Research Advances on the Role of *Helicobacter pylori* CagA Protein in the Pathogenesis of Gastric Cancer

Ronglu Xu[1], Wenchao Zhang[2]

[1]Jinping Kanghe Mental Hospital Company, Jinping, China

[2]Jinping Miao-Yao-Dai Autonomous County Center for Disease Control and Prevention, Jinping, China

Abstract

Helicobacter pylori (*Hp*) infection is a key environmental factor in the development of gastric cancer, with its virulence factor, the cytotoxin-associated gene A (CagA) protein, playing a central role in the pathogenic process. Extensive research data indicate that in regions with a high incidence of gastric cancer, the infection rate of *Hp* is significantly higher than in low-incidence areas, and *Hp* infection shows a clear geographical correlation with the incidence of gastric cancer. Moreover, the occurrence of intestinal metaplasia in *Hp*-positive patients is far more frequent than in *Hp*-negative individuals, suggesting that *Hp* may promote the formation of intestinal metaplasia.

This article systematically reviews recent research advances on the mechanisms by which the CagA protein drives the development and progression of gastric cancer, including interference with host cell signaling pathways, induction of epithelial-mesenchymal transition (EMT), promotion of immune evasion, synergy with environmental carcinogens, and regulation of key metabolic enzymes. As a major virulence factor of H. pylori, CagA can activate signaling networks such as SHP2, PI3K/Akt, MAPK, and Wnt/β-catenin through both phosphorylation-dependent and independent pathways. Current evidence suggests that this disrupts the balance between cell proliferation and apoptosis, although its role in pro-apoptotic effects may be relatively limited.

The aberrant activation of these signaling pathways may ultimately lead to uncontrolled cell proliferation, inhibited apoptosis, genomic instability, and microenvironment remodeling. In-depth investigation of the carcinogenic mechanisms of CagA can provide an important theoretical basis for molecular typing, risk prediction, and targeted intervention of gastric cancer, holding profound significance for its prevention and treatment.

Keywords

Helicobacter pylori, CagA Protein, Gastric Cancer, Pathogenesis

1. Introduction

Gastric cancer (GC) is one of the most common malignancies worldwide with high incidence and mortality rates, posing a serious threat to human health. Numerous epidemiological surveys and experimental studies have confirmed that chronic infection with *Helicobacter pylori* (*Hp*) is a significant environmental risk factor for gastric cancer, and the International Agency for Research on Cancer (IARC) has classified it as a Group I carcinogen [1].

Among the various virulence factors of *Hp*, the CagA protein, located at the end of the cag pathogenicity island (cag PAI), is widely regarded as a key driver in the progression of gastric mucosal lesions to cancer due to its ability to be directly injected into host cells and interfere with critical cellular signaling pathways [2].

With advances in medical research, recent years have witnessed new discoveries regarding the mechanisms by which the CagA protein contributes to the development and progression of gastric cancer. This article integrates the latest research findings to focus on the multi-dimensional mechanisms of CagA in gastric carcinogenesis, providing a systematic review of research progress aimed at offering a more comprehensive theoretical basis for the prevention, diagnosis, and treatment of gastric cancer.

Globally, the incidence of gastric cancer exhibits significant regional variations. Regions such as East Asia, South America, and Eastern Europe have higher incidence rates, while North America and Western Europe have relatively lower rates. This geographical disparity is largely correlated with the distribution of *Hp* infection rates. For instance, in East Asian countries like Japan and South Korea, high *Hp* infection rates correspond to some of the world's highest incidences of gastric cancer [3], further underscoring the close association between *Hp* infection, particularly CagA-positive *Hp* infection, and the development of gastric cancer.

Furthermore, gastric carcinogenesis is a complex, multi-stage process involving multiple factors. The progression from normal gastric mucosa to chronic non-atrophic gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia (intraepithelial neoplasia), and finally to invasive adenocarcinoma is known as the Correa cascade [4]. Throughout this prolonged process, the CagA protein plays a continuous role in various ways, driving the progression of lesions.

In-depth study of the mechanisms of CagA protein not only contributes to a better understanding of the development of gastric cancer but also provides important targets for new prevention and treatment strategies. For example, designing drugs targeting the structure and function of CagA or developing vaccines to prevent *Hp* infection may become potential means to reduce the incidence of gastric cancer [5].

2. Structural Characteristics and Translocation Mechanism of CagA Protein

CagA is a protein with a molecular weight of approximately 120–145 kDa, and its structural features are crucial to its function. The C-terminal region of CagA contains multiple EPIYA (Glu-Pro-Ile-Tyr-Ala) motifs, which vary in number and are key sites for interactions with various signaling molecules within host cells.

Based on differences in the flanking sequences of the EPIYA motifs, they can be classified into different types. Western strains primarily contain EPIYA-A, B, and C types, whereas East Asian strains mainly feature EPIYA-A, B, and D types. Studies have shown that CagA containing multiple EPIYA motifs, particularly the East Asian EPIYA-ABD pattern, is associated with stronger carcinogenic potential [6]. This is because different EPIYA motifs exhibit varying affinities and interaction modes when binding to host cell signaling molecules, leading to differences in the oncogenic capacity of CagA.

To exert its pathogenic effects, the CagA protein must enter the host cell, a process dependent on *Hp*'s type IV secretion system (T4SS). The T4SS is a complex molecular apparatus whose core components are encoded by genes on the cag PAI. These genes work together to form a "molecular syringe" structure capable of translocating CagA across the double-membrane barrier of the bacterium and the host cell into gastric epithelial cells.

The structure and function of the T4SS are essential for CagA translocation. It consists of multiple protein subunits, including channel proteins embedded in the bacterial membrane, adhesion proteins on the bacterial surface, and ATPases that provide energy. During CagA translocation, the T4SS first binds to receptors on the host cell surface, then undergoes a series of conformational changes to push CagA stepwise across the membranes into the host cell [7].

Additionally, certain structural features of CagA itself may facilitate its translocation via the T4SS. CagA may possess specific signal sequences recognized and preferentially transported by the T4SS. Moreover, the molecular weight and spatial structure of CagA may also affect translocation efficiency, with smaller size and more flexible structures potentially favoring passage through the T4SS channel [7].

A deep understanding of the structural characteristics and translocation mechanism of CagA is significant for comprehending its carcinogenic role. Studying the relationship between EPIYA motif types/numbers and carcinogenic potential can provide a basis for assessing the oncogenic risk of *Hp* infection. Research on the structure and function of the T4SS may offer targets for developing drugs that block CagA translocation, thereby inhibiting its pathogenic effects.

3. Core Molecular Mechanisms by Which CagA Drives Gastric Carcinogenesis

3.1 Phosphorylation-Dependent Activation of Signaling Pathways and Cellular Phenotypic Disruption

Upon injection into host cells, tyrosine residues within the EPIYA motifs of CagA can be phosphorylated (pCagA) by host cell Src family kinases (SFKs) or c-Abl kinase. This phosphorylation is a critical step for CagA's oncogenic actions. Phosphorylated CagA can recruit and aberrantly activate the SH2 domain-containing tyrosine phosphatase SHP2.

Under normal conditions, SHP2 activity is tightly regulated; it participates in growth factor signaling and modulates physiological processes such as cell growth, differentiation, and metabolism. However, when pCagA binds to SHP2 forming a complex, it may lead to sustained activation of SHP2. Persistent SHP2 activation can then aberrantly activate the downstream Ras/ERK MAPK signaling pathway.

The Ras/ERK MAPK pathway plays a key regulatory role in cell proliferation, differentiation, and survival. Its aberrant activation can promote cell proliferation, enhance invasive capacity, and inhibit apoptosis, creating conditions for tumorigenesis. Studies indicate that sustained activation of the Ras/ERK MAPK pathway in gastric cancer cells may lead to dysregulated cell cycle control and abnormal proliferation, contributing to tumor formation [8].

Besides activating the Ras/ERK MAPK pathway, pCagA can also bind and inhibit the serine/threonine kinase Par1b (also known as MARK2). Par1b is a key regulator maintaining cell polarity and microtubule stability, achieved through phosphorylation of downstream target proteins.

Inhibition of Par1b by pCagA may result in loss of cell polarity, disruption of tight junctions, and cytoskeletal rearrangement. Loss of polarity prevents cells from maintaining normal morphology and function, while disrupted tight junctions increase intercellular permeability, facilitating tumor cell invasion and metastasis. Cytoskeletal rearrangement can significantly enhance cell migration and invasion, which are critical steps in malignant transformation and metastasis of epithelial cells [9].

In normal epithelial cells, tight and adherent junctions form an orderly tissue structure with defined polarity, enabling specific physiological functions. When Par1b is inhibited, this orderly structure may be disrupted, granting cells enhanced motility and making them more likely to detach from the primary site, invade surrounding tissues and blood vessels, and metastasize.

3.2 Phosphorylation-Independent Interference with Signaling Pathways

Non-phosphorylated CagA can also exert oncogenic effects by interfering with host cell signaling pathways through various mechanisms. Aberrant activation of the c-Met receptor is one important mechanism. Non-phosphorylated CagA can directly bind to the intracellular domain of the hepatocyte growth factor receptor c-Met in host cells. This binding mimics the effect of hepatocyte growth factor (HGF) stimulation, causing c-Met dimerization and autophosphorylation in the absence of ligand.

Dimerization and autophosphorylation of c-Met can activate its downstream PI3K/Akt and Ras/MAPK signaling pathways. These pathways play key roles in cell proliferation, survival, and migration. Research indicates that activation of the PI3K/Akt pathway promotes cell survival and inhibits apoptosis, while activation of the Ras/MAPK pathway may enhance cell proliferation and migration [10]. Therefore, non-phosphorylated CagA, by activating the c-Met receptor and its downstream pathways, may promote the growth and metastasis of gastric cancer cells.

CagA can also interfere with the Wnt/β-catenin signaling pathway by disrupting the E-cadherin/catenin complex. Binding of CagA to the intracellular domain of E-cadherin may destabilize the E-cadherin/β-catenin/α-catenin complex. This complex is crucial for maintaining adherens junctions, tightly connecting adjacent cells and preserving tissue integrity and cell polarity.

Destabilization of this complex can lead to release of β-catenin into the cytoplasm. Cytosolic β-catenin can then translocate to the nucleus, where it binds to TCF/LEF transcription factors, aberrantly activating target genes of the canonical Wnt/β-catenin signaling pathway (e.g., c-Myc, Cyclin D1). Activation of these genes may promote cell proliferation and stemness maintenance, thereby driving gastric carcinogenesis.

The ERM protein family (Ezrin/Radixin/Moesin) may participate in cytoskeletal remodeling and signal transduction during this process, exacerbating disruption of the epithelial barrier and acquisition of malignant behaviors. ERM proteins link the plasma membrane to the cytoskeleton and are involved in cell shape maintenance, migration, adhesion, and signal transduction. When CagA disrupts the E-cadherin/catenin complex, ERM proteins might further promote cytoskeletal rearrangement through interactions with the cytoskeleton, enhancing cell migration and invasion.

Furthermore, CagA's binding to phosphoinositides may also influence PI3K/Akt pathway activation. CagA has the ability to specifically bind phosphoinositides (e.g., PIP2, PIP3) on the host cell membrane. This binding not only anchors CagA to the plasma membrane but may also locally modify the lipid environment or directly activate PDK1 and Akt, leading to hyperactivation of the PI3K/Akt signaling pathway.

Akt, as a key pro-survival kinase, upon activation can significantly inhibit apoptosis and accelerate cell cycle progression and metabolic reprogramming. In gastric cancer cells, hyperactivation of the PI3K/Akt pathway may lead to inhibited apoptosis, aberrant proliferation, and altered metabolism to meet the energy demands of rapidly growing tumor cells.

3.3 Induction of Epithelial-Mesenchymal Transition (EMT)

Epithelial-mesenchymal transition (EMT) is a core process in tumor invasion and metastasis, characterized by loss of epithelial polarity and acquisition of mesenchymal cell morphology, migration, and invasive capabilities. Recent research has deeply elucidated the mechanisms by which CagA induces EMT in gastric cancer cells [11].

CagA can significantly upregulate the expression of mesenchymal markers (N-cadherin, Vimentin, Snail, Slug, Twist) and downregulate the epithelial marker E-cadherin. This change alters the morphology and function of gastric cancer cells, transitioning them from an epithelial to a mesenchymal phenotype, thereby conferring enhanced migration and invasion abilities.

The mechanisms primarily involve activation of multiple pro-EMT signaling pathways. Among these, the TGF-β/Smad pathway is significant. CagA may enhance TGF-β signaling through direct or indirect means, activating Smad transcription factors. Activated Smad factors enter the nucleus and upregulate the expression of EMT-related transcription factors like Snail and Slug, driving the EMT process.

The PI3K/Akt/GSK3β pathway is also an important route for CagA-induced EMT. CagA-activated Akt can phosphorylate and inhibit GSK3β activity. Normally, GSK3β phosphorylates and promotes the degradation of transcription factors like Snail and Slug, maintaining the epithelial phenotype. When GSK3β activity is inhibited, the stability of Snail and Slug increases, allowing them to function persistently and promote EMT.

The MAPK pathway also plays a crucial role in CagA-induced EMT. CagA-activated ERK MAPK can phosphorylate and stabilize transcription factors like Snail, leading to their accumulation and subsequent promotion of EMT-related gene expression.

Additionally, as mentioned earlier, CagA disruption of the E-cadherin complex causes β-catenin nuclear translocation, directly activating the transcription of EMT-related genes, which is another mechanism of CagA-induced EMT. The synergistic action of these signaling pathways may ultimately drive morphological changes and enhanced migration/invasion in gastric cancer cells, laying the groundwork for metastasis.

During tumor progression, EMT enables tumor cells to detach from the primary tumor, invade surrounding tissues and blood vessels, and metastasize to other body parts via the circulatory or lymphatic systems, forming new metastatic lesions. Therefore, CagA-induced EMT plays a significant role in gastric cancer metastasis and is a key factor contributing to poor prognosis in patients.

3.4 Promotion of Immune Evasion

Immune evasion within the tumor microenvironment is a critical aspect of tumor development, allowing tumor cells to escape attack by the host immune system and thus grow and metastasize continuously. CagA plays an important role in immune evasion in gastric cancer.

Studies show that CagA, by activating host cell signaling pathways, can promote the generation and secretion of PD-L1-rich exosomes by gastric cancer cells [12]. Exosomes are small vesicles secreted by cells, containing bioactive substances like proteins and nucleic acids, and can mediate intercellular communication.

These PD-L1-carrying exosomes released into the tumor microenvironment can be taken up by T cells. PD-L1 on the exosome surface binds to PD-1 on T cells, delivering inhibitory signals that lead to T cell exhaustion, reduced proliferation, and decreased cytokine secretion (e.g., IFN-γ), thereby suppressing anti-tumor immune responses [12]. T cells are crucial in the immune system's fight against tumors; their functional impairment prevents the body from effectively recognizing and eliminating tumor cells, allowing their survival and proliferation.

Furthermore, CagA may affect immune cells in the tumor microenvironment through other mechanisms. For example, studies indicate CagA may induce tumor cells to secrete immunosuppressive cytokines like IL-10 and TGF-β, which can inhibit immune cell activity, further promoting immune evasion. Simultaneously, CagA might also affect the function of macrophages, dendritic cells, and other immune cells, shifting them from an anti-tumor phenotype to a pro-tumor growth phenotype.

3.5 Synergistic Effects with Carcinogens

Environmental carcinogens also play a significant role in gastric carcinogenesis, and *Hp* infection (especially CagA+ strains) may act synergistically with them [13]. MNNG (N-methyl-N'-nitro-N-nitrosoguanidine), a nitrosamine compound found in pickled foods, is a potent carcinogen.

Studies using gastric mucosal epithelial cells or animal models treated with both MNNG and CagA-positive *Hp* strains [14] showed that compared to single treatment groups (MNNG alone or *Hp* infection alone), the combined treatment group (MNNG + CagA+ *Hp*) significantly accelerated the malignant transformation of gastric mucosal epithelial cells. Specific manifestations included abnormally vigorous proliferation, blocked apoptosis, markedly increased cellular atypia, significantly enhanced anchorage-independent growth ability (soft agar colony formation), and ultimately a greatly increased tumor formation rate.

The mechanism of this synergy involves multiple aspects. First, CagA, by inducing chronic inflammation (generating ROS/RNS), may cause oxidative damage to cells. ROS/RNS are reactive oxygen and nitrogen species that can attack biological macromolecules like DNA, proteins, and lipids, leading to DNA damage and gene mutations.

Second, CagA may also inhibit DNA repair processes and interfere with P53 function. P53 plays a vital role in DNA damage repair, activating the expression of DNA repair-related genes and promoting damage repair. When P53 function is impaired, cells may fail to efficiently repair DNA damage, leading to accumulation of mutations and accelerated malignant transformation.

Additionally, CagA may alter epigenetic modifications (DNA methylation, histone modification abnormalities). Epigenetic modifications are chemical changes that do not alter the DNA sequence but can affect gene expression, playing important regulatory roles in cell growth, differentiation, and carcinogenesis. Aberrant epigenetic modifications induced by CagA may suppress the expression of tumor suppressor genes and activate oncogenes, further promoting malignant transformation.

MNNG, as a carcinogen, can directly damage DNA and cause gene mutations. When combined with CagA-positive *Hp* strains, CagA may amplify the genotoxicity of MNNG through the above mechanisms, exacerbating cellular damage, accelerating the accumulation of mutations, and thus significantly speeding up the process of malignant transformation.

In daily life, pickled foods are a major source of MNNG. Long-term consumption of pickled foods may increase the risk of gastric cancer, and this risk could be further elevated in individuals with *Hp* infection (especially CagA+ strains). Therefore, reducing the intake of pickled foods and timely eradication of *Hp* infection are crucial for preventing gastric cancer.

3.6 Regulation of Metabolic Reprogramming

Tumor cells exhibit significant metabolic differences compared to normal cells, requiring metabolic changes to meet the energy and biosynthetic demands of rapid proliferation [15]. The CagA protein can influence the metabolic processes of gastric cancer cells by regulating the expression of key metabolic enzymes, thereby promoting tumor development.

Studies indicate that CagA can specifically upregulate the expression of α-enolase (ENO1) in host gastric epithelial cells

[16]. ENO1 is a dual-function protein; as a key glycolytic enzyme, it catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate (PEP), a critical step in glycolysis. Simultaneously, ENO1 can also be expressed on the cell surface, acting as a plasminogen receptor involved in extracellular matrix degradation and remodeling.

CagA-induced high expression of ENO1 may, on one hand, enhance glycolytic flux (Warburg effect) in cells. The Warburg effect is a hallmark metabolic feature of tumor cells, where they primarily rely on glycolysis for energy even under aerobic conditions, rather than more efficient oxidative phosphorylation. This metabolic mode provides ample energy and metabolic intermediates for rapid tumor cell proliferation, meeting biosynthetic needs such as nucleic acid, protein, and lipid synthesis.

On the other hand, cell surface ENO1, by binding plasminogen and promoting its activation to plasmin, may enhance the cell's ability to degrade the basement membrane and extracellular matrix. The basement membrane and extracellular matrix are physical barriers to tumor cell invasion and metastasis; their degradation facilitates tumor cells breaching these barriers, invading surrounding tissues and blood vessels, and metastasizing.

Besides ENO1, CagA may also affect the metabolism of gastric cancer cells by regulating the expression of other metabolic enzymes. For instance, CagA might influence the expression of enzymes related to glucose transport, amino acid metabolism, and lipid metabolism, further optimizing the metabolic environment for tumor cell growth and metastasis.

In-depth research into the regulatory mechanisms of CagA on tumor cell metabolic reprogramming not only aids in better understanding the metabolic characteristics of tumor cells but also provides targets for developing new anti-tumor drugs. For example, inhibiting ENO1 activity or expression might suppress glycolysis and invasive/metastatic capabilities of tumor cells, offering a therapeutic approach for gastric cancer.

4. Role of CagA in Correa's Cascade

Correa's cascade describes the classic pathological progression from *Hp*-associated chronic gastritis to gastric cancer, specifically: normal gastric mucosa → chronic non-atrophic gastritis → chronic atrophic gastritis → intestinal metaplasia → dysplasia (intraepithelial neoplasia) → invasive adenocarcinoma [17]. Throughout this process, CagA-positive *Hp* infection plays a pivotal role, acting as a key factor initiating and driving progression at each stage. The induced persistent inflammatory damage, imbalance between cell proliferation/apoptosis, EMT, and genetic and epigenetic alterations persist throughout.

At the stage of normal gastric mucosa, *Hp* binds to gastric epithelial cells via surface adhesins and injects the CagA protein into host cells. CagA injection triggers a series of cellular responses, first potentially causing local inflammation and attracting infiltration of inflammatory cells like neutrophils and macrophages. These cells release large amounts of inflammatory factors, reactive oxygen species (ROS), and reactive nitrogen species (RNS), damaging the gastric mucosa and initiating chronic non-atrophic gastritis.

With persistent *Hp* infection, the continuous action of CagA may lead chronic non-atrophic gastritis to progress to chronic atrophic gastritis. At this stage, CagA, by interfering with cell signaling pathways, may cause an imbalance between proliferation and apoptosis of gastric mucosal cells, gradual atrophy of gastric glands, and reduced acid secretion. Simultaneously, CagA may affect the differentiation of gastric mucosal stem cells, altering the structure and function of the gastric mucosa.

Further progression of chronic atrophic gastritis may lead to intestinal metaplasia, where gastric mucosal epithelial cells are replaced by intestinal-type epithelial cells. CagA likely contributes to this process through induction of EMT and alterations in epigenetic modifications. As mentioned, CagA can activate multiple pro-EMT signaling pathways, promoting the transition of gastric epithelial cells to a mesenchymal phenotype, laying the foundation for intestinal metaplasia. Meanwhile, aberrant epigenetic modifications caused by CagA may alter the expression of genes related to intestinal cell differentiation, promoting the formation of intestinal metaplasia.

Following intestinal metaplasia, the stage of dysplasia (intraepithelial neoplasia) occurs, which is a precancerous lesion. Here, sustained interference by CagA with cell signaling pathways may lead to uncontrolled proliferation, genomic instability, and significant abnormalities in cell morphology and structure. The synergistic effect of CagA with environmental carcinogens may also become more pronounced at this stage, accelerating malignant transformation.

Ultimately, dysplasia may progress to invasive adenocarcinoma. During this process, CagA-induced EMT grants tumor cells enhanced invasive and metastatic capabilities, enabling them to breach the basement membrane, invade surrounding tissues and blood vessels, and form invasive growth and distant metastases.

The ERM protein family (Ezrin, Radixin, Moesin) are bridge molecules connecting the plasma membrane to the cytoskeleton, involved in cell shape maintenance, migration, adhesion, and signal transduction. Studies show that in *Hp*-associated gastric mucosal lesions (e.g., intestinal metaplasia, dysplasia), especially with CagA+ strains, the expression and/or activity (phosphorylation status) of ERM proteins may be aberrantly altered [18].

These changes may participate in carcinogenesis through various mechanisms. First, ERM proteins might be involved in CagA-induced cytoskeletal rearrangement and loss of polarity. As mentioned, CagA can cause cytoskeletal

rearrangement and polarity disruption, and ERM proteins, as molecules linking the membrane to the cytoskeleton, may mediate this process, exacerbating abnormalities in cell morphology and structure.

Second, ERM proteins may affect the transmission of inflammatory signaling pathways (e.g., NF-κB activation). NF-κB is a key inflammatory transcription factor whose activation promotes the expression of inflammatory factors, exacerbating inflammation. Altered expression or activity of ERM proteins might influence NF-κB activation, further promoting persistent chronic inflammation and accelerating the progression of gastric mucosal lesions.

Moreover, ERM proteins can promote cell migration and invasion, accelerating the progression to higher-grade lesions (e.g., from dysplasia to cancer). Additionally, crosstalk exists between ERM proteins and signaling pathways interfered with by CagA (e.g., PI3K/Akt, Rho GTPase), collectively promoting carcinogenesis. This crosstalk complicates the intracellular signaling network, further enhancing the oncogenic effects of CagA.

5. Potential Diagnostic and Therapeutic Strategies Based on CagA Pathogenic Mechanisms

In-depth understanding of the oncogenic mechanisms of CagA provides new perspectives for the prevention and treatment of gastric cancer. Based on these mechanisms, various potential diagnostic and therapeutic strategies can be developed.

5.1 *Hp* Eradication Therapy

Eradication of *Hp*, especially CagA-positive strains, at the precancerous lesion stage (atrophic gastritis, intestinal metaplasia, low-grade intraepithelial neoplasia) has been confirmed by numerous studies and clinical trials as an effective primary prevention strategy. By eliminating the source of CagA+ *Hp* infection, the progression of the Correa cascade can be blocked, significantly reducing the risk of gastric cancer [19].

Currently, the commonly used clinical regimen for *Hp* eradication is bismuth-containing quadruple therapy: proton pump inhibitor (PPI) + bismuth + two antibiotics [20]. This regimen effectively inhibits *Hp* growth and reproduction, improving eradication rates. However, with increasing *Hp* resistance, some patients may experience treatment failure. Therefore, antibiotics should be chosen rationally based on individual patient circumstances to develop personalized treatment plans.

Furthermore, patients after *Hp* eradication require regular follow-up and monitoring to prevent reinfection and the development of gastric cancer.

5.2 CagA as a Biomarker

Detecting serum anti-CagA antibodies or analyzing the CagA EPIYA genotype of the infecting strain (especially multi-copy EPIYA-C/D) can help identify high-risk populations for gastric cancer, enabling closer monitoring and endoscopic follow-up.

Serum anti-CagA antibody testing is a simple, non-invasive method. Detecting the presence of anti-CagA antibodies in serum can indicate past infection with CagA-positive *Hp*. Patients positive for these antibodies have a relatively higher risk of gastric cancer and require more intensive monitoring.

Analyzing the CagA EPIYA genotype of the infecting strain allows for more accurate assessment of carcinogenic risk [21]. As mentioned, CagA containing multiple EPIYA motifs, particularly the East Asian EPIYA-ABD pattern, is associated with stronger carcinogenic potential. Genotype analysis can identify patients at high risk, enabling personalized prevention and monitoring strategies.

5.3 Drug Development Targeting CagA-Activated Signaling Pathways

Developing inhibitors targeting key oncogenic pathways activated by CagA is a research hotspot, and these inhibitors hold promise as new therapeutic agents for gastric cancer.

SHP-2 inhibitors: e.g., SHP099, which can block the formation of the p-CagA/SHP-2 complex, thereby inhibiting sustained SHP2 activation and the aberrant activation of its downstream pathways, suppressing tumor cell proliferation and invasion.

Immune checkpoint inhibitors: e.g., anti-PD-1/PD-L1 antibodies, which can reverse CagA-induced exosomal PD-L1-mediated immunosuppression, restore T cell function, and enhance anti-tumor immune responses.

Wnt pathway inhibitors: e.g., PRI-724 (targeting β-catenin), which can inhibit the CagA-activated Wnt signaling pathway, reduce β-catenin nuclear translocation and subsequent target gene activation, thereby suppressing cell proliferation and stemness maintenance.

Most of these drugs are currently in clinical trials, requiring further research to verify their efficacy and safety.

5.4 Novel Vaccines

CagA vaccines aim to induce mucosal and systemic immunity to prevent or clear *Hp* infection (especially CagA+ strains), blocking the carcinogenic process at its source. Although challenges remain, vaccine development is an

important direction for preventing *Hp*-associated gastric cancer [22].

Current CagA vaccines under research include, for example, a CagA/UreB Salmonella vector oral vaccine [23]. This vaccine uses a Salmonella vector carrying genes for CagA and UreB (another *Hp* virulence factor) administered orally. After ingestion, the Salmonella colonizes the intestine and expresses CagA and UreB proteins, inducing mucosal and systemic immune responses, producing antibodies and immune cells against *Hp* to prevent or clear the infection.

However, vaccine development faces numerous challenges, such as immunogenicity, safety, and durability. Further optimization of vaccine design (e.g., multivalent vaccines, novel adjuvants, delivery systems) is needed to improve protective efficacy and longevity.

6. Summary and Outlook

The CagA protein, as a key virulence factor of H. pylori, exerts its molecular regulatory effects by interacting with host proteins and interfering with multiple signaling pathways, including SHP2, PI3K/Akt, MAPK, and Wnt/β-catenin. This interference can lead to induction of epithelial-mesenchymal transition (EMT), promotion of immune evasion (particularly through exosomal PD-L1 expression), synergy with environmental carcinogens (e.g., MNNG), and regulation of metabolic reprogramming (e.g., upregulation of α-enolase). These mechanisms work together to drive the malignant transformation of gastric mucosal epithelial cells and accelerate the development of gastric cancer. CagA plays a significant role throughout the Correa's Cascade.

Past research has deeply explored the structural characteristics, translocation mechanisms, and various roles of the CagA protein in gastric carcinogenesis, providing an important theoretical foundation for understanding the pathogenesis of gastric cancer. Based on these findings, potential diagnostic and therapeutic strategies have been developed, such as *Hp* eradication therapy, CagA as a biomarker, drugs targeting CagA-activated pathways, and novel vaccines, offering new hope for the prevention and treatment of gastric cancer.

However, many questions remain requiring further research. For instance, the precise three-dimensional structure of CagA interactions with host target proteins is not fully elucidated, limiting the development of highly specific inhibitors; personalized precision prevention and therapeutic systems based on CagA molecular typing are not yet perfected, needing more research to establish finer risk prediction models; the clinical translation of CagA-targeted therapies and effective vaccines faces numerous challenges, requiring further optimization of efficacy and safety.

Future research should focus on the following areas:

Utilizing advanced techniques like cryo-electron microscopy and X-ray crystallography to reveal the precise 3D structures of interactions between key domains of CagA (e.g., EPIYA motifs, CM sequence) and host target proteins (e.g., SHP2, Par1b, c-Met, E-cadherin). This structural information is crucial for understanding how CagA affects cell signaling and provides a basis for developing highly specific inhibitors.

Developing individualized precision prevention and therapeutic systems based on CagA molecular typing: Integrating the polymorphism of the H. pylori cagA gene 3' terminal sequence (EPIYA-A, B, C, D types and their varying repeat combinations), expression levels, phosphorylation status, and downstream activated signaling pathway profiles to establish more refined risk prediction models and guide personalized drug intervention and monitoring strategies for subpopulations with different carcinogenic mechanisms.

Accelerating the clinical translation of CagA-targeted therapies and effective vaccines: Promoting CagA signaling pathway inhibitors showing promise in gastric cancer treatment into clinical trials; optimizing vaccine design (e.g., multivalent vaccines, novel adjuvants, delivery systems) to improve protective efficacy and durability.

Continued in-depth exploration of the carcinogenic mechanisms of CagA will not only contribute to a more comprehensive understanding of the molecular basis of gastric cancer but also provide key scientific basis and targets for developing more effective prevention, early diagnosis, and personalized treatment strategies, ultimately helping to reduce the incidence and mortality of gastric cancer and improve human health.

References

[1] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum. 2012;100B:1-441.

[2] Hatakeyama M. *Helicobacter pylori* CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. Cell Host Microbe. 2014;15(3):306-316. doi:10.1016/j.chom.2014.02.008

[3] Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249.

[4] Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. Cancer Res. 1992;52(24):6735-6740.

[5] Zeng M, Mao XH, Li JX, et al. Efficacy, safety, and immunogenicity of an oral recombinant *Helicobacter pylori* vaccine in children in China: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;386(10002):1457-1464.

[6] Hatakeyama M. Structure and function of *Helicobacter pylori* CagA, the first-identified bacterial protein involved in human cancer. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93(4):196-219.

[7] Backert S, Tegtmeyer N, Fischer W. Composition, structure and function of the *Helicobacter pylori* cag pathogenicity island encoded type IV secretion system. Future Microbiol. 2015;10(6):955-965.

[8] Zhang X, Zhang P. *Helicobacter pylori* CagA promotes gastric cancer development via the Ras/ERK signaling pathway. Oncol Rep. 2020;43(2):499-508.
[9] Saadat I, Higashi H, Obuse C, et al. *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature. 2007;447(7142):330-333.
[10] Tegtmeyer N, Wessler S, Backert S. Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J. 2017;284(6):858-878.
[11] Yong X, Tang B, Li BS, et al. *Helicobacter pylori* virulence factor CagA promotes tumorigenesis of gastric cancer via multiple signaling pathways. Cell Commun Signal. 2020;18(1):1-15.
[12] Yue X, Zhao Y, Zhang Y, et al. *Helicobacter pylori* CagA promotes immune escape of gastric cancer cells via exosomal PD-L1. J Exp Clin Cancer Res. 2021;40(1):1-15.
[13] Xue L, Forman D. *Helicobacter pylori* and gastric cancer: the role of environmental factors. Curr Opin Gastroenterol. 2019;35(6):511-516.
[14] Toh JWT, Wilson RB. Pathogenesis of *Helicobacter pylori* infection. Scand J Gastroenterol. 2020;55(8):904-910.
[15] Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. Cell Metab. 2016;23(1):27-47.
[16] Zhang Y, Zhang Y, Xia L. CagA promotes glycolysis and proliferation of gastric cancer cells via regulating ENO1 expression. Front Oncol. 2020;10:538. [17] Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis. 2012;13(1):2-9.
[17] Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis. 2012;13(1):2-9.
[18] Hayashi T, Senda M, Suzuki N, et al. Differential mechanisms for SHP2 binding and activation are exploited by geographically distinct *Helicobacter pylori* CagA oncoproteins. Cell Rep. 2017;20(12):2876-2890.
[19] Ford AC, Yuan Y, Moayyedi P. *Helicobacter pylori* eradication therapy to prevent gastric cancer: systematic review and meta-analysis. Gut. 2020;69(12):2113-2121.
[20] Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6-30.
[21] Jones KR, Joo YM, Jang S, et al. Polymorphism in the CagA EPIYA motif impacts development of gastric cancer. J Clin Microbiol. 2009;47(4):959-968.
[22] Sutton P, Boag JM. Status of vaccine research and development for *Helicobacter pylori*. Vaccine. 2019;37(50):7295-7299.
[23] Akhiani AA, Pappo J, Kabok Z, et al. Protection against *Helicobacter pylori* infection following immunization is IL-12-dependent and mediated by Th1 cells. J Immunol. 2002;169(12):6977-6984.