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Features of Helicobacter pylori virulence. Literature review

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Abstract

The severity of chronic diseases of the gastrointestinal tract, especially the stomach and duodenum, caused by the presence of Helicobacter pylori bacteria depends on the degree of pathogenicity of the strains, as well as the presence of certain cytotoxic genes. The authors analyzed the literature data on the composition and properties of the bacteria.

Keywords: Helicobacter Pylori; chronic gastritis; gastric ulcer; duodenal ulcer; gastric mucosa.

The special ability of Helicobacter Pylori (HP) to colonize the gastric mucosa and cause gastritis or ulcer depends not only on the immune status of the host organism but also on the number and presence of a specific strain of the bacterium with specific virulence factors. One of the important virulence factors of HP is the presence of flagella and the ability to chemotaxis to places of accumulation of other bacteria of this species and, as a result, rapid colonization of the gastric mucosa (GMM) [1-3].

The HP flagella are represented by a complex of proteins - flagellins HpaA, FlaA, FlaB, FlaD, FlgK. Of these, HpaA, FlaA, FlaB were detected in strains isolated from patients with gastric diseases. These flagellins are the primary targets of damage in the humoral

immune response after infection. The structure of the flagella includes a complex of proteins consisting of FliM, FliN, FliG, FliY. It was also established that the FliM, FliG, FliY genes are not able to produce flagella, and FliN is able to synthesize flagella, but they are defective, so these strains are immobile. HP moves differently depending on the environment: "swims" - swimming (in a liquid medium), "slides" - spreading (in a semi-liquid agar), "crawls" - swarming (on a dense medium).

Some recent studies indicate an important role in the colonization of the HP regulator of iron absorption, which is involved in the regulation of ion homeostasis, oxidative response and flagellar mechanism, another recently discovered factor - Dsb-like protein HP0231 - is involved in the modulation of motility, redox homeostasis and is important for the colonization of the stomach of HP.

An important property of bacteria is the ability to secrete glycocalyx components, which allows the formation of biofilms and promotes colonization of the epithelium and survival in adverse conditions. A continuous biofilm coating of the gastric mucosa is observed in urease-positive patients, while in urease-negative patients such coating is less than 2% of the surface [4].

Biofilm formation is promoted by various environmental factors, such as: favorable temperature, pH, microaerophilic conditions, low concentrations of antibiotics. It should be noted that in the case of biofilm formation, eradication therapy may be ineffective.

A study examining the effects of the component N-acetylcysteine confirmed its ability to disrupt biofilms, indicating that biofilm-targeted therapy may be successful for the treatment of HP-associated diseases.

Adhesion of H3 to the epithelial cells of the GI tract facilitates the bacteria's access to nutrients and is the most important factor in pathogenicity. Receptors for Hp are molecular structures that are part of mucus: sialic acid residues, sulfo groups of glycoproteins, glycolipids, phospholipids, and residues of Lewis-like antigens, which are characteristic not only of gastric epithelial cells, but also of erythrocytes of blood group I (0).

The ability of the microorganism to bind to connective tissue proteins, in particular collagen, laminin, vitronectin, etc. has also been shown. HP has a large set of surface membrane proteins - Hop (Hp outer membrane proteins), which play an important role in adhesion and adaptation to the macroorganism. The HP genome contains more than 30 omp genes, which can be divided into 2 subgroups: hop (Helicobacter outer membrane proteins) and hor (hop-related groups). The hop subgroup is encoded by 21 genes and includes 2 known adhesins: babA (Lewis blood group antigen-binding adhesion) and sabA (sialic Lewis X

antigen-binding adhesion). These adhesins recognize specific carbohydrate fragments of the gastric mucosal epithelium, which contributes to infection and inflammatory processes in the gastrointestinal tract.

Bab proteins (blood group antigen-binding adhesion), whose genes (babA and babB) are represented in the form of several alleles, cause the adhesion of HP to the Lewis antigen system on epithelial cells of the gastric mucosa. In vitro, it has been shown that bacteria specifically bind to the gastric mucosa, and this process is regulated by fucosylated antigens of this group. Some researchers indicate that strains with high levels of babA expression determine more severe mucosal damage and are more often associated with gastric ulcers and gastric cancer.

At the same time, the diversity of the babA and babB genes may influence the selectivity of adhesion of different HP strains. It is known that babA is indirectly involved in the acid-sensitive process and responds to an increase in pH. The babA protein plays a role in the acid adaptation of the bacteria in response to changes in hydrochloric acid secretion during the progression of the disease. The babA gene binds to glycoconjugates and inhibits proliferation caused by bacterial aggregation, indicating a novel role of mucin in host defense against HP [5, 6].

The outer inflammatory protein oipA is inflammatory and supports inflammation of the GI tract, associated with the secretion of IL-8 and IL-6, the degree of HP seeding, the severity of neutrophilic infiltration, and the development of interstitial metaplasia. Yamaoko Y. et al. (2006) [7] found an association of oipA-positive strains with duodenal ulcer and neutrophilic infiltration, while the sabA genotype was associated with gastric cancer, intestinal metaplasia, and atrophy of the gastric body mucosa. Bacterial adhesion also includes NLBH (neuraminyl lactose binding haemagglutinin), which is a protein (3 kDa) that affects the secretion of hydrochloric acid by parietal cells. HpaA (Hp adhesin A), represented by surface lipoproteins alpA and alpB, ensures attachment of the bacterium to the gastric epithelium.

Another very important factor in the pathogenicity of HP is its ability to produce urease. It is represented by an M2+-dependent enzyme consisting of subunits ureA (26.5 kDa) and ureB (60.3 kDa), which form a complete molecule with a molecular weight of 540 kDa. Urease, consisting of subunits ureA, ureB, ureC, urel, is a marker of HP infection and a factor protecting the microorganism from hydrochloric acid, ensures long-term persistence of the bacterium in the human GI tract, enhances inflammatory reactions by activating the secretion of cytokines, monocytes, neutrophils, the formation of free radicals and nitric oxide. HP

produces a huge amount of this enzyme, which allows it to neutralize the acidic environment and create a microenvironment around the bacterium in the form of a “cloud” of ammonia [8].

Given the strong antigenic properties of urease to bind to antibodies, the antigen-antibody complex formed is removed from the bacterial cell surface, thereby protecting H. pylori from lysis. The leading role of urease in the colonization of the GI tract was proven in animal models, when urease-negative H. pylori strains were unable to colonize the gastric epithelium of gnotobionts, even after neutralization with hydrochloric acid.

The urease enzyme has other important functions, it acts on epithelial cells as a toxin: ammonium ions are formed, which can destroy tight intercellular contacts with damage to the epithelium. It has also been established that it has a cytotoxic effect on the epithelial cells of the gastrointestinal tract in vitro. Urease is an inducer of acute and chronic inflammatory cellular responses of the host [9].

An additional mechanism for the development of the inflammatory response, mediated through its mediators, is the ability of H. pylori urease to induce platelet aggregation with subsequent initiation of arachidonic acid metabolism via the lipoxygenase pathway. Urease participates in the inflammatory response and promotes adhesion by interacting with the CD74 receptors of the gastric epithelium.

Cytotoxicity is the most important factor in the pathogenicity of H. pylori. Recent studies show that this bacterium contributes to damage to epithelial cells through the production of cytotoxins vacA (vacuolating-associated cytotoxin) and cagA (cytotoxin-associated gene) [10].

The vacA toxin (140 kDa) is encoded by the vacA gene, which is present in all strains of HP, and reflects allelic diversity in three main regions: "S" (signal), "I" (intermediate), "M" (middle). The level of toxin secretion is determined by the mosaic structure of the vacA gene. The vacA regions exist in two allelic types - s1 and s2, i1 and i2, m1 and m2, which determines the difference between strains in cytotoxic activity. In s1, subtypes have been identified: s1a, s1b, s1c.

VacA toxin in vitro: causes vacuole formation in cells in vivo with the formation of erosions and ulcers. This cytotoxin increases the permeability of membranes for anions, significantly reduces the rate of re-epithelialization of experimental ulcers and epithelial cell proliferation due to disruption of cell functions associated with the integrity of its cytoskeleton, passive urea transport through gastric epithelial cells affects the survival of H. pylori in host cells, and also reduces the ATP content in epithelial cells, stimulates cell apoptosis.

VacA disrupts protein transport, increases membrane permeability by damaging the cytoskeleton, and inhibits bacterial opsonization, thereby disrupting the normal functioning of the gastrointestinal defense mechanisms. VacA also stimulates the diffusion of urease across the epithelium, making the submucosal layer accessible for the enzyme to act. Purified cytotoxin vacA inhibits epithelial cell proliferation and reduces the rate of ulcer healing (UL) [10].

Strains with the s1 allele secrete active toxin and are associated with a high risk of developing IBD and gastric cancer (GC), and the combination s1/s2 or s2 has been found in patients with GC. The m1 subtype demonstrates stronger vacuolating activity than the m2 subtype and is associated with a high risk of gastric epithelial damage and carcinogenesis. Its allele has also been shown to be associated with gastric adenocarcinoma. Researchers have also found that residents of Latin America, the Middle East, and Africa infected with s1 or m1 strains have an increased risk of developing IBD and GC compared with individuals infected with s2 and m2 strains.

Differences in genotype prevalence among strains by geographic origin have also been found. For example, m1 strains are prevalent in Northeast Asian countries such as Japan and South Korea, while m2 strains are prevalent in Southeast Asian countries such as Taiwan and Vietnam, but the relationship between the development of certain diseases and geographic region has not been determined.

The cag-PAI “pathogenicity island” in HP is a region of chromosomal DNA containing approximately 31 genes encoding proteins of the IV secretory system and divided into 2 regions: cagI and cagll. The cagA cytotoxin, a marker of the “pathogenicity island” of HP, promotes ulcer formation, development of atrophy, destruction of the intercellular matrix and basement membrane, tumor invasion and metastasis by inducing the uPA (urokinase-type plasminogen activator) and uPAR (urokinase-type plasminogen activator receptor) complex in cancer cells in the stomach, stimulating the production of IL-8, and promoting the activity of antral gastritis.

The cytotoxins cagC, cagE, cagH stimulate the production of IL-8, and cagF is involved in the process of recognition and delivery of cagA to T4CC channels (IV secretory system). The function of IV secretory system is to transport effector molecules of HP to eukaryotic cells. After delivery to the host cell, the product of the terminal gene "pathogenicity island" (cagA) undergoes a process of phosphorylation and activates eukaryotic phosphotase, which leads to dephosphorylation of host cell proteins and morphological changes. This phosphorylated protein alters the activity of cytokine genes that

initiate phagocytes and, thus, causes the induction of IL-8, as well as potent activation of neutrophils. The presence of the cagA gene is associated with high levels of inflammation, which through a chain of sequential transformations leads to more serious diseases such as gastric ulcers and gastric cancer. Jang S. et al. (2017) [11] reported that some HP strains are heterogeneous in terms of cagA copies (up to 4 copies) located in the chromosome, the number of which can vary and is directly related to toxicity.

In Western countries, it has been reported that individuals infected with cagA-positive strains are at greater risk of developing ulcers and gastric cancer than those infected with cagA-negative strains of Hp. However, this relationship has not been established in East Asian populations. Researchers also discuss the role of cagA-positive Hp strains in the pathology of other organs and systems, for example, in cardiovascular pathology, autoimmune thyroid disease.

In addition, cagA is a polymorphic gene, which is represented by a different number of repeat sequences located in the 3rd region. Each repeat region of cagA contains Glu-Pro-Ile-Tyr-Ala (EPIYA) profiles, which include tyrosine phosphorylation. According to the deciphered EPIYA sequences of the profile, 4 segments are distinguished: EPIYA-A, EPIYA-B, EPIYA-C, EPIYA-D, each of which contains an EPIYA-A repeat region. However, EPIYA sequence profiles have geographical features, which may explain the differences in the prevalence of colorectal cancer in different countries. Thus, in EPIYA-A, the cagA region of the Western HP isolates is repeated, associated with EPIYA-A, EPIYA-B, EPIYA-C segments (A-B-C type of cagA). The EPIYA-C segment is variably repeated (up to 3 times) among different cagA strains. CagA strains isolated from East Asian HP isolates also contain EPIYA-A and EPIYA-B segments, but without the EPIYA-C segment repeat, instead of which they have an EPIYA-D segment unique to this region. Accordingly, in EPIYA-A, the cagA region of the East Asian bacterial isolates is repeated and is in association with EPIYA-A, EPIYA-B, EPIYA-D segments (A-B-D type of cagA). Western cagA strains that have a repeated EPIYA-C segment are more often associated with the development of precancerous changes and colorectal cancer.

The data obtained from studying the role of the repetitive segment suggest that Hp strains that have these sequences are less resistant to the action of hydrochloric acid, as indicated by their presence in atrophic gastritis, in which its secretion is reduced. The study by Yamaoka Y. et al. (2011) [7] showed that the incidence of Hp is highest in East Asian countries, as well as in some South American countries, such as Colombia and Peru.

The DupA gene (Duodenal ulcer promoting gene) is located in a plastic region of the genome, which was initially described as a marker for the development of duodenal ulcer and a protective factor against gastric cancer. However, later other researchers showed that it may be associated with the development of gastric cancer, so the function of this gene has not been fully understood. It may be associated with increased production of IL-8.

The absence of this gene in HP, as some researchers believe, may be associated with increased susceptibility to low pH values. Thus, Roesler B. et al. (2011) [12] suggested a possible connection between dupA, vacA s1m1 and cagA positive strains and the development of gastric cancer. A study by Wang M. et al. (2013) [13] showed a connection of this gene with a high risk of developing gastric cancer in East Asian countries: all isolates from cancer patients were found to have cagA, and 31% of them had dupA in association with the vacA genotype, which may determine a high risk of developing gastric adenocarcinoma in this region.

However, Schmidt H. et al. (2009) [14] did not find significant differences between the presence of this gene obtained from patients with duodenal ulcer, gastric ulcer and non-ulcer dyspepsia. Meta-analysis and systematic review confirmed the importance of the dupA gene in the development of duodenal ulcer, but did not find a relationship between ulcer and gastric ulcer.

Strains of HP are very diverse. Genetic heterogeneity of HP should be considered in two aspects: microdifference and macrodifference. Microdifference is the result of spontaneous point mutations, in most cases they are “silent”. The accumulation of mutations contributes to the chronic course of Helicobacter infection. It is fundamentally important that the genes responsible for HP adaptation to the human body are more susceptible to mutations, while the genes necessary for the life support of the bacterium are relatively stable.

Macrodifference arises as a result of genetic recombinations Garcia-Vallve S. et al. (2000) [15]. In particular, the genes of the “pathogenicity island” cag-PAI, which HP acquired as a result of horizontal transmission from other bacterial species. However, HP strains are genetically heterogeneous not only at the population level, but also at the level of a single organism and even an organ. A large percentage of mixed genotypes can be correlated with the level of infection of the population: the higher the frequency of infection, the higher the percentage of mixed genotypes, which is confirmed by studies by Portuguese researchers, where the level of infection of the HP population is over 80%, and the percentage of mixed genotypes for vacA is 37% and iceA is 36.7%. Thus, Finger S. et al. (2006) [16] found the presence of more than one strain of Hp in half of the 63 examined patients. Cases of the

simultaneous presence of cagA-positive and cagA-negative isolates in one patient have been described. This phenomenon may be caused by the deletion of part or all of the “pathogenicity island”. The results of genotyping of Hp strains allow us to predict not only the epidemiological indicators of diseases associated with Hp, but also to predict their dynamics as a result of treatment. Of great scientific interest is the study of the sensitivity of Hp to antimicrobial drugs and pathogenicity factors.

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