

Helicobacter pylori infection status and evolution of gastric cancer

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Abstract

Gastric cancer (GC) is a globally prevalent malignancy with a particularly heavy burden in China. *Helicobacter pylori* (*H. pylori*) is a Group I carcinogen for GC, with a higher seroprevalence rate indicating a higher GC incidence. However, only approximately 3% of the individuals with *H. pylori* infection eventually develop GC, and about 2.6% still progress to GC even 10–20 years after the eradication of *H. pylori*. Thus, the pathogenic mechanism of *H. pylori* for GC must be elucidated, and high-risk individuals precisely identified. Furthermore, GC can occur even in individuals who have never been infected with *H. pylori*. As *H. pylori* infection rates decline, the proportion of *H. pylori*-negative GC cases is increasing annually, gaining significant research attention. In this review, potential pathogenic mechanisms of *H. pylori* infection are explored from the aspects of *H. pylori* virulence factors and host factors (genetic susceptibility and immune microenvironment). Possible risk factors for *H. pylori*-negative GC include infections by other microorganisms (e.g., bacteria, fungi, and viruses), autoimmune gastritis, bile reflux, genetic mutations, and environmental factors. We aim to review the potential mechanisms for GC with varying *H. pylori* infection statuses, identify the high-risk individuals, and pose questions that need to be addressed. In the future, as the prevalence of *H. pylori* infection gradually decreases, GC prevention and management must evolve to address host-specific factors and the growing challenge of *H. pylori*-negative GC by integrating multidisciplinary perspectives.

Keywords: Gastric cancer; *Helicobacter pylori*; Host susceptibility; Gastric microbiota; Autoimmune gastritis; Bile reflux; Inflammation-cancer transformation

Introduction

Gastric cancer (GC) is a globally prevalent malignancy that poses a severe threat to human health. GC ranks in the top five malignancies in both incidence and mortality, accounting for 4.9% of all cancer cases and 6.8% of all cancer-related deaths globally. Approximately 1 million new GC cases are diagnosed annually worldwide, with more than 650,000 attributable deaths.^[1] Notably, China has a particularly heavy GC burden. In 2024, approximately 232,310 new GC cases and 171,388 deaths were recorded in China.^[2] Thus, clarifying the risk factors and pathogenesis of GC is crucial for early diagnosis and treatment.

Helicobacter pylori (*H. pylori*) is classified by the World Health Organization as a Group I carcinogen and has a crude global prevalence of over 50%. Globally, nearly 90% of non-cardia GC cases are associated with *H. pylori* infection.^[3,4] Regions with a high incidence of GC, such as South Korea, China, and Japan, tend to have higher *H. pylori* seroprevalence rates. In China, 78.5% of non-cardia

GC and 62.1% of cardia GC cases are attributable to *H. pylori* infection.^[5] *H. pylori* eradication is important for GC prevention. A large-scale study based on the community in China demonstrated that successful eradication reduces GC incidence, whereas failed eradication cases did not show this trend.^[6] Specifically, *H. pylori* eradication can reduce the risk of GC by 43% after over 20 years.^[7] In recent decades, the global incidence and mortality rates of GC have shown a consistent decline in both sexes, similar to the declining trend of *H. pylori* prevalence.^[1,3,4] However, the correlation between *H. pylori* and GC is not always consistent. For instance, India and Bangladesh are known to have a high prevalence of *H. pylori* but a low incidence of GC. Furthermore, while only approximately 3% of individuals with *H. pylori* infection develop GC, approximately 2.6% of the population still develop GC even 10–20 years after *H. pylori* eradication.^[4,7] Therefore, GC induced by *H. pylori* infection is closely linked to host factors, such as genetic background and the immune

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microenvironment, emphasizing the significance of identifying individuals at high risk of developing GC.

However, a considerable percentage of GC cases are not fully associated with *H. pylori* infections. While significantly less common than *H. pylori*-positive GC, *H. pylori*-negative GC occurs in gastric mucosa that has never been infected with *H. pylori*, with a cumulative incidence of approximately 4.2 per 10,000 persons.^[8] Although it accounts for 0.4–3.6% of all gastric dysplasia and GC cases, *H. pylori*-negative GC has shown a gradually increasing trend.^[9] Compared with *H. pylori*-positive GC, *H. pylori*-negative GC tends to occur at a younger age (average age: 50–59 years), with a higher proportion of female patients. Moreover, undifferentiated GC, especially diffuse-type GC (DGC), is more common among *H. pylori*-negative than *H. pylori*-positive populations.^[10] Possible risk factors include genetic mutations, infections with other microorganisms, autoimmune gastritis (AIG), and bile reflux. Therefore, *H. pylori*-negative GC also deserves more attention [Figure 1].

Till now, the diagnostic criteria of *H. pylori*-negative GC remain controversial due to the confusion in distinguishing between *H. pylori*-negative and post-*H. pylori* eradication. Furthermore, the pathogenesis of GC with different *H. pylori* infection statuses has yet to be elucidated. In this review, we explore the recent advances in the potential pathogenesis of GC associated with different *H. pylori* infection statuses and provide some perspectives for the future studies.

Pathogenic Mechanisms of *H. pylori* Infection

Virulence factors of *H. pylori*

Urease and flagellin proteins

Upon infection, the majority (approximately 80%) of *H. pylori* colonize in the gastric mucus layer, with the remainder largely adhering to surface epithelial cells and gastric pits. Flagellin proteins (FlaA/FlaB) of *H. pylori* facilitate bacterial motility within the gastric mucosa. Urease can hydrolyze urea to produce ammonia, neutralize

gastric acid, and create a conducive environment for bacterial survival. In addition, urease can activate oxidative stress responses within host cells, causing cell damage.^[11]

Cytotoxin-associated gene A

Cytotoxin-associated gene A (CagA) was first identified as an immunodominant antigen. It is injected into host cells via the type IV secretion system (T4SS). Once inside the cell, the EPIYA (Glu-Pro-Ile-Tyr-Ala) motifs of CagA are phosphorylated by host cell kinases, activating downstream signaling pathways, such as the nuclear factor-κB (NF-κB) pathway and inducing interleukin-8 (IL-8)-mediated inflammatory responses. Moreover, CagA can disrupt cell junctions, release β-catenin, and thereby promote the proliferation and carcinogenesis of gastric epithelial cells.^[11] We recently demonstrated that the host gene AU-rich element binding factor1 (AUF1), which is activated by *H. pylori* via CagA through the phosphorylated extracellular signal-regulated kinase (p-ERK) pathway and cytoplasmic translocation, could suppress autophagic degradation of CagA and downregulate gastrin 1, a CagA inhibitor. Accumulated CagA activates inflammatory pathways intracellularly and spreads via exosomes to induce widespread inflammation, ultimately exacerbating *H. pylori*-associated gastritis and even GC risk.^[12]

Vacuolating cytotoxin A

Vacuolating cytotoxin A (VacA) is a cytotoxin that induces vacuole formation in host cells and inhibits autophagy and apoptosis. The VacA s1/m1 strain exhibits the strongest vacuolating cytotoxin activity and is significantly associated with increased GC risk. By binding to receptors on the host cell membrane, VacA interferes with intracellular signaling, causing cellular dysfunction and DNA damage. VacA can downregulate the expression of signal transducer and activator of transcription 3 (STAT3) and anti-apoptotic proteins B-cell lymphoma-2 and B-cell lymphoma-extra large, thereby inducing apoptosis in gastric epithelial cells.^[13]

Thioredoxin-1

By comparing the differentially expressed proteins in *H. pylori* strains between patients with GC and benign gastric disease, we found that thioredoxin-1 (Trx1) was significantly overexpressed in *H. pylori* strains isolated from patients with GC.^[14] Moreover, we demonstrated that *H. pylori* strains highly expressing Trx1 significantly reduced the survival rate of gastric epithelial cells while increasing the number of GC cells *in vitro*.^[15] In addition, we successfully established a Mongolian gerbil *H. pylori* infection model and identified that the high-Trx1 *H. pylori* group had earlier GC onset and more severe gastric mucosal lesions.^[16] During GC development, *H. pylori* with high Trx1 expression affects the stress response and function of redox-active proteins (such as 14-3-3α/β, glutathione S-transferase, and heat shock protein 70) in host cells. *H. pylori* with high Trx1 expression can more strongly adhere to gastric mucosal

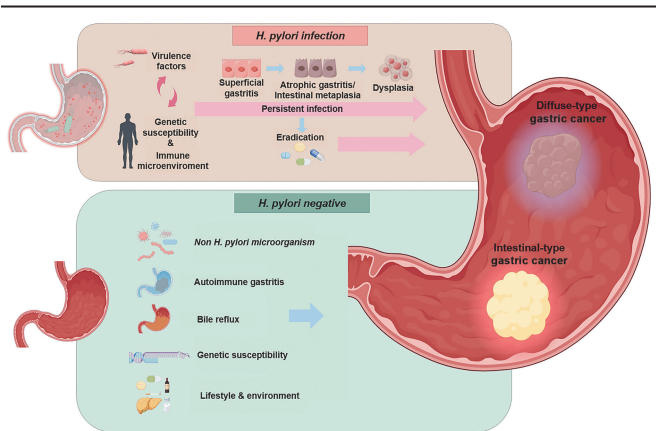


Figure 1: The evolution of GC with different *H. pylori* infection statuses. GC: Gastric cancer; *H. pylori*: *Helicobacter pylori*.

epithelial cells (GES-1), with higher expression levels of related adhesion factors, such as blood agglutination-associated protein A2, outer inflammatory protein A, and sialic acid-binding adhesin A. Mechanically, high-Trx1 *H. pylori* can significantly upregulate the expression levels of IL-23A, IL-17A, IL-8, IL-6, and tumor necrosis factor- α (TNF- α), while activating the NF- κ B signaling pathway, thereby promoting the inflammatory responses and the GC development.^[17]

Host determinants

Although *H. pylori* infection is a major risk factor for GC, not all infected individuals develop GC. This variation in individual susceptibility depends not only on *H. pylori* virulence but also on host factors, primarily genetic susceptibility and the responsiveness of the host immune response.

Host genetic susceptibility

Host genetic variations influence gene polymorphisms, inflammatory reactions, and immune response, thereby altering individual susceptibility to *H. pylori*-associated gastric pathologies, including GC. The next section introduces the polymorphisms for several main genes [Table 1].

Gene polymorphisms of pattern recognition receptor

Host cells recognize *H. pylori* pathogen-associated molecular patterns (PAMPs) through various pattern recognition receptors (PRRs), which induce the rapid production of pro-inflammatory cytokines to facilitate bacterial clearance. The two most important classes of PRRs are Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs).^[18] Single-nucleotide polymorphisms (SNPs) in TLRs are associated with *H. pylori* infection susceptibility and GC risk. The TLR locus 4p14, which contains the genes encoding for TLR1, TLR6, and TLR10, was negatively associated with *H. pylori* serological status in German and Dutch cohorts. Notably, the TLR locus 4p14 was associated with a reduced risk of precancerous gastric lesions and decreased *H. pylori* infection in a Chinese cohort.^[19] Various SNPs, including TLR4 rs11536889, TLR4 rs10759932, TLR1 rs4833095, TLR10 rs10004195, TLR5 rs1640827, TLR5 rs17163737, and TLR9 rs5743836, have been associated with an increased risk of *H. pylori*-related GC in different studies,^[18–20] indicating that genetic susceptibility to *H. pylori* infection indeed promotes carcinogenesis. Disruption of TLR signaling by knocking down myeloid differentiation primary response 88, the adaptor protein for all TLRs except TLR3, significantly reduced inflammation-associated GC in mice, demonstrating the direct impact of inflammatory TLR signaling on carcinogenesis. Although the mechanistic effects of cancer-associated TLR SNPs remain incompletely understood, the aforementioned regulation of TLR expression by TLR locus 4p14 implies that host genetic background may partly explain the increased susceptibility of some *H. pylori*-infected patients to cancer induction.^[19] Thus, genetic background screening may aid in the early identification of individuals at high risk for GC.

Table 1: Host genetic susceptibility factors for *H. pylori*-associated GC.

Categories	Genes	Specific polymorphisms/allele	Risk of GC
TLR	TLR4	rs11536889	↑
		rs10759932	↑
	TLR1	rs4833095	↑
	TLR10	rs10004195	↑
	TLR5	rs1640827	↑
		rs17163737	↑
	TLR9	rs5743836	↑
NLR	NOD1 (NLRC1)	rs2907749 (GG genotype)	↓
		rs7789045 (TT genotype)	↑
	NOD2 (NLRC2)	rs718226 (G allele)	↑
		rs2111235 (C allele)	↓
		rs7205423 (G allele)	↓
	NLRP3	rs10754558	↑
		rs4612666	↑
Cytokines	IL-1β	-511T allele	↑
	TNF-α	-308 A allele	↑
	IL-8	-251A allele	↑
DNA repair	ERCC2 (XPD)	K751Q polymorphism	↑
	XRCC1	R194W polymorphism	↑
	OGG1	S326C polymorphism	↑
	Homologous recombination genes	Pathogenic variants in ATM, BRCA1, BRCA2, PALB2	↑
	Mismatch repair genes	Pathogenic variants in MLH1, MSH2, MSH6	↑
	APC	Pathogenic variants	↑
	CDH1	Pathogenic variants	↑
HLA	HLA-A	HLA-A*02	↑
	HLA-C	HLA-Cw*5	↑
		HLA-Cw*3	↑
	HLA-DRB1	HLA-DRB1*01	↑
		HLA-DRB1*04:05	↑
		HLA-DRB1*04:04	↑
		HLA-DRB1*16:01	↑
	HLA-G	HLA-G*14bp DEL	↑
	MICA	MICA*009	↑
		MICA*A9	↑

ATM: Ataxia telangiectasia mutated; APC: Adenomatous polyposis coli; BRCA: Breast cancer; CDH1: Cadherin 1; ERCC: Excision repair cross-complementation group; HLA: Human leukocyte antigen; *H. pylori*: *Helicobacter pylori*; IL: Interleukin; MICA: Major histocompatibility complex class I polypeptide-related sequence A; MLH: mutL homolog; MSH: mutS homolog; NOD: Nucleotide-binding oligomerization domain; NLR: NOD-like receptor; NLRC: NOD-like receptor family caspase recruitment domain containing; NLRP: NOD-like receptor family pyrin domain containing; OGG: 8-Oxoguanine DNA glycosylase; PALB2: Partner and localizer of BRCA2; TLR: Toll-like receptor; TNF: Tumor necrosis factor; XPD: Xeroderma Pigmentosum Group D; XRCC: X-ray cross-complementation group; ↑: Increase; ↓: Decrease.

NLRs are also crucial in pathogen recognition and clearance. Genetic polymorphisms in NLRs have also been linked to *H. pylori*-associated GC. The NOD1 rs2907749 GG genotype is associated with lower GC risk, while carrying the NOD1 rs7789045 TT genotype is associated with higher GC risk.^[21] The expression levels of both NOD1 and IL8 messenger RNA are upregulated in inflammatory gastric mucosa and gastric tumor tissues, which is consistent with the *H. pylori*-induced inflammation–carcinogenesis sequence.^[22] NOD2 is also upregulated in *H. pylori*-related GC. The NOD2 rs718226 G allele is associated with an increased risk of gastric mucosal dysplasia and GC in Chinese patients, whereas the NOD2 rs2111235 C allele and rs7205423 G allele showed a reduced risk of disease progression in patients with *H.*

pylori infection.^[23] A reduced *NOD1/2*-mediated immune response to *H. pylori* infections contributes to bacterial persistence, while the subsequent hyperactivation of other inflammatory responses may induce inflammation-associated carcinogenesis.

The recognition of multiple *H. pylori* virulence factors by PRRs in gastric epithelial or immune cells triggers downstream NF- κ B signaling. This induces the transcriptional upregulation of NOD-like receptor family pyrin domain-containing 3 (*NLRP3*) and pro-IL-1 β /pro-IL-18, thereby initiating inflammasome formation.^[24] The *NLRP3* and NOD-like receptor family caspase recruitment domain containing (*NLRC4*) inflammasomes have been clearly implicated in *H. pylori* infection. *NLRP3* rs10754558 and *NLRP3* rs4612666, in combination with *H. pylori* infection, increased the risk of GC. Thus, host genetic factors affecting inflammasome formation interact with *H. pylori* infection to influence GC risk.

Cytokine-related genes polymorphisms

Polymorphisms occur in genes encoding cytokine receptors and antagonists, including IL-10, TNF- α , IL-1 β , and IL-1, thereby affecting cytokine expression. Multiple studies indicate a close association between polymorphisms in cytokine-related genes and GC risk. For instance, IL-1 β increases in the gastric mucosa of *H. pylori*-infected individuals. Transgenic mice overexpressing human IL-1 β in parietal cells spontaneously develop gastritis and dysplasia after 1 year of age and exhibit increased dysplasia and carcinogenesis upon *H. pylori* infection. In addition, *H. pylori*-colonized individuals carrying high-expression *IL1 β* polymorphisms had a significantly increased risk of hypochlorhydria, gastric atrophy, and gastric adenocarcinoma compared with those carrying genotypes associated with restricted *IL1 β* expression, as well as an increased GC risk associated with the *IL1 β* -511T allele.^[25] Importantly, these associations were observed in *H. pylori*-colonized individuals, but not in uninfected subjects, highlighting the critical role of host–environment interaction and inflammation in GC progression.

The expression of TNF- α , a pro-inflammatory acid-suppressive cytokine, is also increased in the gastric mucosa of *H. pylori*-colonized individuals. Polymorphisms associated with increased TNF- α expression are correlated with an increased risk of GC and its precursor lesions.^[26] A study by Machado *et al*,^[27] which included patients with chronic gastritis and GC, found that individuals carrying the TNF- α -308 A allele polymorphism had an increased risk of GC. Beyond the significant polymorphisms initially identified in the *IL1* and TNF- α gene clusters, further research has screened for disease-associated SNPs in other cytokines. For example, the early phase of the acute inflammatory response to *H. pylori* infection involves the release of the chemokine IL-8 by epithelial cells, inducing granulocytes to infiltrate the lamina propria to clear the infection. Ohyauchi *et al*^[28] demonstrated that expression of the *IL8*-251A allele was associated with the development of gastric ulcer, gastric atrophy, and ultimately GC.

Human leukocyte antigen polymorphisms

Human leukocyte antigen (HLA) molecules are essential mediators of adaptive immunity that bind foreign antigens or peptides and present them to CD4⁺ T cells, thereby initiating immune responses that target these invaders for removal. The resulting diversity in HLA protein function significantly influences the development and course of both infectious and non-infectious diseases. Notably, specific HLA variants are implicated in the dysregulated immune responses to *H. pylori* infection, a major risk factor for gastric carcinogenesis, potentially through mechanisms involving altered antigen presentation and chronic inflammation mechanisms.^[29] Specific classical HLA class I alleles have been associated with increased disease susceptibility in specific populations: *HLA-A**02 and *HLA-Cw**5 in Turkish cohorts, and *HLA-Cw**3 in Chinese cohorts. Among HLA class II alleles, *HLA-DRB1**01 (Chinese cohorts), *HLA-DRB1**04:05 (Japanese cohorts), *HLA-DRB1**04:04 (Korean cohorts), and *HLA-DRB1**16:01 (Swedish cohorts) were positively correlated with disease progression. Furthermore, the non-classical class I *HLA-G**14bp DEL allele confers increased risk for *H. pylori*-associated GC in Spanish cohorts. Regarding major histocompatibility complex (MHC) class I polypeptide-related sequence A (*MICA*) alleles, *MICA**009 and *MICA**A9 have been identified as susceptibility alleles in Chilean and Chinese cohorts, respectively.^[29]

DNA mismatch repair gene polymorphisms

H. pylori infection induces gastric inflammation and oxidative stress via reactive oxygen species/reactive nitrogen species (ROS/RNS), causing tumorigenic DNA damage. This infection heightens susceptibility to oxidative stress and DNA damage, particularly in DNA repair-deficient models. A nested case–control study investigated 12 polymorphisms in DNA repair genes (mutL homolog 1 [*MLH1*], mutS homolog 2 [*MSH2*], 8-oxoguanine DNA glycosylase 1 [*OGG1*], excision repair cross-complementation group 2 [*ERCC2*], and X-ray cross-complementation group 1 [*XRCC1*]) combined with *H. pylori* seropositivity in 246 gastric adenocarcinoma cases, 1175 matched controls, and 91 chronic atrophic gastritis patients. Ultimately, the *ERCC2* K751Q polymorphism was associated with an increased risk of non-cardia tumors.^[30] Genetic polymorphisms in *XRCC1* R194W and *OGG1* S326C may play significant roles in the evolution of *H. pylori*-related gastric lesions in a high-risk Chinese population.^[31] A recent Japanese study that conducted cohort analyses of the Biobank Japan (BBJ) and the Hospital-based Epidemiologic Research Program at Aichi Cancer Center found that pathogenic variants in nine cancer susceptibility genes were significantly associated with GC risk, including homologous recombination genes (ataxia telangiectasia mutated [*ATM*], breast cancer 1 [*BRCA1*], *BRCA2*, partner and localizer of *BRCA2* [*PALB2*] and mismatch repair genes [*MLH1*, *MSH2*, *MSH6*], as well as adenomatous polyposis coli [*APC*] and cadherin 1 [*CDH1*]). Among these, pathogenic variants in homologous recombination genes (*ATM*, *BRCA1*, *BRCA2*, *PALB2*) showed a significant enhancing interaction with *H. pylori* infection, thereby significantly increasing GC risk.^[32]

Immune microenvironment

Persistent *H. pylori* infection remodels the gastric immune microenvironment into an immunosuppressive niche that accelerates carcinogenesis through the synergistic dysfunction of myeloid and lymphoid cells [Figure 2]. Neutrophils initiate this process by dominating early inflammatory responses, where their excessive ROS/RNS production directly inflicts genotoxic damage on gastric epithelia, while secreted cytokines, such as IL-6, IL-8, and TNF- α perpetuate chronic inflammation.^[11,33] Concurrently, antigen-presenting cells, including monocytes, macrophages, and dendritic cells, exhibit significantly

impaired functionality and downregulated major histocompatibility complex II (MHC-II) expression that suppresses antigen presentation and reduces phagocytic capacity, thereby collectively enabling maintaining bacterial persistence. Macrophages further undergo infection-driven polarization toward an immunosuppressive M2 phenotype via glucocorticoid receptor activation and autophagic disruption. This reprogramming event enhances tumor cell proliferation, migration, and ROS generation while inducing the secretion of the anti-inflammatory IL-10.^[34] T lymphocyte dysfunction critically facilitates immune evasion. Consequently, CD4⁺ T-cell responses become pathologically skewed, with T helper 1

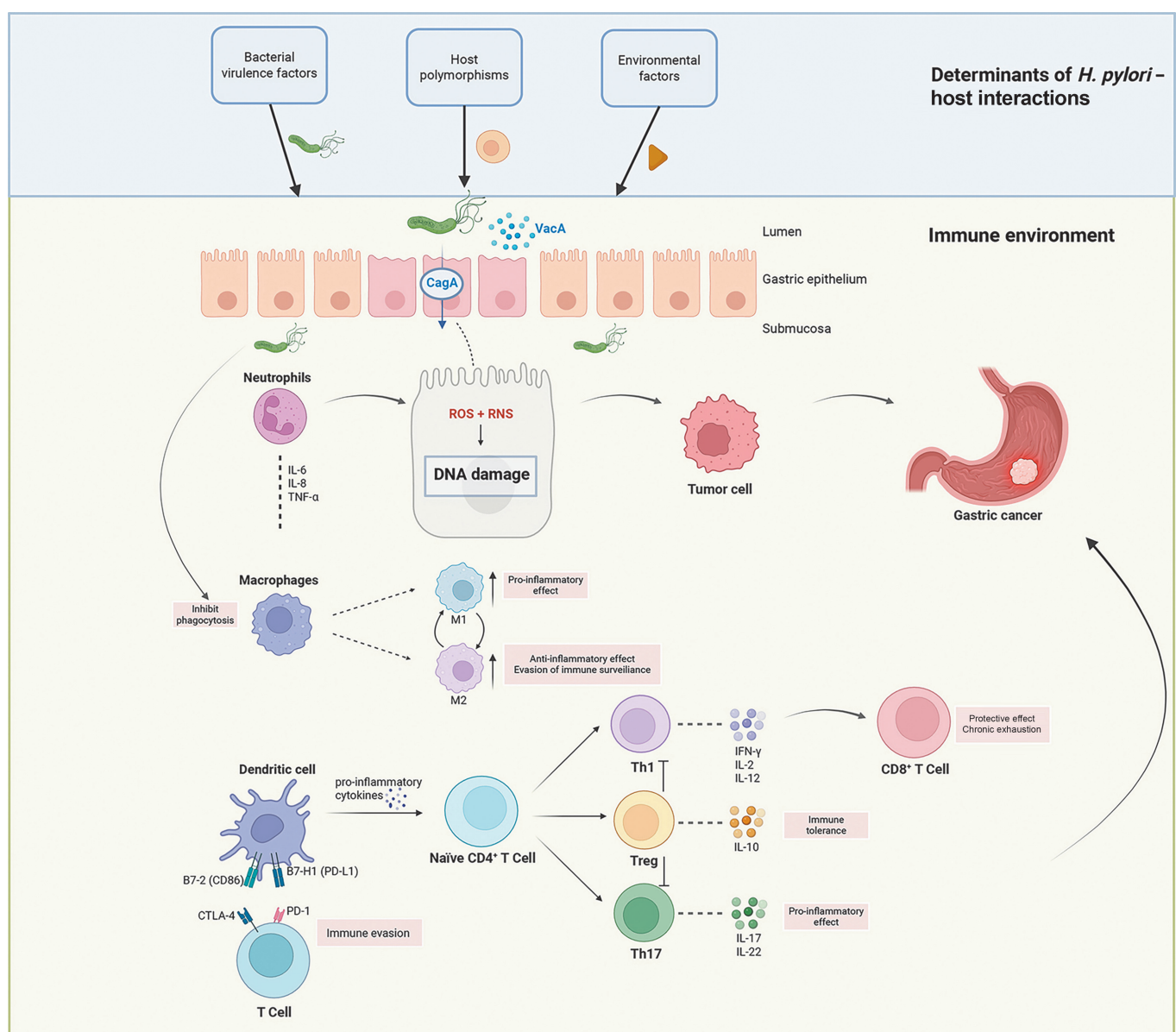


Figure 2: Mechanism of *H. pylori*-induced immune microenvironment remodeling in GC. Bacterial virulence factors (e.g., CagA), host genetics, and environmental cues initiate interactions with gastric epithelium. *H. pylori* triggers innate immunity: Neutrophils generate ROS/RNS, causing epithelial DNA damage and chronic inflammation via IL-6/IL-8/TNF- α . Myeloid cells (macrophages, DCs) dysregulate—macrophages polarize to immunosuppressive M2 (secreting IL-10), and DCs/monocytes downregulate MHC-II, enabling bacterial persistence. Adaptive immunity falters: CD4⁺ T cells skew to Th1/Th17 (amplifying carcinogenic pathways, tissue injury), while CD8⁺ TRM cells exhaust, losing anti-tumor capacity. A CTLA4⁺ T-cell subset suppresses activation, aiding immune escape. Costimulatory molecules (e.g., PD-L1, CD86) dysregulate, reinforcing tolerance. This cascade—initial DNA damage, chronic inflammation, immune evasion—establishes an oncogenic microenvironment, driving gastric cancer development. CagA: Cytotoxin-associated gene A; CTLA: Cytotoxic T-lymphocyte-associated protein; IFN: Interferon; IL: Interleukin; PD-1: Programmed cell death protein 1; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; TNF- α : Tumor necrosis factor- α ; Th: T helper cells; Treg: Regulatory T cells; VacA: Vacuolating cytotoxin A.

(Th1)-derived interferon (IFN)- γ paradoxically amplifying pro-carcinogenic signaling cascades (e.g., lipopolysaccharide [LPS]-TLR4 and CagA-hepatocyte growth factor receptor [CagA-MET] pathways) despite initial bactericidal activity. Furthermore, Th17-secreted IL-17 fuels neutrophil-mediated tissue injury through inflammatory cascades. IL-17A promotes gastric carcinogenesis, partially by regulating the IL-17RC/NF- κ B/nicotinamide adenine dinucleotide phosphate oxidase 1 (NOX1) signaling pathway.^[35] Regulatory T cells (Tregs) suppress anti-*H. pylori* immune responses, establishing immune tolerance that facilitates bacterial persistence, chronic infection, and sustained inflammation. This Tregs-driven immunotolerant milieu and the associated chronic inflammatory state inflict long-term mucosal damage, thereby driving the pathological progression from chronic gastritis to GC.^[36] CagA-specific CD8⁺ tissue-resident memory T (TRM) cells infiltrate the gastric mucosa early in *H. pylori* infection, regulating bacterial growth via cytotoxic molecules and neutrophil recruitment. As infection progresses chronically, CD8⁺ TRM cells become functionally exhausted and lose their protective capacity, permitting CagA-driven malignant transformation, signifying dynamic immune shifts influencing bacterial persistence and mucosal damage. Therefore, therapeutically inducing functional CagA-specific CD8⁺ TRM cells represents a promising strategy to potentially suppress persistent infection and associated inflammation, thereby reducing GC risk.^[37] Notably, a recently identified HLA-DR⁺ cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)⁺ T cell subset engages macrophages via the CTLA4/CD86 inhibitory axis, actively suppressing T-cell activation and representing a novel mechanism of immune escape.^[38]

Costimulatory ligand dysregulation reinforces the tolerance, B7-2 (CD86) upregulation sustains pathogenic T-cell stimulation, while B7-H1 (programmed death-ligand 1, PD-L1) overexpression and B7-H2 (inducible T-cell costimulator ligand) downregulation inhibit effector responses and promote regulatory T cell differentiation. Furthermore, B7-H3 elevation exacerbates inflammation via Th2 polarization. Thus, *H. pylori* subverts host immunity through a triad of interconnected mechanisms, namely myeloid cell dysfunction (neutrophil-mediated DNA damage and APC impairment), T cell dysregulation (Th1/Th17 imbalance, CD8⁺ exhaustion, and CTLA4⁺-mediated suppression), and dominant inhibitory signaling (PD-L1 and CTLA4/CD86 axes), culminating in an immunotolerant microenvironment conducive for neoplastic progression.^[39]

Mechanism of GC After *H. pylori* Eradication

The mechanism for GC development after *H. pylori* eradication is not fully clarified, but some key theories have emerged. The classic “hit-and-run” paradigm suggests that *H. pylori* causes initial, irreversible damage. In this model, CagA acts as the “hit” event, delivering abnormal signals as direct carcinogenic stimuli and inducing genetic instability. The “run” occurs when these initiating events are gradually superseded by successive genetic and/or epigenetic changes (e.g., E-cadherin methylation) that occur in cancer-prone cells even long after eradication.^[40] CagA has been shown to elevate fat mass and

obesity-associated protein (FTO) to induce GC progression via this persistent mechanism.^[41] An alternative or complementary pathway involves continuous impairment of immune function. *H. pylori* infection can induce immune exhaustion of the gastric mucosa, as evidenced by the persistence of immune exhaustion markers, such as low CD8⁺, high CD4⁺/CD3⁺, and PD-L1/CD4⁺ expression, even after eradication. This lasting impairment may explain the development of metachronous GC after endoscopic resection.^[42]

In addition, some medicines can also influence GC development. Studies conducted by the University of Hong Kong demonstrated that long-term use of proton pump inhibitors is associated with increased GC risk (hazard ratio 2.44) even after *H. pylori* eradication therapy. The potential mechanism includes decreased gastric acid, increased gastrin, and changes in the gastric microbiome (e.g., the growth of nitrate-reducing bacteria).^[43] On the contrary, metformin, aspirin, and statins are associated with a lower risk of GC.^[44]

Mechanisms of *H. pylori*-Negative GC

Gastric microorganisms

Pathogenic bacteria

Several genera of bacteria, such as *Streptococcus*, *Fusobacterium*, *Prevotella*, and *Lactobacillus*, have been consistently identified as enriched bacteria in GC tumor tissues.^[45] *Streptococcus anginosus* (*S. anginosus*) and *Fusobacterium nucleatum* (*F. nucleatum*), in particular, have been extensively studied.

S. anginosus is a common commensal bacterium in the oral cavity and gastrointestinal tract that is more likely to colonize the gastric mucosa of individuals who have not been infected with *H. pylori*. *S. anginosus* is significantly more abundant in the tumor tissues and feces of patients with GC than those with gastritis, as well as in cancerous tissues compared with adjacent non-cancerous tissues.^[46,47] Mechanically, *S. anginosus* directly contacts and adheres to gastric mucosal cells via the binding of its surface protein TMPC to the annexin A2 receptor (ANXA2) on gastric mucosal cells. This attachment not only facilitates colonization but also activates the mitogen-activated protein kinase (MAPK) signaling pathway. MAPK activation significantly enhances the proliferation, migration, and invasiveness of GC cells, inhibits apoptosis, increases tumor incidence, and enlarges tumor size.^[48] Furthermore, *S. anginosus* infection activates the pyroptosis pathway mediated by Gasdermin E (GSDME), which involves the cleavage of GSDME, upregulation and cleavage of NLRP3, and an increase in cleaved caspase-3. The N-terminal fragment of cleaved GSDME penetrates the cell membrane to form pores, causing the release of cellular contents and cell death. Cleaved caspase-3 links the apoptotic and pyroptotic pathways. *S. anginosus* infection activates the NLRP3 inflammasome by releasing PAMPs and damage-associated molecular patterns (DAMPs), further promoting the release of inflammatory factors and activation of the pyroptosis pathway.^[49] *S.*

anginosus infection enriches pro-inflammatory Th17 cells, immunosuppressive tumor-associated macrophages, and polymorphonuclear myeloid-derived suppressor cells within tumor tissues. It also expedites the exhaustion of CD8⁺ T cells, inhibits their differentiation and infiltration, and significantly reduces the CD8⁺ T-cell infiltration in tumor tissues. In addition, *S. anginosus* infection can reshape the tumor immune microenvironment by metabolizing arginine to produce ornithine, further promoting development of GC.^[47]

F. nucleatum is a Gram-negative anaerobic bacterium commonly found in the human oral cavity and an opportunistic pathogen associated with various diseases. *F. nucleatum* infection induces chronic gastritis and gastric mucosal dysplasia in mice, and promote the malignant progression of GC, by enhancing its metastatic potential.^[50] *F. nucleatum* is significantly more abundant in GC tissues than in adjacent normal tissues, and is detected in approximately one-third of GC patient samples.^[51] *F. nucleatum* can invade GC cells and survive intracellularly. Co-culture experiments have shown that *F. nucleatum* colonization can dysregulate actin and its regulatory factors, thereby enhancing cancer cell motility and promoting the invasion and metastasis of GC cells.^[51] *F. nucleatum*-infected GC cells secrete exosomes and upregulate the HOXA transcript at the distal tip (HOTTIP), which could promote Ephrin type B receptor 2 expression by sponging microRNA-885-3p, thus activating the phosphoinositide 3-kinase/protein kinase B pathway (PI3K/AKT), subsequently promoting GC progression.^[52] In addition, *F. nucleatum* increases IL-17A secretion by activating the NF-κB/rel homology domain protein B signaling pathway, which in turn recruits tumor-associated neutrophils (TANs) and promotes PD-L1 expression on TANs. This leads to the exhaustion of CD8⁺ T cells, facilitating immune evasion and enhancing the efficacy of anti-PD-L1 antibody therapy.^[50]

Fungi

Similar to bacteria, the diversity and abundance of gastric fungi are decreased in patients with GC. Several studies involving Chinese cohorts have found increased abundance of *Cutaneotrichosporon*, *Malassezia*, *Solicoccozyma*, *Candida albicans* (*C. albicans*), and *Archaeorhizomyces* in GC tissues.^[45] *C. albicans* is the most significantly enriched fungus in GC in the Chinese population, with a significantly higher abundance in cancer tissues than in adjacent non-cancerous tissues and accounting for approximately 22% of the total fungal abundance. Gastric fungal dysbiosis in the stomach can upregulate inflammatory pathways, including cytokine and chemokine signaling pathways, to induce inflammation and GC development.^[53]

Viruses

Epstein-Barr virus (EBV) is a γ-herpesvirus belonging to the Herpesviridae family and is currently the most extensively studied virus associated with GC. It primarily infects humans, and the prevalence of EBV in GC cells is

7.5%, while its infection rate is higher in *H. pylori*-negative than in *H. pylori*-positive GC. EBV infection can increase the risk of GC by more than 18-fold and accounts for approximately 7% to 9% of global GC cases annually. Furthermore, it can induce significant genomic and epigenomic change to promote cancer development.^[54]

Mechanistically, the EBV latent membrane proteins 1 and 2A (LMP1 and LMP2A) promote methylation by activating DNA methyltransferases (DNMTs). LMP2A triggers STAT3 phosphorylation, enabling STAT3 to dock onto the DNMT1 promoter and upregulate its transcription. Concurrently, LMP1 enhances DNMT1 activity via the cellular Jun/c-Jun N-terminal kinase axis, while also engaging NF-κB signaling to transcriptionally upregulate DNMT3A and DNMT3B.^[55] In addition, EBV infection increases the expression of olfactomedin 4 (OLFM4) by activating the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway. EBV infection also mediates OLFM4 secretion via microvesicles, thereby promoting tumor progression by Hippo signaling^[56] [Table 2].

Inflammation caused by autoimmune gastritis and bile acid

Autoimmune gastritis

Chronic inflammation driven by autoimmune processes, particularly AIG, is also a major risk factor for GC development. AIG involves the chronic immune-mediated destruction of gastric parietal cells and is diagnosed based on serological markers (anti-parietal cell and anti-intrinsic factor antibodies), histopathological evidence of corpus-predominant atrophy with inflammation, and functional abnormalities.^[57] While often initially asymptomatic, AIG holds significant clinical issues because of its association with pernicious anemia and an increased risk of gastric neoplasia, particularly neuroendocrine tumors and, more controversially, gastric adenocarcinoma.^[57] The pathogenesis of AIG centers on a breakdown of immune tolerance, primarily through T cell-mediated autoimmunity targeting parietal cells. This results in chronic inflammation, epithelial damage, and reparative metaplastic changes that rarely progress to invasive carcinoma compared with *H. pylori*-associated atrophy. Nevertheless, whether AIG increases the risk of adenocarcinoma comparable to *H. pylori* infection remains controversial. Addressing this uncertainty, a prospective study by Rugge *et al*^[58] followed 211 patients with *H. pylori*-negative AIG for a cumulative 10,541 person-years and observed no significant increase in GC risk. This study suggests that undetected prior *H. pylori* infection may contribute to GC risk in some patients with AIG. Moreover, Hoft *et al*^[59] demonstrated that both *H. pylori* infection and AIG induce a highly similar gastric metaplasia, characterized by a shared precancerous alanyl aminopeptidase (ANPEP⁺) intestinal metaplasia (IM) subtype with transcriptomic signatures indicative of malignant potential. This compelling evidence establishes that both conditions elevate GC risk through analogous molecular pathways. This evidence establishes AIG as a plausible pathogenic mechanism for *H. pylori*-negative GC. AIG initiates a distinct cascade of inflammatory

Table 2: Gastric microorganisms and key oncogenic mechanisms.

Microorganisms	Representative species/virus	Key oncogenic mechanisms
Bacteria	<i>S. anginosus</i>	TMPC-ANXA2 binding → MAPK activation → proliferation, migration, invasion↑; apoptosis↓ Release PAMPs and DAMPs → activates NLRP3 inflammasome → activates GSDME-mediated pyroptosis pathway
Bacteria	<i>F. nucleatum</i>	Intracellular invasion → actin dysregulation → motility, invasion, metastasis↑ Exosomes upregulate HOTTIP → promote Ephrin type B receptor 2 expression → activate the PI3K/AKT pathway → enhance GC progression
Fungi	<i>C. albicans</i>	Gastric mycobiome dysbiosis → chronic activation of cytokine/chemokine pathways → sustained inflammation drives GC
Virus	EBV	LMP2A upregulates DNMT1 via STAT3; LMP1 upregulates DNMT1/3A/3B via JNK/NF-κB → global hypermethylation silences tumor-suppressor genes Activates cGAS-STING pathway → upregulates OLFM4 → OLFM4 secreted via microvesicles → YAP activation → promotes tumor progression

ANXA2: Annexin A2; *C. albicans*: *Candida albicans*; DAMPs: Damage-associated molecular patterns; DNMT1/3A/3B: DNA Methyltransferase 1/3A/3B; EBV: Epstein-Barr virus; *F. nucleatum*: *Fusobacterium nucleatum*; GSDME: Gasdermin E; GC: Gastric cancer; HOTTIP: HOXA transcript at the distal tip; JNK: c-Jun N-terminal kinase; LMP1/2A: Latent Membrane Protein 1/2A; MVs: Microvesicles; MAPK: Mitogen-activated protein kinase; NLRP3: NOD-like receptor family pyrin domain containing 3; OLFM4: Olfactomedin 4; PAMPs: Pathogen-associated molecular patterns; PI3K/AKT: Phosphoinositide 3-kinase/protein kinase B pathway; *S. anginosus*: *Streptococcus anginosus*; STAT3: Signal transducer and activator of transcription 3; STING: Stimulator of interferon genes; TMPC: Surface protein of *S. anginosus*; YAP: Yes-associated protein; ↑: Increase; ↓: Decrease.

and metaplastic changes within the gastric mucosa, ultimately predisposing individuals to malignancy through mechanisms that diverge from, yet share some common inflammatory endpoints with, *H. pylori*-induced pathogenesis.

Bile reflux

Bile acid, which can inflict gastric mucosal damage and chronic inflammation, is another independent risk factor for the development of precancerous gastric lesions and GC.^[60] Bile reflux refers to the backflow of duodenal contents (mainly the bile) into the stomach, which usually occurs after gastric or biliary surgery or as primary biliary reflux. Bile reflux tends to occur in *H. pylori*-negative individuals.^[61] Its incidence in the precancerous lesion and GC groups was significantly higher than that in the chronic gastritis group (36.4% and 57.3% vs. 18.4%).^[62] Bile reflux-induced gastric IM is mainly mediated by bile acids and regulated by several critical molecules and signaling pathways, including farnesoid X receptor (FXR), G protein-coupled bile acid receptor (TGR5), hepatocyte nuclear factor 4α (HNF4α), SRY-box transcription factor 2 (SOX2) DNA/RNA methylation modifications, STAT3 signaling, and the NF-κB pathway.^[60] Treatment of gastric cells with deoxycholic acid increased the expression of the TGR5 receptor and STAT3 phosphorylation, while upregulating the accumulation of phosphorylated-STAT3 in the cell nucleus. It directly bound to the promoter and activated the transcription of Krüppel-like factor 5 (KLF5), promoting tumor cells growth.^[63] Furthermore, the crosstalk between bile acid and gut microbiota may regulate bile acid metabolism and alter gut microbiota composition by reducing the abundance of beneficial bacteria while increasing the proportion of pathogenic bacteria (such as LPS-producing bacteria), contributing significantly to the development of GC.^[64] Moreover, the bile acid metabolomics is a potential biomarker for GC diagnosis. A previous study found that

without considering the state of *H. pylori* infection, the model of six bile acids, including hyocholic acid (HCA), tauroolithocholic acid (TLCA), norcholeic acid, deoxycholic acid 3-glucuronide (DCA-3G), tauroolithocholic acid 3-sulfate (TLCA-3S), and hyodeoxycholic acid/lithocholic acid (HDCA/LCA) could diagnose GC and early GC with the areas under the curve of 0.98 and 0.94, respectively.^[65] Further studies are needed to explore the predictive value of the model for malignant transformation of IM.

Genetic and environmental factors

Genetic background

DGCs are more common in the *H. pylori*-negative population, with many studies exploring gene mutations associated with DGC^[66] [Table 3]. *TP53* is a tumor-suppressing transcription factor. *TP53* mutations are the most frequent somatic mutations in DGC, occurring in up to 46% of cases. *TP53* mutations lead to loss of tumor-suppressive function and acquisition of gain-of-function properties. *CDH1*, which encodes E-cadherin and is located on chromosome 16 (16q22.1), is a key intercellular adhesion protein. *CDH1* mutations are more common in *H. pylori*-negative DGC and may be the driver mutations for intramucosal DGC. In families with *CDH1* mutations, the lifetime risk of DGC is approximately 37–42% for men and 24–33% for women.^[67] The loss of E-cadherin function leads to decreased intercellular adhesion and loss of cell polarity, and promotes tumor cell detachment and metastasis. E-cadherin dysfunction can also lead to the activation of oncogenic signaling pathways (such as the Wnt/β-catenin pathway), thereby causing uncontrolled growth and promoting the DGC development. Catenin Alpha 1 (*CTNNA1*) encodes an important protein that is involved in the cell skeleton formation and cell connection. For individuals negative for *CDH1*, further detection of *CTNNA1* can be performed.^[68] *CLDN18-ARHGAP26* fusion gene is a functional acquired DGC oncogene with

Table 3: Common genetic susceptibility of DGC.

Gene	Location	Mutation rate	Mechanism for DGC
<i>TP53</i>	17p13.1	46%	Loss of tumor-suppressive function and acquisition of gain-of-function properties
<i>CDH1</i>	16q22.1	25%	Loss of E-cadherin → impaired cell adhesion, polarity loss, metastasis Activates Wnt/β-catenin → uncontrolled growth
<i>CTNNA1</i>	5q31.2	low	Dysfunction disrupts cell adhesion → promotes tumor invasion
<i>CLDN18-ARHGAP26</i>	3q22.3/5q31.3	15–25%	Activates RhoA, FAK, YAP pathways → tumor formation
<i>RHOA</i>	3p21.3	10–25%	Overexpression + high GTP binding → YAP1 dephosphorylation → activates Rho-ROCK → migration/invasion
<i>ARID1A</i>	1p36.11	12%	Tumor-suppressor gene silencing and oncogene aberrant activation → cell-cycle dysregulation, epithelial-mesenchymal transition
<i>APC</i>	5q22.2	11%	Truncating mutations → β-catenin stabilization → Wnt pathway hyperactivation → cancer progression
<i>PIK3CA</i>	3q26.32	10%	Persistent PI3K activation → overactivation of the PI3K–Akt–mTOR axis → promote growth and inhibit apoptosis

DGC: Diffuse gastric cancer; FAK: Focal adhesion kinase; GTP: Guanosine triphosphate; mTOR: Mammalian target of rapamycin; PI3K/AKT: Phosphoinositide 3-kinase/protein kinase B pathway; *RHOA*: Ras homolog family member A; Rho-ROCK: Ras homolog family member A-Rho-associated coiled-coil-containing protein kinase; TEAD: TEA domain transcription factor; YAP: Yes-associated protein.

a detection rate of approximately 15–25% in the DGC population. The *CLDN18-ARHGAP26* fusion gene can activate the ras homolog family member A (*RHOA*) signaling pathway and promote tumor formation by activating the focal adhesion kinase (FAK) and yes-associated protein (YAP) signaling pathways.^[69] A recent study has identified a new mutant gene, *RHOA*, and the *RHOA* R129W mutation induces RhoA overexpression and a higher guanosine triphosphate (GTP) binding state. In addition, the *RHOA* R129W mutation leads to a decrease in the phosphorylation level of Serine 127/397 in YAP1, thereby affecting the activity of the Rho-ROCK signaling pathway and promoting cell migration and invasion.^[70] Notably, a study in 2022 found that the genes with high mutation rates in Chinese patients with hereditary DGC are inconsistent with those reported previously. Although the germline variant mutation rate of *CDH1* is low among Chinese patients with hereditary DGC, its somatic variant mutation rate is as high as 25.3%.^[71]

Lifestyle and environmental factors

GC development is closely related to dietary habits. A low consumption of fruits and vegetables and high intake of salty and smoked foods are well-recognized risk factors that exacerbate gastric mucosal inflammation. Moreover, smoking is an independent risk factor for GC, especially for cardia cancer. The smoking index of patients with *H. pylori*-negative undifferentiated early GC is significantly higher than that of patients with *H. pylori*-positive GC.^[72] Compared with non-smokers, the risk of current smokers is 25% higher, which increases to 33% in individuals who have smoked for more than 40 years. Many components in tobacco smoke are carcinogenic, and smoking may promote GC development by activating nicotinic acetylcholine receptors (nAChR) and β-adrenergic receptors (β-AR). Carcinogens in tobacco smoke (such as polycyclic aromatic hydrocarbons and nitrosamines) can lead to the formation of DNA adducts, thereby inducing gene mutations. In addition, alcohol

consumption also increases the risk of GC, especially for the *H. pylori*-negative population. Heavy drinking poses a 3.48-fold higher risk in subjects not previously infected by *H. pylori*, while the association was not observed in the *H. pylori*-positive population.^[73] Alcohol mainly exerts its carcinogenic effects through its metabolite acetaldehyde, which increases oxidative stress and induces gastric mucosal inflammation. Moreover, the combined effect of smoking and drinking is significantly synergistic toward the development of GC.^[74] In addition, metabolic dysfunction-associated fatty liver disease (MAFLD) is also associated with *H. pylori*-negative GC. A multicenter cohort study in Japan found that MAFLD, age, smoking, and alcohol consumption are independent risk factors for *H. pylori*-negative GC, with MAFLD being the strongest independent factor. The risk of GC in individuals with MAFLD is nearly seven times higher than that in healthy individuals.^[75]

Conclusions and Future Perspectives

H. pylori infection and GC

As a group I carcinogen, *H. pylori* infection plays an important role in GC development. First, *H. pylori* eradication is still an effective way of decreasing the risk of GC. Extensive efforts have been made in exploring the most effective and safe eradication therapies for *H. pylori* infection. Currently, many high-quality studies in China have demonstrated the non-inferior efficacy and safety of vonoprazan-based dual therapy, providing further evidence for the effective eradication of *H. pylori*.^[76,77]

As the most fundamental and cost-effective approach to curb *H. pylori* infection, *H. pylori* vaccine research has recently focused on two core goals: preventing primary infection and promoting the treatment of established infections. Recombinant protein vaccines and subunit vaccines dominate current research for their good safety profile. Oral delivery is prioritized for its convenience and ability

to target gastric mucosal immunity, although challenges like acid-induced antigen degradation require formulation optimization such as microencapsulation.^[78] Despite these advances, *H. pylori* vaccines face translational challenges.^[79] Rising *H. pylori* antibiotic resistance complicates combination strategies with eradication therapy. Bacterial immune escape mechanisms (e.g., antigenic variation) reduce vaccine protective duration. Furthermore, large-scale clinical data on long-term GC risk reduction remain limited. Key directions in vaccine development include antigen selection, immune adjuvant optimization, vaccine type innovation, and delivery route improvement. The use of ideal antigens (e.g., urease subunit B, a conserved subunit of *H. pylori* urease critical for bacterial acid resistance; or Trx1, the Chinese population-specific *H. pylori* virulence marker identified by our group) are needed to induce robust cross-reactive immunity, while safe mucosal adjuvants should be explored to enhance local immune responses in the gastric mucosa, such as the CD8⁺ TRM response. Future studies should therefore prioritize developing multi-antigen vaccines (combining urease subunit B with Chinese population-specific markers like Trx1) to improve cross-protection, designing personalized vaccines tailored to individual genetic backgrounds (e.g., HLA polymorphisms) or immune status, and exploring synergies between vaccines, antibiotic eradication, and immune therapies. Regulating the gastric-gut microbiota via probiotics may enhance mucosal immune responses to vaccines.

In addition, although CagA and VacA are the principal pathogenic virulence factors, and exhibit strong associations with *H. pylori*-associated GC, the prevalence (>90%) of CagA-positive *H. pylori* infection is high in the Chinese population. Thus, additional virulence proteins may better identify individuals harboring high-risk, highly pathogenic strains within this demographic.^[80] Our research has identified Trx1 and arginase (RocF) as key virulence markers for prevalent strains in China.^[14] We addressed their pathogenic mechanisms in promoting bacterial colonization, resisting host oxidative stress, and exacerbating gastric inflammation and lesions. Therefore, Trx1 is a promising non-invasive serum biomarker for screening high-risk individuals, while targeting Trx1/RocF could inform novel therapies on inhibiting *H. pylori*-induced lesion progression. Building upon this foundation, subsequent research priorities must include elucidating the precise mechanistic role of the Trx1 in gastric carcinogenesis, advancing the clinical application of virulence markers for identifying highly pathogenic *H. pylori* strains prevalent in the Chinese population, delving deeper into the interplay in host-microbiome interactions, and identifying other virulence factors unique to the Chinese population to provide novel avenues for the precise prevention and treatment of GC.

Considering that not all individuals with *H. pylori* infection develop GC, it is vital to define the high-risk individuals and develop personalized management strategies. Identifying high-risk individuals relies on extending beyond established hereditary syndromes to encompass germline genetic variants, immunogenetic polymorphisms (e.g., in TLRs influencing *H. pylori* persistence), and critically, epigenetic alterations. However, a key knowledge gap remains: no

clinically validated multi-omics risk model currently exists to integrate these factors for GC risk stratification. Future investigations should prioritize the development of such models (e.g., combining TLR4/IL-1 β polymorphisms with serum CagA antibody levels) to guide clinical screening frequency (e.g., annual *vs.* biennial endoscopy).

A critical emerging focus in *H. pylori*-associated gastric carcinogenesis is the role of spasmolytic polypeptide-expressing metaplasia (SPEM), a metaplastic lineage characterized by the expression of trefoil factor 2 (TFF2) and MUC6 in gastric fundic glands. SPEM arises from the trans-differentiation of mature chief cells, mucous cells, and isthmus stem cells into spasmolytic polypeptide-secreting mucous cells, indicating reprogramming of gastric epithelial cells. As a key intermediate in gastric mucosal repair and pathological progression, SPEM can further advance to intestinal IM and ultimately GC, making it a novel and important target for GC prevention and management. Recent studies have underscored the pivotal role of SPEM in gastric dysplasia and GC development, particularly under conditions of persistent inflammation induced by *H. pylori* infection. Our team has shown that luteolin suppresses precancerous lesions via the STAT3/lipocalin-2 axis; translational gaps persist in validating such chemo-preventive agents in large-scale clinical trials, which should thus be a priority for bridging basic findings with patient treatment.^[81] Mechanistically, *H. pylori* infection drives SPEM formation in the context of chronic inflammation and immune responses.^[82] Moreover, *H. pylori*-associated SPEM progression involves the recruitment of M2 macrophages and their secretion of IL-33, which further propels the malignant evolution of SPEM.^[83] These molecular and cellular mechanisms must be clarified for the development of targeted therapeutic strategies for inhibiting GC progression at the pre-malignant stage. However, critical knowledge gaps remain: the precise causal relationships between *H. pylori* infection, SPEM initiation, and subsequent transition to GC have not been fully elucidated. Furthermore, key molecular targets regulating malignant transformation of SPEM remain to be identified.

GC after *H. pylori* eradication

Except in those with *H. pylori* infection-induced GC, there are some individuals who still develop GC even after *H. pylori* eradication, which tends to be associated with the persistent influence of *H. pylori* infection on the gastric mucosa via the “hit-and-run” mechanism. Therefore, post-eradication GC and *H. pylori*-negative GC are two distinct entities with different potential mechanisms that need to be distinguished carefully. However, the confirmation of *H. pylori*-negative status remains confusing. Currently, the following four conditions need to be met simultaneously: no history of *H. pylori* eradication; at least two detection methods showing negative results; no significant atrophy or only mild atrophy of the gastric mucosa on endoscopy; and no gastric mucosal manifestations of *H. pylori* infection with almost no infiltration of inflammatory cells.^[9] Therefore, the accurate diagnosis of *H. pylori*-negative GC relies on the meticulous confirmation of the *H. pylori* infection status. Till now, no biomarkers have been identified for differentiating

between *H. pylori*-negative and past infection. Although a European study confirmed that the CagA antibody can remain serologically positive even 10 years after eradication and may be a promising biomarker,^[84] this finding has not been validated in the Chinese population. Biomarkers for past infection evaluated from peripheral blood, gastric mucosa, or gastric juice should be further explored based on the Chinese population. This will pave the way for the mechanism study of *H. pylori*-negative GC and post-*H. pylori* eradication GC.

H. pylori-negative GC

Previous studies have demonstrated that some non-*H. pylori* microorganisms (including *S. anginosus*, *F. nucleatum*, and EBV) contribute to GC development. Notably, our team also found that as gastric mucosal lesions progress in *H. pylori*-negative individuals, the abundance of *Burkholderiaceae* continuously increases, while the abundance of *Streptococcaceae* and *Prevotellaceae* exhibits a continuous downward trend.^[85] Therefore, it is necessary to further identify the pathogenic microbiota that play a significant role in *H. pylori*-negative GC development. However, some questions remain regarding the microbes and GC. First, the microorganisms enriched in GC tissues vary among different studies, and the carcinogenic mechanisms of certain microorganisms have not been clearly understood. Second, the occurrence of GC is a multi-stage process, and the microbial community may play different roles at different stages. This dynamic change and its impact on GC have not been accurately captured. Third, some studies have found decreased microbiota diversity in GC tissues; however, whether the changes in the microbiota are the cause or the consequence of GC could not be clarified. Although high-throughput sequencing technology has identified the microbial characteristics associated with GC, the utility of gastric microbiome analysis for risk assessment or early screening of GC in clinical practice remains controversial. Large-scale cohort studies are still needed to provide more evidence.

Autoimmune responses and bile reflux may also trigger chronic inflammation that drives gastric carcinogenesis. These processes ultimately promote malignant transformation via sustained immune activation, tissue damage, aberrant repair processes, and genomic instability. The association between AIG and GC risk requires clarification, particularly to distinguish the intrinsic risk of “pure” *H. pylori*-negative AIG from that confounded by infection. In addition, the relationship between *H. pylori* infection and AIG presents intriguing paradoxes and unresolved questions. Future research must prioritize the meticulous ascertainment of *H. pylori* status in AIG cohorts using highly sensitive methodologies, such as serological assessment of past infection markers, to accurately delineate cancer risk attributable solely to autoimmune mechanisms. Equally critical are longitudinal investigations evaluating whether *H. pylori* eradication alters the natural history of AIG or mitigates subsequent GC risk in affected individuals. Mechanistically, comprehensive comparative profiling of genomic, transcriptomic, and epigenomic alterations in gastric lesions from *H. pylori*-negative AIG vs. *H. pylori*-positive gastritis is essential to elucidate

shared and distinct oncogenic pathways. Resolving these interconnected questions will not only clarify the complex interplay between infection and autoimmunity in gastric oncogenesis, but it will also inform targeted surveillance and prevention strategies for *H. pylori*-negative GC. Although many studies have demonstrated the carcinogenic effect of bile reflux, no universally accepted diagnostic criterion for bile reflux gastritis has been established. Diagnosis requires a combination of clinical manifestations, endoscopic findings, and histological characteristics. Furthermore, the relationship between bile reflux and *H. pylori* infection remains controversial. Several studies have demonstrated a negative correlation between *H. pylori* infection and bile reflux, and bile acid may be important in eradicating *H. pylori*.^[61] IM is more commonly observed in the high-concentration bile reflux group with negative *H. pylori* infection than in the *H. pylori*-positive group.^[86] In contrast, some studies suggested that *H. pylori* may induce the bile reflux, thus further exacerbating gastric mucosa damage.^[87] Therefore, both the pathogenic mechanism and the relationship between bile reflux and *H. pylori* infection need further exploration.

In addition, lifestyle and environmental factors are significant elements in the occurrence of various tumors, including GC. During periods of high prevalence of *H. pylori* infection, the impact of lifestyle and environmental factors on GC is often overlooked. Evidence showed that individuals with a high genetic risk but a favorable lifestyle had a lower risk of GC than those with a high genetic risk and an unfavorable lifestyle.^[88] Moreover, various environmental exposures may interact synergistically with defects in DNA damage repair to induce genomic instability and initiate gastric carcinogenesis, indicating possible interactions between genetic and environmental factors. In the future, as the prevalence of *H. pylori* infection gradually decreases, the impact of lifestyle and environmental factors on GC should be explored, with a focus on the prevention and management of GC through multidisciplinary approaches.^[74] Notably, preventing malignant progression at the precancerous stage is of great clinical importance for GC prevention.

In conclusion, GC pathogenesis involves multi-stage and multi-factor interactions. Aside from identifying Chinese high-virulence *H. pylori* strains and the high-risk individuals in the Chinese population, close monitoring of the population should be conducted after the eradication of *H. pylori*. Moreover, future research should prioritize deciphering the unique pathogenetic mechanisms of *H. pylori*-negative GC and identifying potential therapeutic strategies. To advance GC prevention and treatment, future research must prioritize: (1) refining personalized clinical management for Chinese populations (via validated high-virulence *H. pylori* markers and host risk models); (2) addressing critical knowledge gaps (e.g., Chinese-specific biomarkers for *H. pylori*-negative GC, microbiota dynamics, AIG-*H. pylori* interactions); and (3) accelerating the translation of basic findings to clinical practice (e.g., biomarker validation, chemo-preventive trials). These efforts will ultimately reduce the global burden of GC.

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Conflicts of interest

None.

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