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Helicobacter pylori cytotoxin-associated gene A virulence and its association with the epithelial-mesenchymal transition in gastric cancer

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Abstract:
Introduction and objective: Gastric cancer is currently one of the most prevalent malignancies worldwide with a high mortality rate. *Helicobacter pylori* (*H. pylori*) infection significantly contributes to the onset and progression of gastric cancer mainly due to the induction of chronic inflammatory responses. The pathogenicity of *H. pylori* is associated with a vast number of virulence factors among which cytotoxin-associated gene A (CagA) plays a crucial role.
Review methods: We conducted a literature review of PubMed, Web of Science, and Scopus on September 1st, 2021. There were no limits regarding the year and the language of publication. Articles included in this review concerned human and animal studies. The following search string was applied during the search: (gastric cancer) AND (epithelial-mesenchymal transition) AND (Helicobacter pylori) AND (cytotoxin-associated gene A). The final analysis included 135 articles independently reviewed by the authors.

Abbreviated description of the state of knowledge: *H. pylori* CagA-positive strains seem to be more virulent compared to the CagA-negative strains. CagA pathogenicity includes the increased secretion of pro-inflammatory cytokines, induction of cancer stem cell-like properties, apoptosis prevention, or overactivation of particular oncogenic pathways. *H. pylori* might induce epithelial-mesenchymal transition (EMT) via numerous pathways, among which CagA-related pathogenicity is considered to be of high significance.
Summary: Mechanisms associated with CagA action are involved in the maintenance of chronic *H. pylori* infection, subsequent EMT induction, and further onset and progression of gastric cancer. Because of a huge number of *H. pylori* strains with different virulence mechanisms, the clinical outcome of patients is also associated with the particular type of strain that infected a patient.
Keywords: Helicobacter pylori, cytotoxin-associated gene A, epithelial-mesenchymal transition, gastric cancer, carcinogenesis

1. Introduction

Incidental clinical manifestations or symptoms such as stomach discomfort after eating, early post-prandial satiety, recurrent epigastric pain, dysphagia, nausea, or vomiting might all be the first and early symptoms of gastric cancer (GC). Since the above-mentioned symptoms are also usually associated with dyspeptic symptoms they are often underestimated by patients. Currently, GC is one of the most prevalent malignancies worldwide and constitutes the fourth leading cause of death related to cancer [1]. Every year, this malignancy is diagnosed in more than a million of new patients [2]. More than 780,000 patients diagnosed with GC, die each year because of this malignancy, which places GC among one of the infamous leading causes of cancer-related deaths in the world, with the highest incidence and mortality rates recorded in Asia [3]. Common clinical manifestations such as weight loss, lack of appetite, anemia, or palpable epigastric lesion tend to appear relatively late during the course of GC and thus, these symptoms might constitute indicators of advanced cancer. Late occurrence of typical symptoms and therefore a delayed diagnosis of GC is associated with high mortality rates related to this neoplasm. Due to the epidemiological data mentioned above, it is an urgent need for doctors, scientists, and researchers worldwide to explore the molecular mechanisms, as well as causes that contribute to GC onset and progression, that might contribute to the more effective treatment strategies and thus, lower mortality rates. The most frequently used classification of GC is the Lauren classification, which classifies GC into three main types - intestinal, diffuse, and mixed type [4]. The most commonly diagnosed GC is primarily the intestinal type, with a far better prognosis compared to the diffuse type of GC [5,6]. Approximately 90% of GCs are adenocarcinomas that might be subdivided into cardia- and non-cardia tumors, depending on their location within the gastro-esophageal junction. GC treatment includes surgery, chemotherapy, radiation therapy, chemoradiation, or targeted therapy. However, the only method enabling a full patient's recovery is radical surgery involving the removal of the stomach and associated lymph nodes, leaving a margin of healthy tissues. Treatment success can be enhanced by additional chemotherapy, neoadjuvant, or adjuvant, depending on GC severity and healthcare standards in a particular region. In some cases, it is justified to apply the chemoradiotherapy. In locally advanced and generalized GC, palliative chemotherapy is used to prolong the life of a patient and improve its quality [7,8]. Other risk factors of GC include improper diet and lifestyle, genetic background, and family factors, as well as environmental factors [9]. Chronic atrophic gastritis associated with *Helicobacter pylori* (*H. pylori*) infection is critical in the formation of GC. The following review aims to present the relevance of the *Helicobacter pylori* cytotoxin-associated gene A (CagA) in the induction of epithelial-mesenchymal transition (EMT) in gastric cancer patients. The review was based on the original and review articles obtained from PubMed, Web of Science, and Scopus databases on September 1st, 2021. Articles included in this review concerned human and animal studies. There were no limits regarding the year and the language of publication. The following search string was applied during the search: (gastric cancer) AND (epithelial-mesenchymal transition) AND (Helicobacter pylori) AND (cytotoxin-associated gene A). The final analysis included 135 articles independently reviewed by the authors.

2. Gastric cancer classification

2.1 Anatomical

The anatomical classification of GC is vague due to the difficulties with defining the beginning of the gastric cardia and distinguishing GC from cancers of the gastroesophageal junction and esophagus [10]. Histologically, there is a concept of Z line, that indicates the transition of the squamous epithelium of the esophagus to the columnar epithelium of the stomach, however, this location does not overlap with the anatomical transition [11]. Siewert classification is based on the classification of gastroesophageal adenocarcinomas according to the location of the tumor epicenter and distinguishes three types of GC: 1) adenocarcinoma of the distal esophagus, 2) carcinoma of the cardia, and 3) subcardial GC [12,13]. According to

the latest, eighth edition of the TNM classification, GC is defined as a tumor that arises more than two centimeters from the esophagogastric junction [14,15].

2.2 Histological

GC can be assessed based on the macroscopic findings of the tumor in the stomach wall, or the microscopic image, i.e. the cellular structure. The Bormann classification (1926) is used to determine the macroscopic features of GC dividing it into four basic forms: 1) polypoid fungating, 2) ulcerative with elevated distinct borders, 4) ulcerative with indistinct borders, and 4) diffuse with indistinct borders [16,17]. The Bormann classification is also included in the Japanese Classification of Gastric Cancer (JCGC), which distinguishes six types of GC [18]. Among histological classifications, the Lauren classification (1965) is currently the most frequently used [4]. GC classification by WHO distinguishes five types of GC with a predominant image of cellular tissue: papillary, mucinous, tubular, poorly cohesive, and rare variants [19]. The recently released 5th edition of "2019 WHO classification of tumors of the digestive system" further details rarer subtypes such as the fundic-gland type carcinoma and the variability among poorly-cohesive carcinomas. The advantage of the WHO classification is the consistency with the histological classification of cancer in other parts of the intestine, which harmonizes the classification. Moreover, it is constantly updated according to the progress of GC research [20]. Besides, other, less frequently used classifications of GC were also introduced. Goseki et al. classification focuses on the tendency to form glandular structures and the presence of intracellular mucus in cancer cells [21]. However, its prognostic value is questioned, and scientific research is often contradictory to its usefulness [22-26]. Ming proposed GC classification based on the growth and invasiveness of cancer cells, dividing it into an expending type and infiltrative type [27]. It seems to correlate with other classification systems, but cannot constitute an independent prognostic factor [28].

2.3 Molecular

The Cancer Genome Atlas (TCGA) proposed the classification of GC into four molecular subtypes based on a cohort study of 295 tumor tissue samples of patients who were not previously treated with chemotherapy or radiotherapy. According to this classification, four molecular subtypes of GC can be distinguished - Epstein-Barr Virus positive (~ 9%), microsatellite instable (22%), genomically stable (20%), and chromosomally instable (50%) [29]. Additionally, in 2015, the Asian Cancer Research Group (ACRG) conducted gene expression profiling on 300 GC cases, distinguishing microsatellite instable (MSI), microsatellite stable with epithelial/mesenchymal transition (MSS / EMT), MSS / TP53-positive, and MSS / TP53-negative subtypes [30]. The genetic classifications correlate with the histological ones, for example, the diffuse type of GC according to the Lauren classification corresponds to the GS and MSS / EMT subtype, and the intestinal type to the MSI subtype [31].

3. Gastric cancer risk factors

3.1 *Helicobacter pylori* infection

Among all the risk factors, *H. pylori* infection presents the greatest carcinogenic effect, accounting for 90% of the non-cardia subtype of GC [32]. *H. pylori* infection significantly contributes to the onset and progression of gastric carcinogenesis, doubling the overall risk of GC [33]. *H. pylori* can survive for decades in the harsh environment of gastric juice, destroying the mucosa, inducing various histological alterations, destruction of intercellular junctions, and sometimes the progression into the mesenchymal phenotype in an epithelial-mesenchymal transition process, that are all related to the malignant transformation. *H. pylori* is prone to colonize the whole stomach especially when the secretion of the hydrochloric acid is lowered, however, the most prevalent site of *H. pylori* colonization is the pylorus. The average prevalence of *H. pylori* infection amidst the adult population is over 50% and currently constitutes the most common infection in the world [34,35]. Although the majority of the infected individuals remain asymptomatic, nearly all of them develop chronic inflammatory responses, among which 1-3% progress to GC. Further progression of gastric lesions is a multifactorial process that is associated with the environmental, host, and bacterial agents [36]. Even though, the mechanism of *H. pylori*-related gastric carcinogenesis is not deciphered yet.

3.2 Genetic factors

Although most GC cases are sporadic, 10% show familiar aggregation, and 1-3% of patients have hereditary GC [37]. Familial GC is divided into three types: hereditary diffuse gastric cancer (HDGC), gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal gastric cancer (FIGC) [38,39]. In addition, GC might occur due to other hereditary cancer syndromes such as Lynch, Peutz-Jeghers, Li-Fraumeni, breast-ovarian cancer syndromes, juvenile polyposis, or familial adenomatous polyposis. HDGC is an autosomal dominant cancer syndrome caused mainly by the inactivating germline mutation in the *CDH-1* gene encoding E-cadherin that is involved in cell adhesion [40]. *CDH-1* gene mutation primarily predisposes to the onset of a diffuse-type GC. It is estimated that mutations in the *CDH-1* gene occur in 25-30% of HDGC-affected

families [41]. Many family members without germline mutations within the *CDH-1* gene have decreased expression of E-cadherin and *CDH-1* allele-specific expression imbalance in tumor tissue. It is estimated that the lifetime cumulative risk of developing HDGC is 67% -70% in men and 56-83% in women [42]. Due to such a high risk of GC development, usually between the ages of 20 and 30, such patients usually undergo prophylactic total gastrectomy with Roux-en-Y esophagus-jejunal reconstruction [43].

3.3 Alcohol consumption

Despite the fact that alcohol is considered to be an independent risk factor for oral, esophageal, laryngeal, and pharyngeal cancers, its effect on GC development is controversial [44]. Many studies recognize ethanol as a factor associated with GC [45,46]. Based on a meta-analysis describing 22 cohort studies (5,820,431 cases), light or moderate alcohol consumption had no significant effect in the pathogenesis of GC, but heavy alcohol consumption (> 24 g / per day) increased cancer risk independently to gender, country of origin, or physical activity [47]. Similar conclusions have been drawn from other studies regarding the severity of alcohol consumption [48-54].

3.4 Smoking

In 2002, the International Agency for Research of Cancer stated that there was enough evidence to qualify cigarette smoking as a risk factor for developing GC, despite inconsistencies in tobacco research [55,56]. According to a meta-analysis of 27 cohort studies and 5 nested case-control studies, smoking increases the risk of GC without a predisposition to a specific anatomical location by 60% in men and by 20% in women compared to non-smokers [57]. A recent prospective study of 7 cohorts from China, Korea, and Japan where GC rates are the highest, showed that current smoking increased the risk of non-cardia GC by 33%. [OR = 1.33; 95% confidence interval (CI), 1.07–1.65], however, this relationship was only cases of seropositive *H. pylori* GCs [58]. A particularly high interaction was observed in patients who were seropositive to HP0305/HP1564, a strong marker for the presence of precancerous gastric lesions. In those cases, the risk of GC among smokers was increased by 46% compared to HP0305/HP1564 seropositive but without the smoking history.

3.5 Diet

Dietary and nutrition factors play a significant role in the etiology of GC. Zhang et al. (2020) in a systematic review and meta-analysis proved that the implementation of a healthy lifestyle is associated with a substantial risk reduction in cancer morbidity (HR = 0.71; IC 95%, 0.66-0.76) and mortality (HR = 0.48; IC 95%, 0.42-0.54) [59]. Besides, the analysis showed that a healthy lifestyle is associated with a 17-58% decrease in site-specific cancers risk, including GC. Among the different dietary models, the Mediterranean diet has a positive effect on reducing overall cancer risk [60]. Regarding the results of the studies on the consumption of vegetables and fruit, these are highly inconclusive, pointing to the large role of fruit and vegetable consumption in the occurrence of GC [61,62], negating their effect [63], or considering only fruit as a factor reducing the risk of GC [64]. The latest systematic review reported that fresh fruit and vegetables as well as micronutrients such as vitamin C, iron, selenium, zinc, and folate are protective factors regarding GC [51]. Besides, the risk of developing cancer is increased by a high-fat diet, red meat, and high salt intake.

4. Helicobacter pylori infection and gastric cancer onset and progression

H. pylori is a Gram-negative microaerophilic facultative bacterium that is an example of one of the most invasive microorganisms that is responsible for one of the highest prevalence of chronic inflammations worldwide. *H. pylori* is classified as a “group 1 carcinogen” according to the World Health Organization (WHO) and International Agency for Research on Cancer (IARC) report [65]. As a gastric pathogen, *H. pylori* colonizes nearly 50% of the world’s population gastric mucosa [66]. Besides, the prevalence of *H. pylori* infection is much higher in the developing countries compared to the highly developed countries. Even though approximately 80% of the infected individuals remain asymptomatic, *H. pylori* infection significantly increases the incidence of gastric and duodenal ulcer disease, as well as the risk of GC.The incidence of *H. pylori* detection is slightly higher in the male population (46,3%) compared to females (42.7%) [67]. The association between *H. pylori* infection and GC is observed as chronic gastritis that can not only lead to peptic ulcers, but also progress into GC, gastric lymphoma (MALT), and other gastrointestinal cancers [68]. Factors that are involved in *H. pylori*-mediated carcinogenesis include urease, carbonic anhydrase, Lewis antigen, cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), outer inflammatory protein A (OipA), duodenal ulcer promoting gene A (DupA), sialic acid-binding adhesin, (SabA), neutrophil-activating protein (NAP), blood-group antigen-binding adhesin (BabA), and heat shock proteins such as Hsp10 and Hsp60. Besides, host-dependent factors and gastric microenvironment promote this process, among others, by inducing an inflammatory response and a release of β-catenin or EGFR however, not every *H. pylori* vector will induce the development of GC.The malignant transformation affects approximately 1-3% of patients infected with *H. pylori* [69]. The whole process from *H. pylori* infection to GC begins with the formation of atrophic gastritis, followed by the intestinal metaplasia,

ultimately leading to dysplasia and adenocarcinoma. Oxidative stress, free radical production, the influx of neutrophils, macrophages, and tumor necrosis factor (TNFα) are important factors thatmodulate the gastric microenvironment in this process [70,71]. Cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2) pathway and activation of NF-κB or Stat3 are considered to be the main pathways for the tumor-promoting inflammatory response [72].Other diseases in which *H. pylori* infection might be of importance include neurological, dermatological, and hematological diseases, as well as diseases related to the organ of vision, cardiovascular system, or allergies [73].Eradication of *H. pylori* could probably reduce the occurrence of GC [74].Research suggests that eradication therapy is cost-effective especially for MALT lymphoma [75]. What's intriguing, is that the opposing results were observed for esophageal cancer. According to some research, *H. pylori* was recognized as a factor preventing the occurrence of adenocarcinoma of this organ [76].

5. Helicobacter pylori virulence factors

5.1 Urease

H. pylori produces a significant amount of urease that enables the colonization of this bacterium in the gastric mucosa inducing inflammatory responses. Urease hydrolyze urea into ammonia and carbamate, increasing the pH of the acidic environment within the stomach. Despite alterations that are induced by the urease in the gastric mucosa, it is also involved in the activation of neutrophils in vivo and in vitro in the gastric microenvironment, as well as impairs gastric epithelial tight junctions [77,78]. Virulence of the urease is associated with the induction of the pro-inflammatory responses and platelet- and neutrophil-activating properties, however, it also exhibits pro-angiogenic properties that significantly enhance the progression of GC [79]. Urease might be used as a marker of *H. pylori* infection at the same time enabling *H. pylori* colonization and persistence in the acidic stomach environment [80-83].

5.2 Cytotoxin-associated gene A

H. pylori strains can be divided into *cagA*-positive and *cagA*-negative depending on the presence of the *cagA* gene that encodes cytotoxin-associated gene A (CagA) protein. The prevalence of *cagA* presence in *H. pylori* strains is estimated at approximately 70% [84]. Numerous adhesions enable the adhesion of *H. pylori* to the surface of gastric mucosa and the formation of type IV secretion system (TFSS) that further provides the delivery of CagA into the host cells [85]. CagA enhances inflammation within the gastric mucosa which further increases the pro-oncogenic activity of CagA inducing neoplastic transformation of the epithelial cells of the gastric mucosa. *H. pylori cagA*-positive strains promote inflammation by numerous mechanisms such as the activation of signal transducer and activator of transcription 3 (STAT3) and further induction of Janus kinase (JAK)/STAT3 signaling pathway, over-activation of the PI3K/Akt/NF-κB signaling pathway, or imbalances between SHP2/ERK and JAK/STAT3 pathways [86,87]. Besides, infection by *cagA*-positive *H. pylori* strains enhances the oxidative DNA damage via increased H_2O_2 production [88]. CagA might affect the cellular shape and motility via forming a complex with the CT10 regulator of the kinase (Crk) adaptor protein, Src homology 2 phosphatase (SHP-2), Abl kinase and a splice variant of Crk, CrkII [89]. CagA along with vacuolating cytotoxin A (VacA) is considered to play an underlying role in gastric carcinogenesis.

5.2 Vacuolating cytotoxin A

Vacuolating cytotoxin A (VacA) plays a role in the formation of anion-selective channels that enhance the release of urea and bicarbonate from host cells, promoting *H. pylori* growth [90]. Further, VacA increases paracellular epithelial permeability by the reduction of the transepithelial electric resistance of polarized cells. VacA is involved in the induction of apoptosis of intoxicated cells as well, primarily due to the activation of caspase-3 [91]. Gastric mucosa damage is promoted by the inflammatory responses induced by VacA such as enhanced release of inflammatory cytokines (IL-1β, IL-6, IL-10, IL-13, and tumor necrosis factor α (TNFα)), overproduction of prostaglandin E_2 (PGE2), or overactivation of p38, ERK, autophagy, and β-catenin signaling pathways [92-94]. Besides, VacA is involved in the mitochondrial dysfunctions, alterations of the epithelial barrier, or the inhibition of the T cells and B cells [95].

5.3 Adhesins

H. pylori adhesins enable the identification of particular peptidoglycans on the surface of gastric epithelial cells promoting bacterial colonization within the gastric mucosa. Among them, blood-antigen binding protein A and B (BabA and BabB), sialic acid-binding adhesin (SabA), *H. pylori* outer membrane protein (HopZ), adherence-associated lipoprotein A and B (AlpA and AlpB), outer inflammatory protein A (OipA), and lacdiNAc-binding adhesin (LabA) are so far the most commonly described in the literature [96]. *H. pylori* adherence to the gastric epithelial cells is associated with the enhanced inflammation as well as more the severe course of GC [97].

5.4 Neutrophil-activating protein

Neutrophil-activating protein (NAP) is involved in the neutrophil and monocyte stimulation inducing enhanced oxygen radical production and increased neutrophil adhesion to the gastric epithelial cells. NAP stimulated the release of numerous cytokines, primarily IL-12 and IL-23, from neutrophil granulocytes and monocytes, as well as dendritic cells [98]. NAP constitutes crucial *H. pylori* virulence factor mainly by facilitating bacterial colonization due to its adherence properties as well as enable bacterial protection primarily due to the prevention of DNA damage from oxidative stress. Besides, NAP is also involved in the intensification of the inflammation induced by *H. pylori* [99].

5.5 Heat shock proteins

Heat shock proteins (HSP) constitute a family of proteins that enable bacterial survival, particularly in stressful environmental conditions. HSP60 might be involved in the progression of gastric carcinogenesis via the release of significant amounts of proinflammatory cytokines, as well as enhanced angiogenesis and metastatic properties. HSP60 also stimulates the increased production of IL-8 by monocytic cells and might act as a molecular chaperone in the gastric environment [100,101] HSP27 is considered to be involved in the *H. pylori* survival as well as intensified damage of the gastric mucosa [102]. HSP70 contributes to the altered gastric epithelial cells proliferation [103].

6. Epithelial-mesenchymal transition and gastric carcinogenesis

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells undergo numerous biochemical reactions that eventually lead to the mesenchymal phenotype transformation (Figure 1).

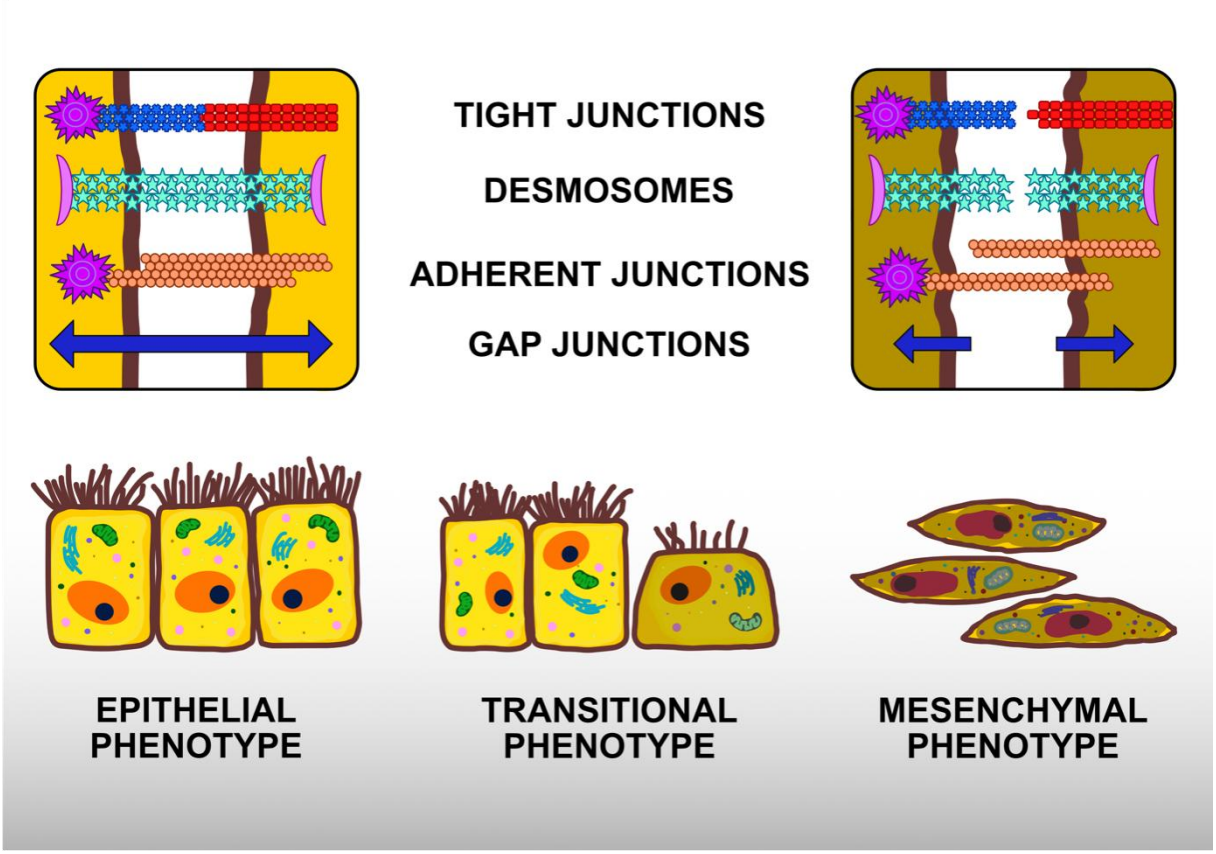

Figure 1. Epithelial-mesenchymal transition – a gradual transformation from the epithelial to mesenchymal phenotype and accompanying alterations in the intercellular connections.

During EMT, epithelial cells gain greater migratory properties, enhanced invasiveness, as well as resistance to apoptosis [104]. EMT, in order to be initiated, requires several molecular processes to occur including overactivation of particular transcription factors, production of extracellular-matrix-degrading enzymes, or expression of specific cytoskeletal and cell-surface proteins [105]. However, EMT is primarily initiated by the alterations within the adherens junctions as well as disturbed adhesions between the cell and extracellular matrix [106]. Besides, transforming growth factor-β (TGF-β) is considered to be an underlying inducer of EMT; other inducers include hepatocyte growth factor (HGF), tumor necrosis factor-a (TNF-a), hypoxia-inducible factor 1a

(HIF1a), Twist1, Zeb1, Zeb2, Snail 1, or Snail2 [107]. Even though EMT is a physiological process that enables proper tissue regeneration and fibrosis as well as maintenance of several processes during embryogenesis, under pathological conditions, it might be a process associated with such cancers as gastric cancer, colorectal cancer, cholangiocarcinoma, breast cancer, or thyroid cancer [108].

During the EMT process, epithelial cells lose their polarity as well as proliferative capacities. Due to the loss of epithelial cells' polarity and the adherence of the tight junctions, invasiveness, and metastatic properties are significantly increased. The whole cell cytoskeleton becomes progressively impaired resulting in the cell's elongated, spindle-like shape and altered cellular junctions. A crucial aspect of EMT is the reversal of the amounts of specific cadherins – a significant E-cadherin downregulation (epithelial marker) and N-cadherin upregulation (mesenchymal marker) which further promotes EMT process itself and stimulates the induction of cancer stem cell-like characteristics [109] (Table 1).

Epithelial markers (decrease)	**Mesenchymal markers (increase)**
E-cadherin	N-cadherin
α-catenin	Vimentin
γ-cadherin	Fibronectin
Zonula occludens-1	Snail1 and Snail2
Cytokeratin	zinc-finger E-box binding homeobox1 and zinc-finger E-box binding homeobox2
Entactin	Twist-related protein 1 and Twist-related protein 2
Laminin-1	*β-catenin*
Desmoplakin	α-smooth muscle actin
Syndecan	Fibroblast-specific protein 1
Mucin 1	OB-cadherin
Type IV collagen	Type I and type III collagen
Claudin	Lymphoid enhancer factor 1

Table 1. Changes in the levels of the epithelial and mesenchymal markers during epithelial-mesenchymal transition.

GC is a tumor in which microenvironment is characterized by enhanced oxidative stress, whereas hypoxic conditions significantly promote EMT by lowering E-cadherin and increasing N-cadherin, vimentin, Snail, or octamer-binding transcription factor 4 (Oct4) levels, resulting in enhanced alterations within the cytoskeleton structure [110]. A large number of patients with dysplasia or early GC present with lowered E-cadherin levels and increased amounts of mesenchymal markers since GC onset is significantly associated with the induction of the EMT process [111]. A significant decrease in E-cadherin levels is also associated with higher invasiveness of GC and enhanced metastatic properties [112]. The incidence of EMT in patients with GC is associated with correlated with poorer prognosis and usually more advanced TNM stages [113]. There is a strong relationship between chronic inflammation, EMT induction, and further GC progress, whereas one of the most crucial pro-inflammatory factors is the infection by *H. pylori*.

7. Helicobacter pylori cytotoxin-associated gene A and its influence on EMT in gastric carcinogenesis

Many researches indicate that *H. pylori* pathogenicity is highly associated with the expression of CagA which was presented to induce a higher risk of GC or peptic ulcer in cases of CagA-positive *H. pylori* infections. The injection of *H. pylori* CagA into gastric epithelial cells is considered to be major causation and trigger of EMT in the stomach. The presence of CagA induces the transition of the gastric epithelial cells into a more invasive phenotype as well as promotes a significant elongation of cells that constitute characteristics typical for EMT [114]. Within GC microenvironment, EMT could be initiated by *H. pylori* CagA by downregulation of the E-cadherin expression primarily by inhibiting programmed cell death 4 (PDCD4); besides, CagA promotes vimentin and Twist-related protein 1 (TWIST1) upregulation [115]. CagA induces a significant upregulation of the mesenchymal and stem cell markers as well as overexpression of CD44, which is believed to be involved in the gastric dysplasia and GC onset [116]. Moreover, chronic *H. pylori* CagA-positive infection stimulates the increase of CD44 cells with stem cell-like properties that present greater tumorigenic potential. CagA-positive *H. pylori* strains induce the overexpression of the *IL-23A* gene which further stimulates the release of IL-23 that is

involved in the induction of the STAT3 pathway affecting the EMT process within gastric mucosa [108]. Since IL23A might be detectable in the sera of patients with GC, it is hypothesized that it might constitute a potential marker enabling the assessment of GC prognosis [117]. Cellular elongation, enhanced metastatic properties, as well as resistance to apoptosis might be induced by the activation of the c-Abl which is responsible for CagA phosphorylation in gastric epithelial cells [118]. Increased levels of c-Abl are observed in cases of gastritis induced by *H. pylori*, primarily CagA-positive. Another mechanism of EMT enhancement by CagA-positive *H. pylori* strains include the overactivation of Yes-Associated-Protein Pathway (YAP) which further activates several other oncoproteins such as *MYC* oncogene, connective tissue growth factor (CTGF), or cysteine-rich angiogenic inducer 61 (CYR61) [119]. It was demonstrated that gastric cancerous tissues express higher YAP and TAZ (YAP paralog) which is associated with poorer prognosis and greater invasiveness of GC [120,121]. Shi et al. (2019) demonstrated that CagA-positive *H. pylori* strains incudes the overexpression of miR-543 which promotes cell migration, invasion, and apoptosis resistance, which all constitute the components of the EMT process [123]. MiR-543 by targeting SIRT1 inhibits autophagy mediated by CagA, further enhancing the above-mentioned processes. Yoon et al. (2014) observed the re-expression of epithelial markers (E-cadherin, Slug, Snail, vimentin, p-Akt, and β-catenin) as well as the inhibition of the reactive oxygen species production, genetic alterations, and EMT progress, in the presence of gastrokine 1 (GKN1) [124]. The results of the study indicate that GKN1 might constitute a potential therapeutic modality involved in the significant reduction of malignant transformation and further GC progression. CagA-positive *H. pylori* strains also induce the overexpression of *CDX1* [125]. *CDX1* is an oncogene that enhances EMT via increased cell proliferation, induction of stem cell-like phenotype in gastric epithelial cells, as well as resistance to chemotherapeutic agents to GC. Besides, *CDX1* stimulates the differentiation of gastric epithelial cells into intestinal epithelial cells via overexpressing transcription factors - SALL4, KLF5, and peroxisome proliferator-activated receptor γ (PPARγ) [126,127]. CagA stimulates the fibroblast differentiation into cancer-associated fibroblasts (CAFs) as well as increased myofibroblast formation; CAFs stimulate the elevated release of HGF, TGFβ, IL-6, HIF1a, and stromal cell-derived factor 1 (SDF-1) that are involved in reprogramming the phenotype of gastric cells from epithelial to mesenchymal [128]. IL-6, IL-8, and fibroblast activation protein (FAP) released by CAFs significantly contribute to macrophage differentiation and M2 polarization. Fibroblast-associated inflammatory responses are promoted by NFκB and STAT3 signaling pathways, as well as Snail1 overexpression contributing to the development of EMT microenvironment in gastric mucosa [129]. These processes are promoted either directly by CagA or indirectly by the activation of TLR2 and TLR4. Huang et al. demonstrated the decreased expression of miR-134 during CagA-positive and penicillin-binding protein 1A mutation-positive *H. pylori* infection, which induced greater cell proliferation, invasiveness, and EMT progression [130]. CagA-positive and VacA-positive *H. pylori* strains might also promote EMT progression in GC by the upregulation of ZEB1 transcription [131]. CagA-positive *H. pylori* strains promote EMT progression because of CagA binding with glycogen synthase kinase-3 (GSK-3) resulting in its decreased activity of the two forms of GSK - GSK-3α and GSK-3β - and the induction of other oncogenic pathways [132]. CagA-positive *H. pylori* strains might contribute to the p53 degradation altering cellular differentiation of the gastric epithelial cells and the impaired apoptotic processes. Such *H. pylori* strains are also capable to induce cancer stem cell-like properties [133]. The loss of the polarity by gastric epithelial cells in the mechanism of CagA action is considered to involve the interaction of the apoptosis-stimulating protein of p53 (ASPP2) and partitioning-defective polarity (PAR) complex. CagA regulates EMT processes by inhibiting programmed cell death protein 4 (PDCD4) in gastric epithelial cells at the same time increasing TWIST1 and vimentin expressions and decreasing E-cadherin levels [134]. Sougleri et al. (2015) reported that CagA promotes the increased transcriptional activation of metalloproteinases (MMP) - MMP-3 and MMP-3, which are EMT markers as well [135].

8. Conclusions

H. pylori CagA-positive strains alter gastric microenvironment via numerous processes that lead to the enhanced proinflammatory responses induced by activated neutrophils, macrophages, natural killer cells (NK cells), or T-cells. The pathogenicity of CagA-positive *H. pylori* strains includes several mechanisms that alone or primarily overlapping each other, induce EMT in gastric epithelial cells reversing phenotype into mesenchymal. This, in turn, significantly increases GC progression and is usually related to poorer prognosis and clinical outcome of patients. In the following review, we presented CagA-related mechanisms in the induction of EMT in the gastric mucosa that are, to our knowledge, so far most widely described in the literature. The mechanisms of CagA pathogenicity in the induction of EMT and further gastric carcinogenesis include among others, induction of the cancer stem cell-like properties, prevention of apoptosis, activation of the YAP pathway, aberrant expression of *CDX1* gene, enhanced release of pro-inflammatory cytokines, activation of CAFs, reduction of GSK-3 activity, the formation of the CagA-ASPP2 complex, or downregulation of PDCD4 protein. Researchers worldwide are continually seeking new molecular targets that might act as potential therapeutic targets in the treatment of GC patients. *H. pylori* eradication leads to an increase in E-cadherin levels at the same

time downregulating mesenchymal markers such as TGF-β, Snail, Slug, Twist1, and vimentin which confirms its significant involvement in EMT.

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