteins has a critical role in the pathogenesis of *Helicobacter pylori,* and it can be stated that the increased activity of Bax could lead to apoptosis. It is indicated that increased activity of Bcl-2 increases the proliferation and cancer. Soleimani et al., in an investigation, investigated the impacts of recombinant protein OipA on tumor cells and showed that recombinant OipA caused induction of apoptosis and death of cancer cells (28, 74). Due to the vacuole genesis in the cell membrane, the VacA factor can also be considered a compelling candidate, requiring detailed studies in this field. Studies have demonstrated that when human stomach epithelial cells are polluted by multiple toxin doses, cell death occurs after two days. Nevertheless, there was no death in the immortal cell lines exposed to the toxic agents. Oral administration of VacA to rats caused acute ulcers in the epithelium of gastric mucosa cells and led to the destruction of cells.

The role of Hp-NapA *Helicobacter* protein in cancer treatment

Neutrophil-activating factor (HP-NAP) is one of the critical proteins of *H. pylori*. During *H. pylori* growth, HP-NAP molecules are released by the bacterium. Some molecules of this protein remain in the outer membrane of *H. pylori* by attaching to the cell wall. The connected HP-NAP to the bacterial cell surface can be the mediator of bacterial binding to the host cell surface carbohydrates (17, 18). This protein stimulates leukocytes in the lower area of epithelial cells and triggers neutrophils- and macrophages-mediated inflammatory responses. Recent studies show that HP-NAP is capable of stimulating the innate immune system on the one hand and acts as a chemotactic factor, and causes the production of free radicals and chemokines like CXCL8, CCL3, and CCL4 of neutrophils, on the other hand (20, 75). This protein can activate the immune system. As an agonist of TLR2, HP-NAP has immunomodulatory properties and the ability to induce expression of IL-12 and IL-23 in the monocytes and neutrophils. By having the potential to alter immune system responses, the protein alters the phenotype of Th2 to Th1 through the production of interferon-gamma and tumor necrosis factor-alpha. The immune responses against cancer predominantly begin with dendritic cells. Dendritic cells express MHC-I, MHC-II and stimulatory molecules on their

cell membrane and direct immune responses by producing cytokine and chemokine molecules. Soleimani et al. used trimethyl chitosan nanoparticles carrying Hp-NAPA recombinant protein as a candidate for treating breast cancer metastatic model tumor (76, 77). HP-NAPA is recently recognized as a new tool for modulating the immune system and a practical immunotherapy gadget.

Conclusion

H. pylori is an important pathogen and can be investigated from two aspects. On the one hand, the bacterium is the cause of cancer and has pathogenic factors that induce cell proliferation and reproduction. On the other hand, some virulent factors in *H. pylori* stimulate cell apoptosis. In these conditions, factors that induce apoptosis can be used to destroy cancer cells directly. The factors that stimulate the immune system can activate the immune system to attack the cancer cells via cell-mediated processes indirectly. This study results testified that some virulence factors of *H. pylori* might be used as a new tool for cancer treatment strategies in the future.

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