Review Article

Roles of *Helicobacter pylori* in the Epithelial-Mesenchymal Transition of Gastric Cancer

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Abstract

Gastric cancer (GC) is one of the most frequent malignant tumors in humans, with over 50% of patients after treatment suffering from recurrence and peritoneal metastasis. *Helicobacter pylori* (*H. pylori*) infection is critical to the development of GC. The phenomenon of epithelial-mesenchymal transition (EMT) in GC is linked with development of the invasive phenotype, which is very likely regulated by *H. pylori* through altering signaling pathways in the gastric cells. In this review, we conclude the current studies on how *H. pylori* affects the EMT of GC, thus contributing to its initiation and metastasis.

Keywords: *Helicobacter pylori*; Epithelial-mesenchymal transition; Gastric cancer; *cag* pathogenicity island (*cag* PAI); Cytotoxin-associated gene A antigen (CagA); TNF-α-inducing protein (Tipα); matrix metalloproteinases (MMPs); tumor microenvironment (TME)

1. Introduction

Gastric cancer (GC) is the fifth most commonly diagnosed malignancy and the third most common cause of cancer-related death worldwide [1, 2]. Over 70% of GC cases occur in developing countries with half the global total cases occurring in Eastern Asia [3]. On the basis of compelling evidence, the World Health Organization (WHO) has confirmed that the incidence of GC, particularly gastric adenocarcinoma (GAC), is closely related to the presence of a class I carcinogen, namely, *Helicobacter pylori* (*H. pylori*) [4, 5]. Since Marshall and Warren first identified *H. pylori* in 1983, a diverse spectrum of gastrointestinal diseases has been found to link with this causative agent, including gastric and duodenal ulceration, GAC, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric non-Hodgkin's lymphoma [6]. On the basis of regional prevalence estimates, approximately 4.4 billion individuals were infected with *H. pylori* globally in 2015. That is, *H. pylori* affected more than half the world's population [7]. Among the patients with *H. pylori*, approximately 10% ends up with peptic ulcer disease, 1-3% develops GC, and 0.1% suffers from gastric MALT lymphoma [8]. Due to unsuccessful eradication-related reinfection and recidivation, intrafamilial transmission associated with low socioeconomic status (e.g., more crowded living conditions) or iatrogenic infection by means of endoscopes, the amount of *H. pylori* infected population has persisted or even increased over the past three decades throughout the world [9].

Metastasis is the main cause of death for GC patients, with over 50% of patients suffering from recurrence and peritoneal metastasis after treatment [10]. The major mechanism of metastasis is the epithelial-mesenchymal transition (EMT) [11]. EMT is a developmental process during which epithelial cells acquire the properties of motility and migration like mesenchymal cells. EMT is relevent to the development of invasive phenotype of GAC [12]. Evidences also suggest that cells undergoing EMT obtain stem cell-like characteristics [13]. EMT-induced cancer stem cell phenotype is conductive to the initiation of GC [14]. Recent studies have indicated that *H. pylori* promotes EMT in gastric cancer [11, 15]. For example, eradication of *H. pylori* reduces the expression of TGF-β1 and increases E-cadherin expression. This indicates that *H. pylori* is a trigger of TGF-β1-induced EMT [16]. Lee et al. pointed that cytotoxin-associated gene A (CagA), the major virulence factor of *H. pylori*, leads to Snail-mediated EMT by reducing GSK-3 activity [17]. Besse`de et al. also demonstrated that *H. pylori* induces the EMT-like variations in gastric epithelial cells, which unveil CSC-like properties [18]. Hence, it is critical to understand the molecular mechanisms of *H. pylori*-induced EMT in order to develop new strategies against GC.

In this review, we will illustrate epidemiology of *H. pylori*-related gastric malignancies, discuss the factors influencing the EMT of GC, and elaborate on recent developments in the molecular mechanisms of *H. pylori*-induced EMT in GC.

2. Epidemiology of *H. pylori*-related gastric malignancies

H. pylori is micro-aerophilic Gram-negative bacillus which is spiral-shaped and flagellated. This kind of bacillus colonizes the gastric mucosa of more than 50% of human beings, while developing countries have the highest prevalence [19]. As a class I carcinogen, *H. pylori* is a causative factor in the cascade leading to GAC, especially non-proximal cancers [8, 9]. A recent large retrospective cohort research, including 371,813 patients in the US with a diagnosis of *H. pylori* infection, found that the cumulative incidence rate of GC at 5, 10, and 20 years after detection of infection was 0.37%, 0.5%, and 0.65%, respectively [4]. This study also showed that treatment of *H. pylori* infection hardly reduce the risk of GC unless eradication of *H. pylori* [4]. Analogously, a systematic review and meta-analysis of six randomised controlled trials (RCTs) suggested that searching for and eradicating *H. pylori* infection were useful tools in reducing the subsequent incidence rate of GC in healthy asymptomatic infected Asian individuals, with a pooled relative risk of 0.66 (95% CI: 0.46-0.95) [20]. This data is confirmed by a meta-analysis by Lee et al. in 2016. They reported that after *H. pylori* eradication, GC risk was decreased by about 35% [21]. Yet, studies also show that infection alone is not adequet for carcinogenesis, proved by high *H. pylori* prevalence and low GAC occurance in sub-Saharan Africa (the "African enigma"), or different incidence rates of GAC throughout Middle Eastern countries despite high *H. pylori* burden [7, 22].

3. Factors affecting the EMT of GC

EMT is produced by complex molecular and cellular procedures, through which the epithelial cells dedifferentiate, loose intercellular adhesion and apical-basal polarity, and acquire mesenchymal characteristics, including motility, invasiveness, and a heightened resistance to apoptosis. Theoretically, epithelial cells obtain the phenotype of mesenchymal cells, such as fibroblasts, which is the generation of EMT [23]. EMT is crucial in the tumorigenic process, contributing to invasion, motility, and a heightened resistance to apoptosis. Abnormal biological behaviors in the EMT of adult epithelial cells inhibit cell adhesion molecules, resulting in a decrease in cell adhesion ability, thereby allowing tumor cells to spread in the body and ultimately promoting tumor metastasis [24]. Therefore, EMT is considered as the beginning of invasion and metastasis, and it also indicates that the tumor cells have a strong ability of invasion and metastasis. In the process of EMT, the cells shut down the expression of epithelial biomarks, like cytokeratins and E-cadherin, and lead to the expression of mesenchymal markers, including vimentin, fibronectin, N-cadherin and integrin, and the expression of other regulatory molecules like SNAIL, TWIST and SLUG, which change obviously [25-27]. Oncogenic pathways inducing EMT include transforming growth factor β (TGF-β), Src, Ets, Ras, Wnt/β-catenin, Notch, nuclear factor-κB and integrin [28-30].

Many factors influence the EMT in GC. One of the classic oxidative stress-related malignances is GC [31], indicating that certain redox-sensitive factors may be important EMT modulators. Taking SENP3 as an example, it is a redox-sensitive SUMO2/3-specific protease. SENP3 induces and promotes the EMT of GC cells by de-conjugating SUMO2/3 and activating an EMT-inducing transcription factor

called FOXC2 [32]. Hypoxia causes the decrease of E-cadherin, and leads to the increase of N-cadherin, Vimentin, Snail, Sox2, Oct4, and Bmi1, In other words, the hypoxic microenvironment facilitates the generation of EMT, together with cytoskeleton remodeling [33]. Cytokines, chemokines and matrix metalloproteinases (MMPs) are the inflammatory mediators which also participate in the EMT of GC [11]. All constituents of the tumor microenvironment can secrete cytokines, such as TNF-α, IL-8, TGF-β, TGF-α, and IL-6, which seem to change the EMT of GC cells [34]. CXCR4 and CCR7 are the most important two chemokine receptors in GC. Actin polymerization is activated after CXCR4 binding its ligand CXCL12, inducing cell motility and the EMT [35-37]. Activation of CCR7 signaling leads to the initiation of EMT in GC cells, by transforming the expression of E-cadherin, MMP-9, and Snail. Thus, cells metastasize toward lymph vessels successfully [38, 39]. The MMP family degradates the extracellular matrix (ECM) and basement membrane barriers, for which this family becomes one of the most important inducers of the EMT [40]. Recent studies have also suggested some other mechanisms for inducing GC EMT. Erythropoietin-producing hepatocellular A2 (EphA2) upregulation, a common event in GC, promotes EMT through activation of Wnt/β-catenin signaling [41]. In human GC tissues, when Aquaporin 3 (AQP3) is overexpressed, which will promote the induction of EMT via the PI3K/AKT/Snail signaling pathway [42].

Recently, researchers found that coculturing *H. pylori* with gastric epithelial cell lines (AGS, MGLVA1, and ST16) contributed to the upregulation of the expression of EMT-associated genes like Snail, Slug, and vimentin [12]. It is reported that treating the human gastric cancer cells with *H. pylori* induces cytoskeletal reorganization through activation of Rac [43] and phosphorylation of focal adhesion kinase (FAK) [44]. As a result, *H. pylori* infection seems to act as an inducer of cell adhesion and motility. Thus, *H. pylori* infection may induce or facilitate EMT process in the GC microenvironment [45].

4. Molecular Mechanisms involved in the EMT of *H. pylori*-related GC

H. pylori is related to the development of gastric adenocarcinoma and lymphoma. *H. pylori* metastasizes to host cells, thereby regulating cell proliferation, affecting the normal apoptotic pathway, influencing cell shape, eliminating connection activity, and promoting EMT phenotype [46, 47]. In the environment of gastric cancer, the bacterial virulence factors involved include cytotoxin-related gene A antigen (CagA), vacuolar cytotoxin (VacA) and outer membrane protein (OMP) [48]. In the following, we will summarize the key mechanisms through which *H. pylori* induces the EMT of GC.

4.1 *cag* PAI

The virulence of *H. pylori* is closely related to the *cag* pathogenic island (*cag* PAI) locus encoding the type IV secretion system (T4SS) and the bacterial oncoprotein CagA [49]. The cag pathogenic island of the pathogenic *H. pylori* type I strain, with a genetic element of approximately 40 kb, encodes a type IV secretion system for exporting virulence determinant. What's more, virulence determinants is closely related to gastric malignant progression [51]. The T4SS forms a syringe-like fimbria structure through which CagA can be injected into target cells [8]. *H. pylori* containing *cag* PaI increases the expression of matrix metallopeptidase 7 (MMP-7) by up-regulating

gastrin secretion via activating gastrin releasing peptide (through the T4SS), leading to increased levels of soluble heparin-binding epidermal growth factor (HB-EGF), thereby triggering the expression of key EMT proteins (e.g., Snail, Slug and Vimentin), which may eventually play a role in the GC development [12].

4.2 CagA protein

A major virulence factor for *H. pylori* is the cytotoxin-related gene a (CagA), which encodes the cagA protein in *cag* PAI. CagA deliveres into gastric epithelial cells via the T4SS and results in cellular transformation [52, 53]. Injecting CagA into gastric epithelial cells induces EMT, which might be the critical triggers of carcinogenesis [54]. CagA+ *H. pylori* infection of normal human gastric epithelial cells increases the expression of EMT symbols Slug and Snail, thus increasing invasion and migration [55]. CagA induces epithelial cells to transition from a polarized state to an invasive phenotype, which is the cellular characteristic of EMT, depending on the signaling triggered by the CagA C-terminal EPIYA motif and the N-terminal mediated CagA localization in the intercellular junction [56]. Whole-genome expression arrays reveal that the intracellularly translocated CagA regulates the expression of EMT-related genes, regardless of the phosphorylation status of CagA [57]. *H. pylori* CagA, as a pathogenic scaffold protein, binds GSK-3 in a similar manner to Axin to make it insoluble, resulting in reduced GSK-3 activity, thereby stabilizing E-cadherin transcription repressor Snail which finally induces the EMT of GC [17]. *H. pylori* CagA, acting as a pathogenic protein, promotes oncogenic YAP pathway, which leads to EMT and gastric cancer [58]. In addition, *H. pylori* CagA can induce gastric cancer cells to produce TWIST1 or vimentin, and inhibit the expression of epithelial cadherin. CagA-induced EMT partly depends on PDCD4 regulation. TWIST1 and PDCD4 are involved in EMT of GC [59]. Studies have showed that microRNAs (miRNAs) play a key role in GC associated with *H. pylori* [60, 61]. Compared with *H. pylori*-negative cancer tissue samples, miRNA microarrays display that miR-543 expression was remarkably increased in *H. pylori*-positive gastric cancer tissue [62]. Shi et al. reported that in GC which associated with *H. pylori*, the overexpression of miR-543 is induced by CagA, causing the translational repression of SIRT1 and suppressing autophagy. Subsequently cell migration and invasion are caused by increased expression of EMT [63]. CagA- and penicillin-binding protein 1A (PBP1A) mutation-positive *H. pylori* (CagA+/P+) strain promotes EMT in GC via the suppression of microRNA-134 [64].

H. pylori CagA also influences the cells surrounding GC, mainly activated cancer-associated fibroblasts (CAFs), which create molecular microenvironment promoting tumorigenesis and cancer invasion [65, 66]. *H. pylori* CagA can induce the activation and differentiation of gastric fibroblasts, mediated by transcription factors NFκB and STAT3 signaling leading to rapid Snail1 protein expression, which may finally activate the secretome responsible for fibroblasts inflammatory and EMT-inducing microenvironment serving for GC development [10]. Normal fibroblasts which induced by *H. pylori* (cagA+vacA+) strain were differentiated into CAFs, which may initiate the EMT process in normal RGM-1 epithelial cell line [67]. Jin et al. also found that *H. pylori* (cagA+vacA+) upregulates the transcription of ZEB together with expression of claudin-2 and CDX-

2, by this way the EMT of AGS cells is promoted [68].

4.3 Tipα

H. pylori in the gastric epithelium releases a carcinogenic factor called the tumor necrosis factor-α (TNF-α)-inducing protein (Tipα) [69], resulting in induction of EMT in human gastric cancer cell lines [70]. Tipα protein composes of 172 amino acids with 19 kDa and plays a role of homodimer with 38 kDa, which is one of the strong TNF-α inducers [70]. Large amounts of Tipα can be secreted by *H. pylori* isolated from gastric cancer patients. Tipα combines with gastric cancer cells by directly binding to nucleolin on the cell surface, during which nucleolin is the receptor of Tipa [70-72]. Tipα is shuttled from membrane to nuclei by surface nucleolin [71], leading to the expression of TNF-α gene through activating NF-κB [69], inducing the process of EMT [73]. Researchers reported that Tipα resulted in formation of filopodia in gastric cancer cell lines, suggesting invasive morphological changes and reducing the Young's modulus of gastric cancer cells, the latter represented that cell stiffness falls and cell motility increases [70]. In human gastric cancer cells, the morphological changes induced by Tipα are crucial phenotypesc of EMT. In terms of molecular mechanisms, Tipα enhances phosphorylation of cancer-related proteins, and increases the expression of vimentin (a significant marker of EMT) with activation of MEK-ERK1/2 signal cascade [70]. Tipα also accelerates tumor aggressiveness in GC by promoting EMT through the way of activating IL-6/STAT3 signaling pathway [74].

The protein Lpp20 (*hp1456*) is one of the key 344 genes contributing to *H.pylori* survival and host colonization [75] locating in the cell envelope or being released inside membrane vesicles in the culture medium [76], is a structural homologue of Tipα and promotes EMT of *H.pylori* [77]. It is proved by researchers that *in vitro*, Lpp20 induces the down-regulation of E-cadherin in gastric cancer cells, besides promotes the migration and proliferation of cells together with the formation of filopodia [77].

4.4 MMPs

H. pylori infection upregulates the expression of matrix metalloproteinase (MMP) family for the reason that the proteins needed to be secreted by pathogens to help their adherence to epithelial gastric cells [78, 79]. The MMPs are one of the most important inducers of the EMT, which induces the EMT by means of the degradation of the extracellular matrix (ECM) and decompose basement membrane barriers [40]. Researchers found that the invasion ability of gastric cancer cells is enhanced by increased expression of MMP-2 and MMP-9, which assioated with the metastasis of GC [80-82]. Upregulation of MMP-7 expression is a biological marker of *H. pylori*-associated GC, potentially regulating the progression of GC through the EMT [12, 83, 84].

4.5 TME

Tumor cells and stroma, a network of blood vessels and a variety of infiltrating inflammatory cells consitituted the tumor microenvironment (TME). These cells significantly promote the progression of GC [85, 86]. *H. pylori* infection mainly targets on gastric fibroblasts and promotes to the paracrine interactions between *H. pylori*, gastric fibroblasts, and epithelial cells. Gastric fibroblasts activated by *H. pylori* can secrete TGF-β, which prompting their

differentiation toward CAF-like phenotype and the EMT-related phenotypic shifts in normal gastric epithelial cell populations, which is the prerequisite for GC development [87]. *H. pylori*-infected fibroblasts show enhanced expression of Snail1 and Twist mRNA[88]. Twist1 is a key regulator of EMT in the GC microenvironment, making an influence on transisting normal fibroblasts (NFs) to CAFs with CXCL2 which acts as the target for transcription [89]. Infected with *H.pylori* in GC may induce a signaling pathway which is called cyclooxygenase-2/prostaglandin E2(COX-2/PGE2) [90]. In CAFs the hyper-methylation of miR-149 is induced by PGE2, contributing to the enhanced secretion of IL-6 [91], which may induce EMT through activating the JAK2/STAT3 pathway in GC [92]. Mesenchymal stem cells (MSCs), which have multipotent differentiation potential. At the sites of cancer and inflammation, MSCs shows its tropism [93, 94]. MSCs are key components of the *H. pylori* infection-associated GC microenvironment, which may be of big importance for GC cell migration [45]. *H. pylori*-infected MSCs obtain the pro-inflammatory phenotype by secreting a combination of multiple cytokines, which are NF-κB-dependent and enhancing the migration of GC cells by promoting EMT [45].

4.6 Other signalings

An actin-binding protein called Afadin is associated with nectins at adherens junctions meanwhile connected with ZO-1 instantly, and regulates the formation and stabilization of the junctional complexes [95, 96]. The expression of Afadin is downregulated when *H. pylori* infection happens, resulting in the emergence of EMT and the acquisition of an aggressive phenotype of gastric cells. This may contribute to the occurrence of GC [97]. Zhou et al. reported that infection with *H. pylori* promotes EMT of gastric cells by upregulating lysosomal-associated protein transmembrane 4β (LAPTM4B) [98]. *H. pylori* infection triggeres the EMT pathway which is induced by TGF-β1, and causes the appearance of gastric cancer stem cells, for example, CD44v8-10 [99]. Meanwhile, Chang et al. points out that the EMT pathway which is induced by TGF-β1only upregulates the TGF-β1when cagE-positive *H. pylori* infection occurs,thereby promoting EMT [100]. A protein specifically localized in the Golgi apparatus called PAQR3, is markedly down-regulated in human GC, and is related to *H. pylori* infection negatively. PAQR3 expression level is tightly in relation to the progression and metastasis of GC [101]. *H. pylori* infection is the cause of GC but host factors are also implicated. IQGAP1 is a scaffolding protein of the adherens junctions interacting with E-cadherin and regulating cellular plasticity and proliferation, whose deficiency favours the acquisition of a mesenchymal phenotype and CSC-like properties induced by *H. pylori* infection [102].

5. Conclusions and Perspectives

In all types of cells, EMT phenomenon is closely related to tumor invasion and metastasis. This article focuses on the EMT process of gastric cancer. Due to infection with *H. pylori*, GC EMT is characterized by transient structural changes, loss of polarity, reduced contact with surrounding cells and matrix, enhanced cell migration, and altered cell phenotypes. This review demonstrates detailedly how *H. pylori* induces the EMT of GC through a variety of different mechanisms. Based on the key targets such as *cag* PAI, CagA, Tipα, MMPs, etc., we can develop

strategies to inhibit gastric cancer metastasis. By blocking the factors that affect the occurrence of EMT and exploring new regulators, we are able to clarify the relationship between EMT and GC, and provide theoretical basis for the development of new drugs for GC invasion and metastasis.

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