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Review Article
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Performance of DNA Methylation on the Molecular Pathogenesis of *Helicobacter pylori* in Gastric Cancer; targeted therapy approach

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Gastric cancer (GC) is a significant cause of cancer mortality which has led to focused exploration of the pathology of GC. The advent of genome-wide analysis methods has made it possible to uncover genetic and epigenetic fluctuation such as abnormal DNA methylation in gene promoter regions that is expected to play a key role in GC. The study of gastric malignancies requires an etiological perspective, and *Helicobacter pylori* (*H. pylori*) was identified to play a role in GC. *H. pylori* infection causes chronic inflammation of the gastric epithelium causing abnormal polyclonal methylation, which might raise the risk of GC. In the last two decades, various pathogenic factors by which *H. pylori* infection causes GC have been discovered. Abnormal DNA methylation is triggered in several genes, rendering them inactive. In GC, methylation patterns are linked to certain subtypes including microsatellite instability. Multiple cancer-related processes are more usually changed by abnormal DNA methylation than through mutations, according to current general and combined investigations. Furthermore, the amount of acquired abnormal DNA methylation is heavily linked to the chances of developing GC. Therefore, we investigated abnormal DNA methylation in GC and the link between methylation and *H. pylori* infection.

Keywords: dna methylation, molecular pathogenesis, *helicobacter pylori*, gastric adenocarcinoma

INTRODUCTION

Adenocarcinomas which originate from glandular tissue, are the most common form of gastric cancer (GC) affecting the cells of the innermost layer of the stomach wall or mucosal layer. GC has a high prevalence and mortality due to its rapid progression and that diagnosis of the disease is often late [1]. From an epidemiological perspective, GC diagnosis and treatment differs significantly relative to geographical area. GC is a multifactorial disease caused by environmental and hereditary factors, which promote the development and progression of cancer. These include the genetic characteristics of the host, infectious agents, diet, and smoking [2].

Helicobacter pylori (*H. pylori*) was discovered by Marshall and Warren in 1983 and is now recognized as the most common human acquired infection. Infection with this bacteria in developing countries often occurs before adolescence, but the probability of infection in developed countries increases with

age [3]. *H. pylori* can cause duodenal ulcers, gastric ulcers, GC, and mucosa-associated lymphoid tissue. Among the diseases caused by this bacterium, cancer is of special importance. If each of the pathogenic factors of *H. pylori* are examined in the laboratory, different perspectives on the effects and applications in the treatment of disease, vaccine production, and defense mechanisms against this microorganism can be elucidated [4]. The study of this bacterium can be performed in two ways; the first aspect related to the pathogenesis of *H. pylori* as a carcinogen in the stomach environment and secondly, a practical perspective in the field of medicine and pharmacy. Therefore, *H. pylori* has characteristics as a pathogen that can potentially be used to treat cancer, by applying genetic engineering and biotechnology strategies and techniques.

More than 80% of people infected with *H. pylori* are asymptomatic suggesting that it might play an important role in the natural ecology of the stomach further validated by the fact that more than 50% of the world's population has *H. pylori* present in their upper gastrointestinal system [5]. *H. pylori* and gastritis are two risk factors for GC. *H. pylori* causes chronic gastritis which can develop into atrophic gastritis and intestinal metaplasia resulting in the formation of adenocarcinoma [6]. *H. pylori* is an oxidase-positive and catalase-positive organism that is specially adapted for survival in acidic gut conditions, by producing high levels of urease enzyme to increase pH levels as well as having a unique morphology allowing for greater motility [7].

According to the World Health Organization, *H. pylori* is classified as a type 1 carcinogen. This gram-negative spiral bacterium has a diameter of 0.5 micrometers and contains a variety of genes responsible for its pathogenesis such as vacA, cagA, hpaA, babA, sabA, alpA, alpB, dupA, lbp and cagPAI that cause different immune patterns in the body, and increase the risk of developing GC. *H. pylori* is found in the mucous layer and produces significantly higher levels of urease compared to other bacteria. This enzyme breaks down urea into ammonia and bicarbonate, producing an alkaline atmosphere in an acidic stomach environment [8]. *H. pylori* appears to play a more important role as a gastric carcinogen however, not all people with the infection develop cancer. *H. pylori* is colonized in gastric mucus, where it causes inflammation and immune reactions that lead to mucus destruction such as atrophy and intestinal metaplasia and dysplasia [9]. Gastric adenocarcinoma is usually found in the distal region of the stomach and is associated with *H. pylori*. Proximal cancer has different pathological and epidemiological features and is usually seen in areas where *H. pylori* is not very common. The most common association of GC with *H. pylori* is seen in two intestinal subtypes. Intestinal GC is common in developing countries and results from the transformation of precancerous lesions such as chronic superficial gastritis to atrophic gastritis, intestinal metaplasia, and dysplasia into cancer. Diffuse GC is a non-differentiated form in which the tumor cells are disorganized, have less glandular structure, and are not associated with precancerous lesions [10]. The pathogenicity of *H. pylori* is increased due to factors that induce apoptosis and cell proliferation. These factors that induce apoptosis can directly drive cancer cell death or stimulate the immune system to attack cancer cells indirectly [11].

The interaction of *H. pylori* with the host is the most important event leading to disease, which involves the initial binding of bacterial receptors to the surface of the host cell. The interaction between the bacterium and the cell not only establishes the bacterium, but some of these targeted connections trigger the host cell's internal signaling pathways, leading to damage to the cell and host tissue. *H. pylori* initially attach to collagen IV of the host cell, and this causes the invasion of bacteria through the lamina propria tissue [12]. Another important protein that bacteria can bind to is the host laminin which is the main protein of the basement membrane. *H. pylori*, after damaging the cell, is exposed to the basement membrane and binds to laminin surface receptors that include 25 and 67 kDa lipopolysaccharide (LPS) proteins. This interaction allows the bacteria to settle better in the affected areas and ulcers [13]. After the bacterium binds and settles on the cell surface, *H. pylori* can infect the host cell and is mediated by the bacterial secretory system that promotes pathogenesis.

MECHANISMS OF PATHOGENIC FACTORS IN *H. PYLORI*

Studies based on experimental infection of Mongolian gerbil with different strains of *H. pylori*, have shown that type 1 strains in the genome containing the cag pathogenicity island (cagPAI) are more likely to lead to cancer. The cagPAI encodes elements that function to stimulate immune reactions, thereby leading to the activation of some transcription factors. These transcription factors promote the expression of several genes responsible for carcinogenesis, chemokine secretion, and activating anti-apoptotic cycles [14]. The type IV secretory system of *H. pylori* injects the CagA protein directly into the

host cell during infection [15]. Upon entering the cell, CagA is phosphorylated and increases cell proliferation as well as the destruction of adhesive connections between adjacent cells. The presence of CagA increases the risk of peptide ulcers or GC by 50-70% [16]. The cagA gene is 40 kb in size and is located at the most downstream end of the pathogenicity islands, encoding the 140 kDa CagA protein that has high immunogenicity. Various studies have shown that strains of *H. pylori* with the cagA gene have the potential to cause long-term ulcers. CagA is also a highly antigenic protein that is associated with prominent inflammatory reactions produced by the secretion of interleukin-8 (IL-8). A study examining immune reagents for the diagnosis of CagA *H. pylori*, produced and identified the first set of monoclonal antibodies against CagA [17]. In addition, chronic infection with *H. pylori* was found to be a major risk factor for developing GC. The cagA gene has a key oncogenic role in the pathogenesis of *H. pylori* and some biological activity of the CagA protein requires tyrosine phosphorylation by host cell kinases [18]. Recent studies have shown relatively high resistance of *H. pylori* to antibiotic treatments therefore, blocking CagA presents itself as a therapeutic target. Indeed, recent therapies to combat *H. pylori* infection now includes the use of specific antibodies that block CagA protein. Studies have shown successful identification of a *H. pylori* CagA subunit that can be targeted with a recombinant protein [19].

VacA is aother factor that promotes pathogenesis of *H. pylori*. VacA is a pore-forming toxin that creates a hole in the host cell membrane leading to the formation of a vacuole in the cell, allowing *H. pylori* to damage the host cell. All *H. pylori* strains express the vacA gene of which only 50-60% show cytotoxic activity *in vivo*. VacA is a 95 kDa protein that consists of two distinct domains (37 and 58 kDa) that are actively secreted [20]. Outside of the bacterial cell, the subunits of VacA protein can form hexamers or dodecamers resembling flowers. Upon acid exposure, the vacuolating activity of VacA is greatly increased in *H. pylori* leading to host cell toxicity, inflammation, and apoptosis. The method by which VacA protein disrupts the intracellular membrane is by forming pores as the protein does not function enzymatically within the host cell. After binding to the host cell through receptor-dependent endocytosis, VacA internalizes and forms selective anion channels in the endosomal membrane [21]. This stimulates the production of vacuoles and eventually causes cell death through apoptosis. VacA channels also increases the permeability of epithelial cells, which allows for *H. pylori* to receive nutrients for bacterial growth. The toxin also causes epithelial cell erosion. The 34 kDa subunit has no toxic activity, while the 58 kDa subunit is involved in binding to the target cell. The vacA gene consists of two main coding regions (S and M) both of which are highly heterogeneous in different strains as well as in different parts of the world. The S region of the vacA gene is divided into two alleles, S1 and S2 while the M region is split to form M1 and M2 alleles. S1/M1 strains express high levels of toxin while S2/M2 strains do not produce any active toxin [22]. Studies suggest that the presence of a gene walk in the *H. pylori* genome increases the risk of GC by approximately 4% which is significantly higher than any other bacterium [23]. The outer membrane proteins of *H. pylori* are involved in its pathogenicity and are important for the density of *H. pylori*, gastric asymmetry, high levels of IL-8, and neutrophil secretion at the host site of inflammation. The oipA gene encodes an outer membrane protein that is an inflammation-related gene, located 100 kb from the cag and pathogenicity island on the *H. pylori* chromosome. The cytotoxic function of this protein was linked to a phenotype with positively expressed CagA and VacA. It has been suggested that the function of oipA is related to the rate of cell death and that OipA also acts as an adhesive protein. Indeed, OipA protein is one of the *Heliobacter* outer membrane proteins, which is a first-class family of *H. pylori* outer membrane proteins. Expression of this protein is associated with duodenal ulcers, GC, and neutrophil accumulation. OipA protein plays a role in host adaptation and its expression is under the control of the slipped-strand repair mechanism. The N-terminal region of OipA contains a signal sequence that is particularly affected by the addition of CT and this is thought to deactivate expression in *H. pylori* after several passages in the laboratory environment. In relation to host cell effects, studies have demonstrated that *H. pylori* OipA promotes apoptosis by modulating Bax and Bcl2 expression levels. The expression of this protein has also been reported to be related to CagA factor [13].

OipA has a synergistic effect with CagE in the production of IL-8. The functional or activated mode of oipA in *H. pylori* induces the secretion of IL-8 however, the secretion of the cytokine is not only dependent on the cagPAI as most strains lacking this genetic locus also induce low levels of interleukin expression which suggests that there are likely to be other mechanisms by which interleukins secretion is induced. Isolates that have a cagPAI also contain oipA, which is associated with duodenal ulcers, GC, and in particular the induction of IL-8 secretion. Negative cagA strains are almost all negative for babA2,

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oipA inactive, and have both S2 and M2 vacA alleles. In contrast, cagA positive were babA2 positive, oipA active and also have both S2 and M2 vacA alleles. The mechanism of intracellular signaling of IL-8 production by oipA protein has been investigated in recent years. The OipA protein is involved in the activation of IRF-1 to the interferon-stimulated responsive element (ISRE), at the IL-8 promoter which results in STAT-1 phosphorylation. Activated oipA promotes STAT-1 expression which subsequently activates IRF-1, which results in ISRE activation. Several transcriptional factors such as ISRE, AP-1, CRE, and NF-κB are activated by oipA. In addition, oipA and the associated p38 pathway are involved in the induction of IL-8 in the gastric mucosa of hosts infected with *H. pylori*, resulting in promoted gastric ulcers. OipA is one of the outer membrane proteins that has been shown to be important in inducing inflammation and increasing the production of IL-8 however, there are many uncertain aspects of the mechanisms by which OipA interacts with the host cell.

The outer membrane of *H. pylori*, like other gram-negative bacteria, contains endotoxin or LPS which is important for maintaining bacterial structure as well as the interaction of bacteria with the environment [24]. Compared to other gram-negative bacterial LPS, the immunogenic activity of *H. pylori* LPS is lower. *H. pylori* has an abnormal LPS that is related to the fatty acid composition that forms the hydrophobic part of lipid A in the presence of 3-hydroxy octadecanoic acid. The LPS of this bacterium is important in reducing the thickness of the gastric mucosal layer which is to prevent the glycosylation of gastric mucosa, further converting high molecular weight structures into structures with lower molecular weight. Also, the secretion of pepsinogen is stimulated, which is unique to this bacterium, inhibiting parietal cells and reducing gastric acid secretion [25]. The LPS of *H. pylori* stimulates the release of IL-8, IL-6, IL-10, IL-12, and also induces the production of TNF-α and PGE2. *H. pylori* has several virulence factors that play a role in pathogenicity [26]. Factors including BABA, HOPP, adherence lipoprotein, HspA, HspB, disulfide reductase, purines, alcohol dehydrogenase, PPK, RO53, FLdA, and amma-glutamyl transpeptidase promote carcinogenesis by increasing pathogenicity of *H. pylori* [27].

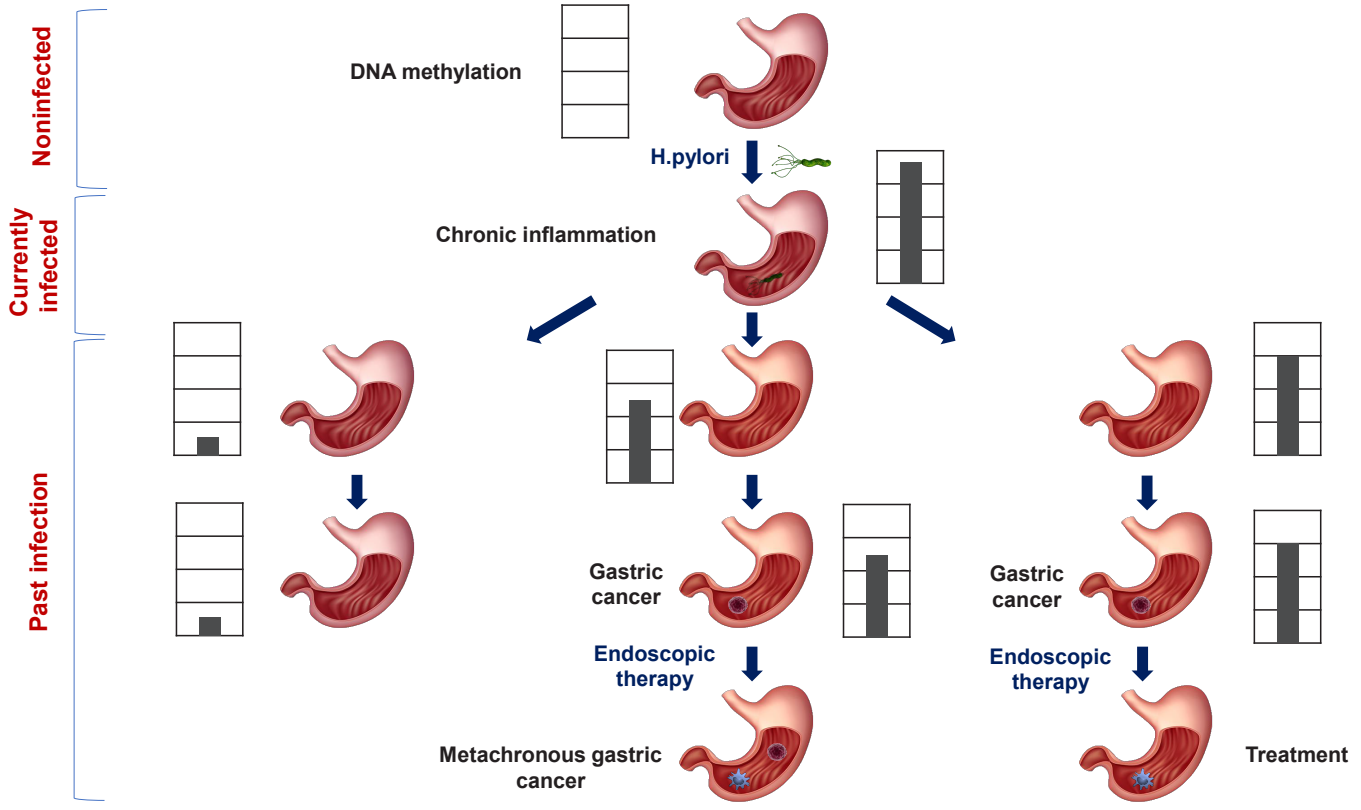

Figure 1. The explanation of *H. pylori* infection, DNA methylation, and GC in patients infected with H.

THE FUNCTION OF DNA METHYLATION IN CARCINOGENESIS

Cancer formation and development have been associated with hypermethylation of tumor suppressor genes. Cytosine-guanine dinucleotides (CpG) are found at the promoter site of DNA repair genes and multiple tumor suppressors. The hypermethylation of gene promoter areas prevents transcription mediators from binding, resulting in the repression of gene expression. Tumor suppressor gene inhibition interrupts the normal cell cycle equilibrium, potentially leading to increased cell reproduction and cancer. Hypermethylation of unmethylated tumor suppressor gene promoter areas, interrupts the gene by inhibiting transcription and the capacity to control aberrant cell reproduction, therefore driving cancerous transformation [28, 29].

DNA METHYLATION CAUSED BY *H. PYLORI* IN GC

Previous research has found a link between aberrant DNA methylation of *H. pylori* and the progression of GC. As *H. pylori* infection is eradicated from the host, it was shown that DNA methylation levels decreased and high methylation levels were maintained in the majority of cases despite *H. pylori* free status [30]. Immunosuppressive drug therapy prevents hypermethylation which demonstrates that *H. pylori* mediated inflammation plays a role in DNA methylation initiation [31]. Infection with *H. pylori* causes a strong inflammatory response in the stomach mucosa, resulting in the overexpression of various inflammatory cytokines such as IL-1β and creating abnormal DNA methylation patterns (Fig. 1). Macrophages infected with *H. pylori* induces the production of nitric oxide which causes hypermethylation of RUNX3 in epithelial cells [32]. *In vivo* investigations have also revealed that *H. pylori* related inflammatory responses are active during DNA methylation, which drives GC. Furthermore, elevated levels of DNA methylation

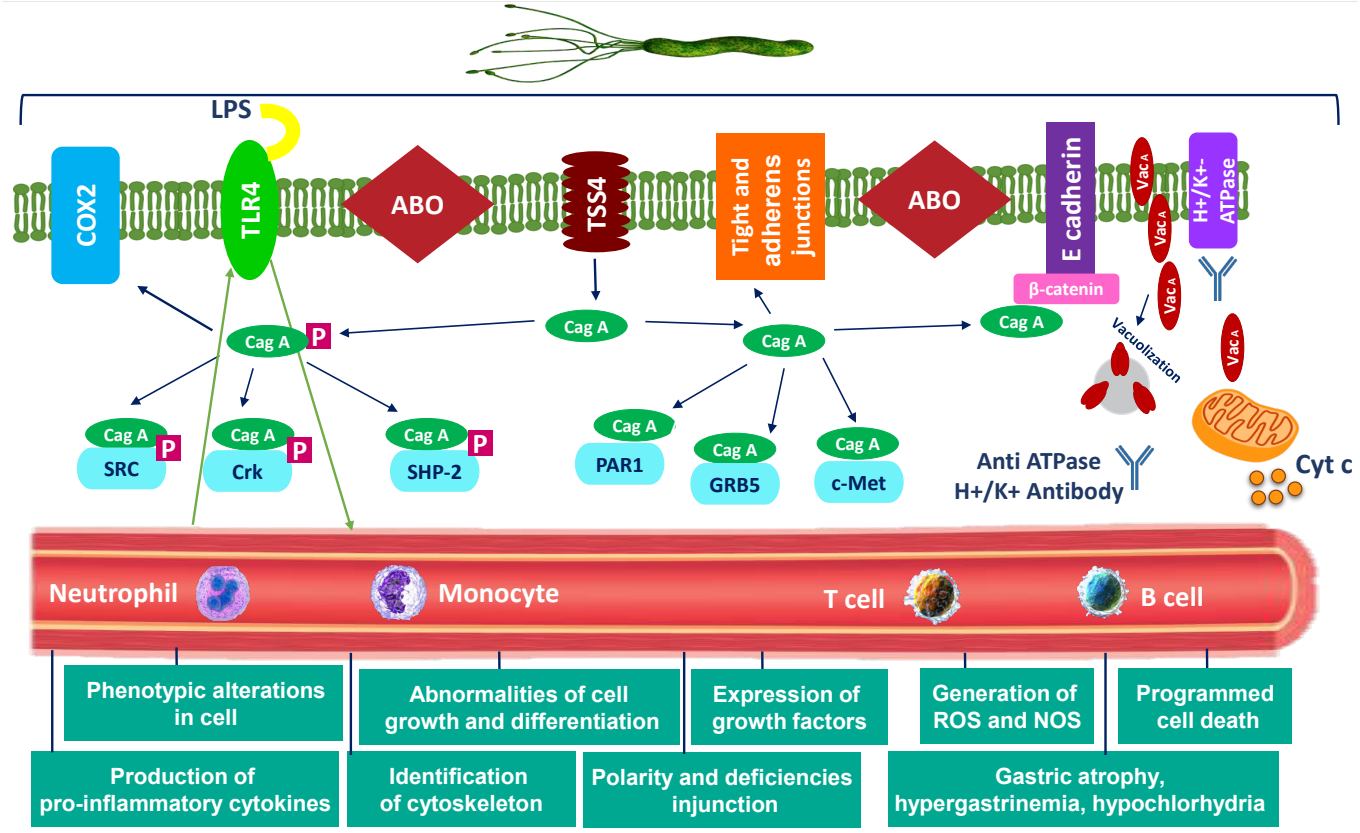

Figure 2. Neutophil, Monocyte,T cell and B cell activities with other genes in GC.

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were discovered in specific gene promoters, and these methylation levels were linked to the intensity of stomach inflammation. Gastric biopsies with abnormal DNA methylation were linked to a higher chance of developing GC, implying that persistent inflammation due to by *H. pylori* can cause gastric tissue hypermethylation [33].

Some bacterial virulence factors have been linked to dysregulation of intracellular signaling pathways and gastritis, which can contribute to cancer progression. The cagPAI has been linked to increased DNA methylation [34]. Type IV secretion systems encoded by cagPAI that allow for bacteria to transfer CagA protein, bacteria DNA, and macromolecules like peptidoglycans to the host cells (Fig. 2). CagA positive *H. pylori* infection results in greater methylation levels of some genes compared to CagA negative *H. pylori* infection infection [35]. CagA is intimately linked to cytotoxic activities of VacA inducing several cellular processes that disturb endosomal cells to drive epithelial cell vacuolation. The most virulent strains of *H. pylori* in conjunction with vacA and cagA expression, are thought to cause serious epithelial damage which correlates with GC progression. However, it is unknown whether these agents contribute to epigenetic modifications in GC [36].

DNA methylation is the most prevalent epigenetic fluctuation. The methylation of cytosine bases in the 5-position at CpG dinucleotides such as 5-methylcytosine, is widely documented. Histone modification regulates gene expression while CpG islands in promoter regions are largely unmethylated. Transcriptional silence is caused by erroneous methylation of promoter CpG islands, which leads to the production of downstream genes. One of the key causes of malignancy is the methylation-

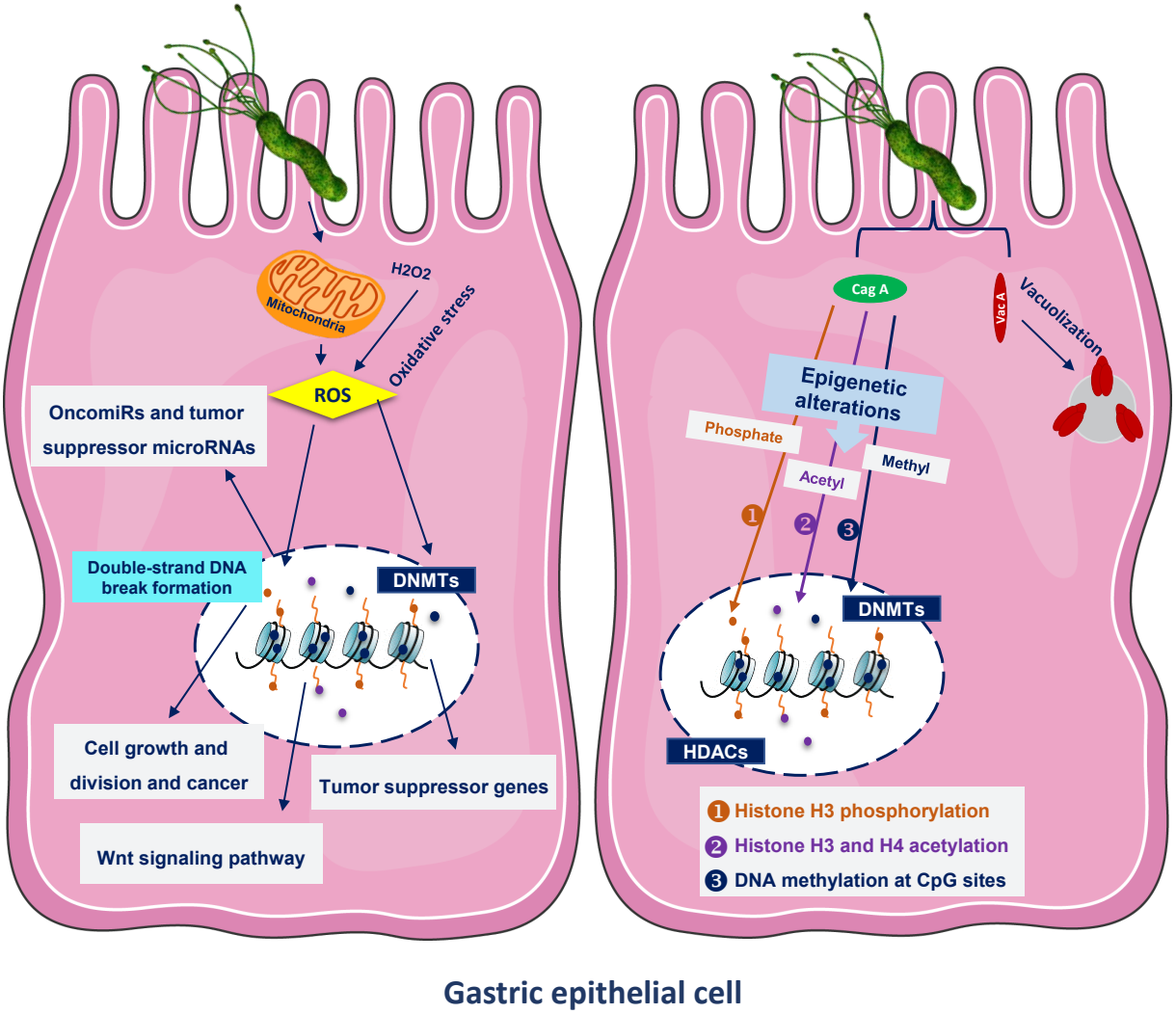

Figure 3. Involvments of oncomiRs and tumor suppressor microRNAs anlong side with other elements in GC.

induced suppression of tumor suppressor genes [37]. Environmental influences have an impact on DNA methylation levels with aging and smoking contributing to abnormal methylation in CpG islands. Chronic inflammation has also been shown to promote abnormal methylation in non-malignant tissues. Inflammation is caused by reflux esophagitis, chronic hepatitis, and ulcerative colitis for instance, resulting in abnormal CpG island methylation. Nevertheless, DNA methylation of the CpG island was found in the colonic mucosa of mice with colitis, caused by dextran sodium sulfate. Infection with *H. pylori* results in abnormal promoter methylation, which inhibits the production of tumor-suppressor genes including CDH1, LOXa and RUNX3. Chronic inflammation produced by *H. pylori* infection as opposed to other cellular changes mediated by *H. pylori*, may also lead to abnormal DNA methylation [38].

REGULATION OF RELATED GENES THROUGH *H. PYLORI*-INDUCED DNA METHYLATION

The activation and deactivation genes applies epigenetic mechanisms like DNA methylation which is a crucial and effective regulator of intracellular signaling systems (Fig. 3). Epigenetic fluctuations are well studied and can be used to explain the effects of environmental agents on the genome, which may promote tumor development. Some of the genes and their roles associated with *H. pylori*-induced DNA methylation of GC are shown in Table 1 [39-58].

THE ROLE OF MICRORNAS AND *H. PYLORI* IN GC

miRNAs are single-stranded, non-coding RNAs that play a pivotal role in regulating cellular and developmental processes. miRNAs are responsible for the expression of various

Table 1. Classification of the genes associated with *H. pylori*-induced DNA methylation in GC

Genes	Roles	Refs
PTEN	Tumor suppressor, AKT/PKB signaling pathway regulator	[39]
p41ARC	Actin polymerization is controlled by the p41 subunit of the Arp2/3 complex	[40]
THBD	Calcium ion binding and activation of transmembrane signaling receptors	[41]
MAP1LC3A	Linked to the autophagosomes accumulation	[41]
USF1, USF2	Transcriptional agent	[42]
CX32, CX43	Gap junction channels are formed, allowing ions and tiny molecules to flow freely across cells	[43]
TFF2	Regulates the mucus layer in the stomach and influences epithelial repair	[44]
HAND1	A transcription factor that has a role in the formation and differentiation of cells	[45]
RUNX3	Tumor suppressor and transcription factor	[46]
FLN	The cytoskeleton remodels, altering cell shape and movement	[47]
WWOX	Tumor suppressor and apoptosis	[48]
CDH1	Versatility, epithelial cell generation and regulation of cell-cell adhesions	[49]
HRASLS	Calcium-independent phospholipase activity	[50]
CDKN2A	Tumor suppressor and cell cycle regulators	[49]
CYLD	Cell survival regulator by NF-κB activation	[51]
LOX	Tumor suppressor which links to collagen and elastin	[52]
MGMT	Related to the protection of the cell against mutation and alkylating factors	[53]
COX-2	Prostaglandin biosynthesis's essential enzyme	[54]
GATA4, GATA5	Transcription factor activity that binds to DNA and chromatin	[55]
FOXD3	Transcriptional inhibitor and transcriptional activator	[56]
ATG16L1	Section of a large protein complex that is required for autophagy	[57]
MHL1	DNA mismatch repair and tumor suppressor	[49]
VEZT	Adherens junctions in the epithelial establishment, maintenance, and remodeling	[58]

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target genes at the post-transcriptional level. miRNAs can act as inducer or suppressor genes by regulating programmed cell growth and death. miRNAs are used to treat various diseases however, little is known about the role of miRNAs in *H. pylori*-induced GC [59]. One of the components that change in *H. pylori* infected hosts are miRNAs. miRNAs were first discovered in 2000 and have attracted the attention of many researchers. miRNAs are the most well-known class of regulatory RNAs in terms of production and function. miRNAs are expressed in various tissue types and uniquely in different diseases, which has led researchers to use miRNAs as a diagnostic marker. Numerous studies have shown that in patients infected with *H. pylori*, the expression of several specific miRNAs increases [60]. *H. pylori* infection of a host results in the production of miR-155 via the NF-κB pathway which subsequently inhibits IL-8. Furthermore, studies have shown that miR-146a has a regulatory role in *H. pylori*-associated GC [61]. It has also been reported that polymorphisms of miR-146a is associated with GC [62]. Increased expression of miR-584, miR-187, miR-22, miR-17, miR-21a, and miR-233 are also reportedly induced in *H. pylori*-associated GC [63]. The expression levels of miR-204 which is a tumour suppressor and the miRNA precursor let-7 are increased after hosts become *H. pylori*-free. miRNAs that are associated with *H. pylori* are listed in Table 2. After eradication of *H. pylori* using antibiotic therapy for seven days, the levels of miR-93, miR-25, and miR-21 did not show significant changes. Increased expression of miR-155 by induction of autophagy indicates anti-*H. pylori*c activity [60].

THE ROLE OF *H. PYLORI* IN THE TREATMENT OF CANCER

Despite various treatments, surgery is considered to be preferred cancer treatment for GC. Although common treatments are able to successfully reduce the size of the tumor, these treatments do not have a positive overall effect on patient survival and there is the possibility of tumor recurrence. Conventional therapies target rapidly proliferating and differentiating cells however, established solid tumors may contain a population of slow proliferating cells as well as highly resistant cancer stem cells that evade both chemo and radiotherapies. Cancer cells often develop resistance to common therapies and the tumor tissue contains various cell populations that are responsible for tumor growth, metastasis, disease recurrence, and are more capable than other cells of inducing a tumor or immune system defect [64]. Due to the aggressive nature of certain cancers as well as the complex mechanisms involved in tumor development, common treatments such as surgery, chemotherapies, and radiotherapies are ineffective in many cases due to the high frequency of side effects, low specificity, and the possibility of disease recurrence. For this reason, alternative types of therapies targeting proteins and bacterial toxins have also been considered. Research on several pathogens of *H. pylori*, each separately, shows their cytotoxic or immunomodulatory effects directly on cancer cells and immunity. Factors such as urease, HP-NAP, and flagella activate the immune system, which in turn triggers internal cell signaling to produce cytotoxins leading to activation the immune system, thus causing the death of cancer cells indirectly [65]. These peptides, proteins, or immunomodulatory properties can increase the activity of cellular endoplasmic reticulum systems to activate macrophages and dendritic cells. Activated macrophages and dendritic cells swallow and process factors that may promote cellular changes, as these bacteria-associated proteins can increase immunity as well as to guide the system towards a specific type of response [66]. This strategy potentially enhances the effectiveness of treatments by stimulating an immune response against the tumor, which is important in advancing practical goals on top of treating cancer. VacA, OipA and LPS factors also have cytotoxic effects on cancer cells. The LPS proteins of *H. pylori* that can bind to laminin, also causes acute gastritis and induces gastric epithelial cell apoptosis, of which caspase-8 and mitochondria play an important role in induced apoptosis. There are several mechanisms involved in interfering with the cell cycle and causing apoptosis or proliferation of *H. pylori*. Intracellular factors that are altered by this bacterium and disrupt the normal

Table 2. Some of the microRNAs involved in *Helicobacter pylori*-associated GC

microRNAs regulation in *H. pylori* positive GC	
Up-regulation	miR-99b, miR-223, miR-222, miR-146a, miR-584, miR-22, miR-187, miR-21a, 135b-5p, miR-21-5p, miR-18a-5p, miR-196a-5p, miR-146b-5p, miR-142-3p, miR-233, miR-17, miR-22
Down-regulation	miR-204, miR-375, miR-31-5p, miR-125a-5p, miR-145-5p

process of the cell cycle include mutations in the p53 protein, increased telomerase activity, increased Fas receptor expression, an effect on BCL2 proteins, and increased NF-κB activity [67].

H. pylori is a cause of cancer and due to its pathogenicity, it induces and multiplies. *H. pylori* also has factors that induce cell apoptosis. In these cases, the factors that induce apoptosis can be used directly to kill cancer cells as they are able stimulate an immune response. Consequently, some of these pathogenic proteins of *H. pylori* could be used as a new tool for cancer treatment strategies.

DNA METHYLATION AS A BIOMARKER IN THE DIAGNOSIS AND DEVELOPMENT OF GC

Recent research is looking into the methylation of key gene promoters, can be used as biomarkers for GC and disease stage prediction. Gastric lavage, plasma, and serum were among the substances examined in a study that identified LINC00643, GUSBP5, JAM2, FLT3, ELMO1, ZNF3, RPRM, RIMS1, and BHLHE22 as epigenetic markers for classifying the risk of GC, after *H. pylori* elimination. The methylation status of the BARHL2 gene was evaluated using gastric lavage DNA or gastric juice exosomal DNA, to determine if is a suitable marker for early GC identification. BARHL2 methylation was discovered at significant raised levels in gastric lavage generated DNA from early GC in one research study. Additional research is needed to determine if gastric wash-derived DNA or gastric juice-derived exo-DNA can be used in clinical settings for early cancer detection [68]. The expansion of histopathologic lesions marks the establishment of the intestinal subtype of GC. In another study, it was found that ZNF610, PCDH10, SORCS3, MPH, RSPO2, and SORCS3 gene methylation levels were able to promote development of gastric lesions regardless of the duration of *H. pylori* infection, baseline diagnostics, gender, mononuclear leukocytes, polymorphonuclear leukocytes, or intraepithelial lymphocytes [69]. Following endoscopic tumor removal, metachronous GC (MGC) can arise. It has also been reported that patients with enhanced miR-34b/c, SFRP2, and DKK2 methylation in the stomach, had a significantly greater prevalence of MGC. MiR-34b/c was found to have the strongest link to MGC development. Similarly, miR-124a-3 has been proposed as a strong indicator of probable MGC development [70].

DNA METHYLATION AND POLYMORPHISMS OF GC

GC formation is linked to gene polymorphisms. The existence of host gene polymorphisms and *H. pylori* cagA/vacAs1m1 strains appear to alter DNA methylation in GC. Interleukin polymorphisms in GC tissues during *H. pylori* infection were examined. Methylation of the COX-2 promoter has been linked to the IL-1RA Allele 2 genotype in cardia tumors, and the associated genotypes IL1B511T + IL-1RA Allele 2 appear to be crucial in the methylation of the COX-2 gene, notably in *H. pylori* strains carrying cagA and vacAs1. Along with cagA-positive *H. pylori*, the related genotypes appear to be involved in CDKN2A unmethylation. The consequences of the NF-κB1 polymorphisms -94 insertion and deletion, and -449 C > G on altering methylation during *H. pylori* infection have been extensively reviewed [71]. In the stomach mucosa, the methylation position of the CDH1, p16INK4a, p14ARF, and DAPK gene promoters was examined and it was discovered that -94 deletion/deletion homozygosity polymorphism was related to the frequency of CpG island methylation. Furthermore, the frequency of methylated genes in deletion/deletion polymorphisms was greater than in insertion/deletion polymorphisms, in elderly *H. pylori*-infected patients compared to those who had a much-increased inflammation level. As a result, the NF-κB1 -94 insertion/deletion ATTG polymorphism is linked to a higher likelihood of age-related gene methylation in non-cancerous stomach mucosa through *H. pylori* [72].

CONCLUSION

Abnormal promoter methylation in GC acts a significant performance through tumor-suppressor genes inactivation. While more research is needed to clarify the specific molecular pathways behind the development of aberrant promoter methylation in response to infection with these pathogens, *H. pylori* may contribute to malignancy by inducing abnormal methylation in gastric epithelial cells. Identifying these systems could shed light on how stomach carcinogenesis occurs, and applying this data to therapeutic use could further help with diagnosis, risk assessment, and treatment. Abnormal DNA methylation is therefore an effective and useful marker for the early diagnosis of malignant tumors. Epigenetic irregularities can be acquired during the early stages of malignancy, leading to genomic mutations in polyclonal tissues and as a result, unusual DNA

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methylation is an efficacious biomarker for the early discovery of malignant tumors. Furthermore, because of its reversible nature, abnormal DNA methylation could be an appealing target from a preventive or therapeutic standpoint. Multiple genetic abnormalities, in contrast to DNA methylation, have a role in GC. The prevalence and targets of genetic abnormalities differ based on the epigenotype. The genome and epigenome are believed to connect and assist in GC in a synergistic manner, and extensive analysis of those in GC may aid in elucidating the process of carcinogenesis.

AUTHOR CONTRIBUTIONS

SV wrote the primary draft of the manuscript. EM, SEN and MA completed many other parts of the manuscript. AAS wrote and completed the manuscript and also revised and edited the article comprehensively. All the authors read and confirmed the final edited version of the manuscript.

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REFERENCES

1. Rawla P, Barsouk A. Epidemiology of gastric cancer: global trends, risk factors and prevention. Prz Gastroenterol. 2019; 14(1):26-38.
2. Pucułek M, Machlowska J, Wierzbicki R, Baj J, Maciejewski R, Sitarz R. *Helicobacter pylori* associated factors in the development of gastric cancer with special reference to the early-onset subtype. Oncotarget. 2018;9(57):31146-62.
3. Nicolescu F. Trends in *Helicobacter pylori* infection. 1st ed. London (United Kingdom): IntechOpen; c2014. Chapter 6, Particulars of the *Helicobacter pylori* infection in children; p. 177.
4. Huang Y, Wang QL, Cheng DD, Xu WT, Lu NH. Adhesion and invasion of gastric mucosa epithelial cells by *Helicobacter pylori*. Front Cell Infect Microbiol. 2016;6:159.
5. Tilahun M, Gedefie A, Belayhun C, Sahle Z, Abera A. *Helicobacter pylori* pathogenicity islands and *Giardia lamblia* cysteine proteases in role of coinfection and pathogenesis. Infect Drug Resist. 2022;15:21-34.
6. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. *H. pylori* infection and gastric cancer: state of the art (review). Int J Oncol. 2013;42(1):5-18.
7. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, et al. *Helicobacter pylori* virulence factors-mechanisms of bacterial pathogenicity in the gastric microenvironment. Cells. 2020;10(1):27.
8. Toh JWT, Wilson RB. Pathways of gastric carcinogenesis, *Helicobacter pylori* virulence and interactions with antioxidant systems, vitamin C and phytochemicals. Int J Mol Sci. 2020;21(17):6451.
9. White JR, Winter JA, Robinson K. Differential inflammatory response to *Helicobacter pylori* infection: etiology and clinical outcomes. J Inflamm Res. 2015;8:137-47.
10. Zhang Y, Zhang PS, Rong ZY, Huang C. One stomach, two subtypes of carcinoma-the differences between distal and proximal gastric cancer. Gastroenterol Rep (Oxf). 2021;9(6):489-504.
11. Pourzardosht N, Hashemi ZS, Mard-Soltani M, Jahangiri A, Rahbar MR, Zakeri A, et al. Liothyronine could block the programmed death-ligand 1 (PDL1) activity: an e-Pharmacophore modeling and virtual screening study. J Recept Signal Transduct Res. 2022;42(1):34-42.
12. Graham DY. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. Gastroenterology. 2015;148(4): 719-31.e3.
13. Matsuo Y, Kido Y, Yamaoka Y. *Helicobacter pylori* outer membrane protein-related pathogenesis. Toxins (Basel). 2017;9(3): 101.
14. Yamaoka Y, Graham DY. *Helicobacter pylori* virulence and can-

cer pathogenesis. Future Oncol. 2014;10(8):1487-500.
15. Tegtmeyer N, Ghete TD, Schmitt V, Remmerbach T, Cortes MCC, Bondoc EM, et al. Type IV secretion of *Helicobacter pylori* CagA into oral epithelial cells is prevented by the absence of CEACAM receptor expression. Gut Pathog. 2020;12:25.
16. Matos JI, de Sousa HA, Marcos-Pinto R, Dinis-Ribeiro M. *Helicobacter pylori* CagA and VacA genotypes and gastric phenotype: a meta-analysis. Eur J Gastroenterol Hepatol. 2013;25(12): 1431-41.
17. Amin M, Shayesteh AA, Serajian A. Concurrent detection of *cagA*, *vacA*, *sodB* and *hsp60* virulence genes and their relationship with clinical outcomes of disease in *Helicobacter pylori* isolated strains of southwest of Iran. Iran J Microbiol. 2019;11(3):198-205.
18. Queiroz DM, Silva CI, Goncalves MH, Braga-Neto MB, Fialho AB, Fialho AM, et al. Higher frequency of cagA EPIYA-C phosphorylation sites in *H. pylori* strains from first-degree relatives of gastric cancer patients. BMC Gastroenterol. 2012;12(1):107.
19. Oluwasola A, Otegbayo J, Ola S, Ebili H, Afolabi A, Odaibo G. Correlation of serum anti-*Helicobacter pylori* immunoglobulin a (IgA) with histological parameters of chronic gastritis in ibadan, Nigeria. Ann Ib Postgrad Med. 2012;10(1):18-24.
20. Foegeding NJ, Caston RR, McClain MS, Ohi MD, Cover TL. An overview of *Helicobacter pylori* VacA toxin biology. Toxins (Basel). 2016;8(6):173.
21. Abbasi O, Mashayekhi F, Mirzajani E, Fakhriyeh Asl S, Mahmoudi T, Saeedi Saedi H. Soluble VEGFR1 concentration in the serum of patients with colorectal cancer. Surg Today. 2015; 45(2):215-20.
22. Baj J, Korona-Głowniak I, Forma A, Maani A, Sitarz E, Rahnama-Hezavah M, et al. Mechanisms of the epithelial-mesenchymal transition and tumor microenvironment in *Helicobacter pylori*-induced gastric cancer. Cells. 2020;9(4):1055.
23. Dewayani A, Fauzia KA, Alfaray RI, Waskito LA, Doohan D, Rezkitha YAA, et al. The roles of IL-17, IL-21, and IL-23 in the *Helicobacter pylori* infection and gastrointestinal inflammation: a review. Toxins (Basel). 2021;13(5):315.
24. Meliț LE, Mărginean CO, Mărginean CD, Mărginean MO. The relationship between toll-like receptors and *Helicobacter pylori*-related gastropathies: still a controversial topic. J Immunol Res. 2019;2019:8197048.
25. Shimomura H, Wanibuchi K, Hosoda K, Amgalanbaatar A, Masui H, Takahashi T, et al. Unique responses of *Helicobacter pylori* to exogenous hydrophobic compounds. Chem Phys Lipids. 2020;229:104908.
26. Mirzaei R, Sabokroo N, Ahmadyousefi Y, Motamedi H, Karampoor S. Immunometabolism in biofilm infection: lessons from cancer. Mol Med. 2022;28(1):10.
27. Hernández-Rubio A, Sanvisens A, Bolao F, Pérez-Mañá C, García-Marchena N, Fernández-Prendes C, et al. Association of hyperuricemia and gamma glutamyl transferase as a marker of metabolic risk in alcohol use disorder. Sci Rep. 2020;10(1): 20060.
28. Vahidi S, Samadani AA. TERRA gene expression in gastric cancer: role of hTERT. J Gastrointest Cancer. 2021;52(2):431-47.
29. Vahidi S, Norollahi SE, Agah S, Samadani AA. DNA methylation profiling of hTERT gene alongside with the telomere performance in gastric adenocarcinoma. J Gastrointest Cancer. 2020;51(3):788-99.
30. Muhammad JS, Eladl MA, Khoder G. *Helicobacter pylori*-induced DNA methylation as an epigenetic modulator of gastric cancer: recent outcomes and future direction. Pathogens. 2019;8(1):23.
31. Huang FY, Chan AO, Lo RC, Rashid A, Wong DK, Cho CH, et al. Characterization of interleukin-1β in *Helicobacter pylori*-induced gastric inflammation and DNA methylation in interleukin-1 receptor type 1 knockout (IL-1R1(-/-)) mice. Eur J Cancer. 2013;49(12):2760-70.
32. Na HK, Woo JH. *Helicobacter pylori* induces hypermethylation of CpG islands through upregulation of DNA methyltransferase: possible involvement of reactive oxygen/nitrogen species. J Cancer Prev. 2014;19(4):259-64.
33. Polakovicova I, Jerez S, Wichmann IA, Sandoval-Bórquez A, Carrasco-Véliz N, Corvalán AH. Role of microRNAs and exosomes in *Helicobacter pylori* and Epstein-Barr virus associated gastric cancers. Front Microbiol. 2018;9:636.
34. Rizzato C, Torres J, Obazee O, Camorlinga-Ponce M, Trujillo E, Stein A, et al. Variations in cag pathogenicity island genes of *Helicobacter pylori* from Latin American groups may influence neoplastic progression to gastric cancer. Sci Rep. 2020;10(1):6570.
35. Hayashi Y, Tsujii M, Wang J, Kondo J, Akasaka T, Jin Y, et al. CagA mediates epigenetic regulation to attenuate let-7 expression in *Helicobacter pylori*-related carcinogenesis. Gut. 2013; 62(11):1536-46.
36. Ricci V. Relationship between VacA toxin and host cell autophagy in *Helicobacter pylori* infection of the human stomach: a few answers, many questions. Toxins (Basel). 2016;8(7):203.
37. Norollahi SE, Alipour M, Rashidy-Pour A, Samadani AA, Larijani LV. Regulatory fluctuation of WNT16 gene expression is associated with human gastric adenocarcinoma. J Gastrointest Cancer. 2019;50(1):42-7.
38. Whyte JM, Ellis JJ, Brown MA, Kenna TJ. Best practices in DNA methylation: lessons from inflammatory bowel disease, psoriasis and ankylosing spondylitis. Arthritis Res Ther. 2019;21(1):133.
39. Zhang B, Zhang X, Jin M, Hu L, Zang M, Qiu W, et al. CagA increases DNA methylation and decreases PTEN expression in

human gastric cancer. Mol Med Rep. 2019;19(1):309-19.
40. Pizarro-Cerdá J, Chorev DS, Geiger B, Cossart P. The diverse family of Arp2/3 complexes. Trends Cell Biol. 2017;27(2):93-100.
41. Demidchik V, Shabala S, Isayenkov S, Cuin TA, Pottosin I. Calcium transport across plant membranes: mechanisms and functions. New Phytol. 2018;220(1):49-69.
42. Costa L, Corre S, Michel V, Le Luel K, Fernandes J, Ziveri J, et al. USF1 defect drives p53 degradation during *Helicobacter pylori* infection and accelerates gastric carcinogenesis. Gut. 2020;69(9):1582-91.
43. Wang Y, Huang LH, Xu CX, Xiao J, Zhou L, Cao D, et al. Connexin 32 and 43 promoter methylation in *Helicobacter pylori*-associated gastric tumorigenesis. World J Gastroenterol. 2014;20(33):11770-9.
44. Hoffmann W. Trefoil factor family (TFF) peptides and their diverse molecular functions in mucus barrier protection and more: changing the paradigm. Int J Mol Sci. 2020;21(12):4535.
45. Funato N, Taga Y, Laurie LE, Tometsuka C, Kusubata M, Ogawa-Goto K. The transcription factor HAND1 is involved in cortical bone mass through the regulation of collagen expression. Int J Mol Sci. 2020;21(22):8638.
46. Lu XX, Yu JL, Ying LS, Han J, Wang S, Yu QM, et al. Stepwise cumulation of RUNX3 methylation mediated by *Helicobacter pylori* infection contributes to gastric carcinoma progression. Cancer. 2012;118(22):5507-17.
47. Uehara S, Udagawa N, Kobayashi Y. Non-canonical Wnt signals regulate cytoskeletal remodeling in osteoclasts. Cell Mol Life Sci. 2018;75(20):3683-92.
48. Zhu ZJ, Teng M, Li HZ, Zheng LP, Liu JL, Yao Y, et al. Virus-encoded miR-155 ortholog in Marek's disease virus promotes cell proliferation via suppressing apoptosis by targeting tumor suppressor WWOX. Vet Microbiol. 2021;252:108919.
49. Hatzistergos KE, Williams AR, Dykxhoorn D, Bellio MA, Yu W, Hare JM. Tumor suppressors RB1 and CDKN2a cooperatively regulate cell-cycle progression and differentiation during cardiomyocyte development and repair. Circ Res. 2019;124(8):1184-97.
50. Murakami M, Sato H, Taketomi Y. Updating phospholipase A_2 biology. Biomolecules. 2020;10(10):1457.
51. Lork M, Verhelst K, Beyaert R. CYLD, A20 and OTULIN deubiquitinases in NF-κB signaling and cell death: so similar, yet so different. Cell Death Differ. 2017;24(7):1172-83.
52. Ye M, Song Y, Pan S, Chu M, Wang ZW, Zhu X. Evolving roles of lysyl oxidase family in tumorigenesis and cancer therapy. Pharmacol Ther. 2020;215:107633.
53. Alvarez MC, Santos JC, Maniezzo N, Ladeira MS, da Silva AL, Scaletsky IC, et al. MGMT and MLH1 methylation in *Helicobacter pylori*-infected children and adults. World J Gastroenterol. 2013;19(20):3043-51.
54. Ye Y, Wang X, Jeschke U, von Schönfeldt V. COX-2-PGE_2-EPs in gynecological cancers. Arch Gynecol Obstet. 2020;301(6):1365-75.
55. Alvarez MC, Fernandes J, Michel V, Touati E, Ribeiro ML. Effect of *Helicobacter pylori* infection on GATA-5 and TFF1 regulation, comparison between pediatric and adult patients. Dig Dis Sci. 2018;63(11):2889-97.
56. Cheng AS, Li MS, Kang W, Cheng VY, Chou JL, Lau SS, et al. *Helicobacter pylori* causes epigenetic dysregulation of FOXD3 to promote gastric carcinogenesis. Gastroenterology. 2013;144(1):122-33.e9.
57. Tanaka S, Nagashima H, Uotani T, Graham DY, Yamaoka Y. Autophagy-related genes in *Helicobacter pylori* infection. Helicobacter. 2017;22(3):e12376.
58. Miao R, Guo X, Zhi Q, Shi Y, Li L, Mao X, et al. VEZT, a novel putative tumor suppressor, suppresses the growth and tumorigenicity of gastric cancer. PLoS One. 2013;8(9):e74409.
59. Mashayekhi S, Saeidi Saedi H, Salehi Z, Soltanipour S, Mirzajani E. Effects of miR-27a, miR-196a2 and miR-146a polymorphisms on the risk of breast cancer. Br J Biomed Sci. 2018;75(2):76-81.
60. Prinz C, Weber D. MicroRNA (miR) dysregulation during *Helicobacter pylori*-induced gastric inflammation and cancer development: critical importance of miR-155. Oncotarget. 2020;11(10):894-904.
61. Yang Y, Huang Y, Lin W, Liu J, Chen X, Chen C, et al. Host miRNAs-microbiota interactions in gastric cancer. J Transl Med. 2022;20(1):52.
62. Xie WQ, Tan SY, Wang XF. MiR-146a rs2910164 polymorphism increases risk of gastric cancer: a meta-analysis. World J Gastroenterol. 2014;20(41):15440-7.
63. Ebrahimi Ghahnavieh L, Tabatabaeian H, Ebrahimi Ghahnavieh Z, Honardoost MA, Azadeh M, Moazeni Bistgani M, et al. Fluctuating expression of miR-584 in primary and high-grade gastric cancer. BMC Cancer. 2020;20(1):621.
64. Chu DT, Nguyen TT, Tien NLB, Tran DK, Jeong JH, Anh PG, et al. Recent progress of stem cell therapy in cancer treatment: molecular mechanisms and potential applications. Cells. 2020;9(3):563.
65. Peek RM Jr, Fiske C, Wilson KT. Role of innate immunity in *Helicobacter pylori*-induced gastric malignancy. Physiol Rev. 2010;90(3):831-58.
66. van Putten JPM, Strijbis K. Transmembrane mucins: signaling receptors at the intersection of inflammation and cancer. J Innate Immun. 2017;9(3):281-99.
67. He Y, Wang C, Zhang X, Lu X, Xing J, Lv J, et al. Sustained exposure to *Helicobacter pylori* lysate inhibits apoptosis and au-

tophagy of gastric epithelial cells. Front Oncol. 2020;10:581364.
68. Yamamoto H, Watanabe Y, Oikawa R, Morita R, Yoshida Y, Maehata T, et al. BARHL2 methylation using gastric wash DNA or gastric juice exosomal DNA is a useful marker for early detection of gastric cancer in an *H. pylori*-independent manner. Clin Transl Gastroenterol. 2016;7(7):e184.
69. Schneider BG, Mera R, Piazuelo MB, Bravo JC, Zabaleta J, Delgado AG, et al. DNA methylation predicts progression of human gastric lesions. Cancer Epidemiol Biomarkers Prev. 2015;24(10):1607-13.
70. Asada K, Nakajima T, Shimazu T, Yamamichi N, Maekita T, Yokoi C, et al. Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. Gut. 2015;64(3):388-96.
71. Zhao R, Liu Z, Xu W, Song L, Ren H, Ou Y, et al. *Helicobacter pylori* infection leads to KLF4 inactivation in gastric cancer through a TET1-mediated DNA methylation mechanism. Cancer Med. 2020;9(7):2551-63.
72. Tahara T, Tahara S, Horiguchi N, Kato T, Shinkai Y, Okubo M, et al. Prostate stem cell antigen gene polymorphism is associated with *H. pylori*-related promoter DNA methylation in nonneoplastic gastric epithelium. Cancer Prev Res (Phila). 2019;12(9):579-84.