Priming the seed: *Helicobacter pylori* alters epithelial cell invasiveness in early gastric carcinogenesis

Silvia Molina-Castro, Vanessa Ramírez-Mayorga, Warner Alpízar-Alpízar

**Abstract**

*Helicobacter pylori* (*H. pylori*) infection is a well-established risk factor for the development of gastric cancer (GC), one of the most common and deadliest neoplasms worldwide. *H. pylori* infection induces chronic inflammation in the gastric mucosa that, in the absence of treatment, may progress through a series of steps to GC. GC is only one of several clinical outcomes associated with this bacterial infection, which may be at least partially attributed to the high genetic variability of *H. pylori*. The biological mechanisms underlying how and under what circumstances *H. pylori* alters normal physiological processes remain enigmatic. A key aspect of carcinogenesis is the acquisition of traits that equip preneoplastic cells with the ability to invade. Accumulating evidence implicates *H. pylori* in the manipulation of cellular and molecular programs that are crucial for conferring cells with invasive capabilities. We present here an overview of the main findings about the involvement of *H. pylori* in the acquisition of cell invasive behavior, specifically focusing on the epithelial-to-mesenchymal transition, changes in cell polarity, and deregulation of molecules that control extracellular matrix remodeling.

**Key words:** *Helicobacter pylori*; Plasminogen activation system; Invasion; Epithelial-to-mesenchymal transition;
Cell polarity; Gastric carcinogenesis

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Helicobacter pylori (H. pylori) infection induces chronic inflammation in the gastric mucosa that, in the absence of treatment, may progress through a series of steps to gastric cancer (GC). GC is only one of several clinical outcomes associated with this bacterial infection, which may be at least partially attributed to the high genetic variability of H. pylori. Accumulating evidence implicates H. pylori in the manipulation of cellular and molecular programs that are crucial for conferring the cells with invasive capabilities, including reprogramming of the epithelial-to-mesenchymal transition signaling programs, changing of the cell apicobasal polarity, and remodeling of the extracellular matrix.

Molina-Castro S, Ramirez-Mayorga V, Alpizar-Alpizar W. Priming the seed: Helicobacter pylori alters epithelial cell invasiveness in early gastric carcinogenesis. World J Gastrointest Oncol 2018; 10(9): 231-243 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i9/231.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i9.231

INTRODUCTION

Persistent Helicobacter pylori (H. pylori) infection induces chronic inflammation in the gastric mucosa, which in susceptible individuals may progress to gastric cancer (GC)[1,2]. The final clinical outcome of the infection depends on complex interactions among the infecting strain of the bacterium, the host, and the environment[3]. The biological mechanisms underlying how and under what circumstances H. pylori alters normal physiological processes in such a way that sequential events culminate in the development of GC remain largely unknown.

A key feature of malignant transformation and progression is the invasion of malignant cells locally and then to distant sites (metastasis)[4]. Invasion and metastasis occur through a series of events in which several processes take place, including reprogramming of signaling pathways that drive the epithelial-derived malignant cells into a mesenchymal-like phenotype, the so-called epithelial-to-mesenchymal transition (EMT), changing of the cell polarity, and remodeling of the extracellular matrix (ECM)[5,6]. Several of these events are activated in gastric epithelial cells by H. pylori directly or as a result of the inflammatory reaction mounted in response to this bacterial infection. This review summarizes the current evidence implicating H. pylori in the activation of molecular and cellular mechanisms related to invasion in the early stages of the pathogenic series of events leading to GC. Specifically, we address the role of H. pylori in the deregulation of molecules that control EMT, cell polarity, and ECM remodeling.

EPIDEMIOLOGY

GC is the fifth most common and the third death-causing cancer worldwide[7]. Incidence rates vary considerably depending on age and sex; however, the most substantial variation is connected to geographic location, with very well-established high- and low-risk areas across the world[8,9]. GC incidence is steadily declining worldwide; and although the reasons are not clear, this may be at least partially linked to the concomitant decrease in H. pylori prevalence[8]. The decrease, however, is not of the same magnitude in GC of different histological subtype or anatomical location[10]. Similarly, mortality rate varies geographically, being particularly high in developing countries but declining globally[8,9]. The 5-year survival rate remains below 30% in most countries, which is mainly connected to the fact that most of the cases are diagnosed at advanced stages, when therapeutic interventions are likely to fail.

HISTOPATHOLOGY

Several schemes are used for classifying GC according to microscopic and histological characteristics. The Lauren classification system is probably the most commonly used[11,12]. The Lauren system divides GC into intestinal, diffuse and mixed subtypes, with important differences at the epidemiological, pathological and molecular levels[11,13].

Marked epidemiological and etiological differences have been revealed for malignant tumors located in the distal part of the stomach and those of the proximal region[14,15]. Therefore, anatomical location of the lesions is regarded as an important parameter in the classification of GC.

PATHOGENESIS

The pathogenesis of GC is a complex and multifactorial process in which environment and lifestyle, host genetics, and H. pylori infection play a role[2,16-21]. As already mentioned, the pathogenesis of GC substantially differs depending on the histological and anatomical subtype. The intestinal subtype of GC, for instance, arises through a sequential series of steps known as the Correa cascade[22], in which H. pylori plays a pivotal role. The infection is usually established early in life and persists lifelong in the absence of treatment, which in combination with environmental factors leads to sustained chronic inflammation characterized by infiltration of inflammatory cells in the gastric mucosa and expression of inflammatory mediators.

Intriguingly, most of the infected individuals remain asymptomatic, while others develop pathologies that are not related to GC. In a minority of infected people, the inflammation evolves into a chronic atrophic gastritis, which is regarded as a pre-neoplastic lesion[22,23]. This may subsequently progress to intestinal metaplasia, dysplasia, and invasive carcinoma[24]. Much less is known.
about the pathogenesis of the diffuse subtype of GC\textsuperscript{[24,25]} and the malignant lesions arising in the most proximal segment of the stomach\textsuperscript{[26]}.\textit{H. pylori}

Infection with \textit{H. pylori} is one of the most prevalent bacterial infections worldwide\textsuperscript{[27]}. This bacterium utilizes several strategies for colonizing and surviving in the hostile environment of the stomach. Some of these are common bacterial mechanisms of acid resistance, such as proton pump activation, decarboxylases, and membrane lipid modification\textsuperscript{[28]}. More specific adaptations to the acidic environment include the enzyme urease, which is encoded by the \textit{ure} gene cluster and catalyzes the conversion of urea into ammonium and carbon dioxide. Urease was, in fact, the first protein identified in \textit{H. pylori} with a role in neutralizing gastric acid, and it is considered a virulence factor\textsuperscript{[29]} since it has proven essential to the survival of the bacterium in the gastric mucosa\textsuperscript{[30,31]}. Besides the \textit{ure} gene cluster, transcriptional regulation in response to acid extends to other genes related to motility, chemotaxis, and virulence\textsuperscript{[32]}.

The genetic variability of \textit{H. pylori} is high, and it probably explains in part the association of this infection with several gastric and extra-gastric pathologies, in addition to GC. Some strains, however, are more strongly associated with GC, namely those harboring particular polymorphic variants in the gene encoding the vacuolating cytotoxin A (VacA) and the ones expressing the Cag pathogenicity island (Cag-PAI)\textsuperscript{[3,33-36]}. Despite no physical or functional relation known for vacA and cag-PAI loci, strains that express virulent VacA usually contain functional Cag-PAI\textsuperscript{[35,37]}. In addition to VacA and Cag-PAI, other virulence factors of \textit{H. pylori} have been associated with gastric pathology, including BabA, SabA, OipA, and DupA (Figure 1)\textsuperscript{[3,36]}.

INVASION AND METASTASIS

The dissemination of cancer cells from primary lesions to form new tumor colonies at distant sites is a key feature of cancer\textsuperscript{[4]}. This occurs in a multistep process, termed the invasion-metastasis cascade: cancer cells locally invade, intravasate into the vascular system, travel in the circulation, extravasate at distant sites, form micrometastatic nodules of cancer cells, and, finally, grow into overt metastatic lesions\textsuperscript{[5,6]}. Importantly, early in this series of events, malignant cells acquire traits that equip them with the ability to invade, leave, and travel to distant tissues. A centrally important process that confers epithelial-derived malignant cells with increased mobility and invasiveness is the EMT program\textsuperscript{[38,39]}.

In order to become invasive, cells commonly lose their apico-basal polarity due to rearrangements in the cytoskeleton, which maintains the shape and internal organization of the cells, and modifications in the intercellular unions that hold them together\textsuperscript{[39,40]}. Also, the degradation of ECM components is essential in several phases of the invasion-metastasis cascade. ECM remodeling is primarily mediated by proteases that belong to the plasminogen activation (PA) system and the matrix metalloproteinase (MMP) family\textsuperscript{[41,42]}. The cellular and molecular mechanisms underlying these processes, as well as their regulation, have been reviewed in depth\textsuperscript{[30,40,43-45]}. Accumulating experimental evidence has implicated \textit{H. pylori} in all these aspects (Figure 2), as discussed below.

EMT and \textit{H. pylori}

EMT is an evolutionary conserved, reversible process in which polarized epithelial cells acquire a mesenchymal phenotype through phenotypical and biochemical changes, thereby resulting in increased capacities of migration, invasion, and apoptosis resistance as well as ECM production and remodeling\textsuperscript{[38]}. Transcription factors such as Snail, Slug, zinc-finger E-box binding (ZEB1/2) and FOXC2 are activated at the beginning of the process. This is accompanied by the expression of specific microRNAs (miRs), for instance the miR-200 family, changes in the expression of particular cell surface proteins, cytoskeletal reorganization, and activation of Wnt/\textit{l}-catenin and Notch signaling\textsuperscript{[38,46,47]}.

A critical feature of EMT is the down-regulation of E-cadherin\textsuperscript{[48]}, a surface glycoprotein expressed in epithelial cells that is a key component of the adherent junctions in epithelial tissues\textsuperscript{[49]}. Expression of E-cadherin can be repressed directly or indirectly by multiple transcription factors, including ZEB1/2, Snail, Slug, nuclear factor-kappa B (NF-\textit{k}B), E47 and KLF8, but also by the proteins SIX1 and FOXC2\textsuperscript{[50-52]}. Furthermore, various signaling pathways can influence the expression of E-cadherin, including TGF\textit{b}, hypoxia-induced response, Wnt/\textit{l}-catenin, Notch and PI3K/Akt, and therefore play a role in EMT\textsuperscript{[53,54]}. Although EMT is usually depicted as a binary switch that shifts cells from a fully epithelial to a fully mesenchymal state, this is a misrepresentation of this process. Frequently, the EMT program drives cells from a fully epithelial state to a partially mesenchymal one in which some epithelial markers are retained. Nonetheless, this subset of mesenchymal traits has profound effects on the cell biology\textsuperscript{[55]}.

Activation of EMT programs in neoplastic cells is usually connected to their dedifferentiation and acquisition of stem cell-like properties\textsuperscript{[56]}. The existence of cancer cells with stem-like properties, the so-called cancer stem cells (CSCs), was first described in breast cancer and subsequently documented in various malignancies, including GC. One the first studies about CSCs in GC showed that these cells have enhanced capability of invasion and tumorsphere formation. Also, it was found that CSCs have distinctive features of the EMT, such as reduced expression of E-cadherin and increased levels of vimentin and MMP2\textsuperscript{[27]}. In primary GC tissue, it was demonstrated by immunohistochemistry that the combination of Snail-1, vimentin, E-cadherin and CD44 predicts tumor aggressiveness\textsuperscript{[58]}. Furthermore,\textit{Molina-Castro S et al. H. pylori alters cell invasive properties
and CD44<sup>high</sup> cell population was dependent on the CagA oncprotein. CagA induces the EMT in a number of ways, as exemplified by a recent in vitro study showing that this bacterial protein up-regulates MMP3, which is also part of the EMT program, through EPIYA motifs in a phosphorylation-dependent manner [62].

Immunohistochemistry staining of human and murine gastric tissue have confirmed that <i>H. pylori</i> infection is correlated with high expression of CD44 and EMT markers [61]. Presumably, ERK and JNK are involved in the described EMT-like changes, CD44 overexpression and the ability to form tumorspheres in vitro that is triggered by <i>H. pylori</i> cagA-positive strains [61].

Other studies addressing the potential induction of CSC-like properties by <i>H. pylori</i> concluded that Wnt/
Figure 2: Role of *Helicobacter pylori* in the reprogramming of cellular and molecular programs related to invasive behavior. Upon infection, *H. pylori* induces a number of intracellular processes in gastric epithelial cells, some of them can result in the reprogramming of cellular and molecular mechanisms underlying the invasive cell behavior. Several of these events take place after phosphorylation of CagA, others may be independent of the translocation and phosphorylation of CagA, and a portion are not connected to the Cag-PAI at all. Although not exclusively, CagA plays an important role in the activation of transcription factors, such as \( \beta \)-catenin, Snail, ZEB1/2, NF-\( \kappa \)B, and AP-1. Some of these transcriptional regulators (e.g., \( \beta \)-catenin, Snail, ZEB1/2) modify the expression of genes encoding for key effectors of the EMT and stem cell programs, including up-regulation of TGF\( \beta \) and CD44 and down-regulation of E-cadherin. Changing of the gastric epithelial cell polarity is another downstream intracellular event connected to *H. pylori*, which is primarily attributed to the physical interaction of components of the T4SS (especially CagL) and \( \beta \)1 integrins. In addition to this translocation-independent mechanism, alteration of the cell polarity can also result from CagA translocation and phosphorylation. Another consequence of the manipulation of the invasive properties by *H. pylori* is the induction of the expression of ECM remodeling effectors, namely uPAR, uPA, MMP7, MMP2, MMP3, and MMP9. This is presumably linked to the activation of the MAPK signaling pathway, which in turn leads to the activation of NF-\( \kappa \)B and AP-1. It is not yet clear whether plasminogen and MMP activation (dashed arrows) takes place, or what are the functional implications of the enhanced expression of these ECM-related molecules in this particular context. Cag-PAI: Cag pathogenicity island; ECM: Extracellular matrix; EMT: Epithelial-to-mesenchymal transition; *H. pylori*: *Helicobacter pylori*; MMP: Matrix metalloproteinase; NF-\( \kappa \)B: Nuclear factor-kappa B; T4SS: Type IV secretion system; uPA: Plasminogen activator; uPAR: Plasminogen activator receptor.
Cell polarity alterations induced by H. pylori

Epithelial cells in the gastrointestinal tract are normally found in organized layers or epithelia. Their polygonal shape and functional organization in an apico-basal polarized manner allow them to lay in an orderly fashion, and the unions they form with each other and with the basal membrane give the epithelium a barrier function. As already mentioned, invasiveness is enhanced when cells lose their polarity due to alterations in the cytoskeleton and intercellular unions. H. pylori has been implicated in changing the polarity of the gastric epithelial cells, which may have important consequences in the context of gastric carcinogenesis. Of the three types of filaments that compose the cytoskeleton, the actin microfilaments are the most affected during H. pylori infection.

The actin cytoskeleton is a very dynamic structure whose assembly is finely tuned by complex signaling networks and involves numerous regulatory proteins. Actin microfilaments form a wide variety of structures: contractile rings, phagocytosis- and endocytosis-related structures, microvilli, cortex, adherens belts (associated with adherens junctions), filopodia, lamellipodia, and stress fibers. The ability of H. pylori to promote rearrangements of the actin cytoskeleton is well-established.

The most evident demonstration of this is the so-called hummingbird phenotype, comprising a change in the epithelial cell shape to the characteristic elongated morphology of H. pylori-infected cells in vitro. This phenotype is thought to be linked to cancer cell migration and invasive growth in vivo.

The hummingbird phenotype involves the formation of stress fibers and protrusions, the disruption of cell-to-cell adhesions, and the deregulation of focal adhesions between the cell and the ECM.

The basic mechanisms by which H. pylori changes the dynamics of the actin cytoskeleton during cell migration have been reviewed in depth by Wessler et al. Briefly, H. pylori, via cag-PAI type-4 secretion system (T4SS; especially CagL) and CagA, is able to modify the host cell's signaling networks. On the one hand, CagL binds β1 integrins, thereby stimulating the focal adhesion kinases (FAKs) and the Src-family kinases (SFKs); meanwhile, CagA activates the Abl-kinase. FAK, SFK, and Abl activate Crk, which in turn activates Rac1, which then promotes the assembly of actin filaments via activation of the Arp2/3 complex, contributing to cell motility. On the other hand, upon injection into the host-cell cytosol, CagA is phosphorylated by SFK (c-Src) and binds Shp-2 and Csk, which then inhibit SFK in a negative feedback loop. Inhibition of SFK induces dephosphorylation of actin regulatory proteins, such as ezrin, vinculin, and cortactin. Cortactin stimulates the actin nucleation activity of Arp2/3 and, upon H. pylori-induced dephosphorylation, accumulates at the tip of the cellular protrusions and colocalizes with F-actin.

The serine/threonine kinase polarity-regulating kinase partitioning-defective 1b (PAR1b) participates in the CagA-mediated remodeling of the actin cytoskeleton. PAR1 inhibits the formation of stress fibers and cortical actin in the cell periphery. Kikuchi et al. showed that the physical interaction between the CagA multimerization sequence and PAR1b, the isoform present in gastric epithelial cells, is crucial for the stable binding of CagA and Shp-2. In fact, a second study found that CagA indirectly activates RhoA-dependent formation of stress fibers by impairing PAR1b-mediated inhibition of RhoA. These results were elegantly combined in a model that proposes a link among cell polarity regulation, the hummingbird phenotype, and actin cytoskeleton. More specifically, upon cell polarity loss of the epithelial cell, PAR1b and aPKC are relocated, resulting in the establishment of a front-to-rear polarity in which these two molecules are asymmetrically distributed, with PAR1b localized in the rear part of the migrating cell.

The binding of CagA to PAR1b modifies this program by perturbing PAR1b localization, which translates into loss of its kinase activity, lifting of the repression of RhoA, and formation of stress fibers; the salient manifestation of this is the hummingbird phenotype.

The affinity of CagA for PAR1b and formation stress fibers increases proportionally to the number copies of the CagA-multimerization (CM) domain present in CagA, which is seemingly higher in East Asian CM than in Western CM and differs in five amino acid residues.

Podosomes are dot-like structures of densely packed F-actin and serve as regulatory proteins by their capacity to degrade ECM components due to the presence of MMPs within. It has been shown in a model of primary hepatocytes and hepatoma cell lines that H. pylori can enhance the formation of podosomes by the induction of inflammatory cytokines such as TGFβ, thus providing additional evidence of the capacity of H. pylori to modify actin structures. Actin-remodeling activity has also been described in another Helicobacter species, H. pullorum. Its cytotoxic distending toxin, responsible for the cytopathological effects observed upon infection, induces actin cytoskeleton remodeling that is accompanied by delocalization of vinculin and up-regulation of cortactin in large, cortical actin-rich lamellipodia.

Another important component of the cellular cytoskeleton is the microtubules. Structurally, they are formed by tubulins and regulated by microtubule-associated proteins. The microtubular network organizes the cell movement of organelles and is part of specialized structures such as cilia, flagella, mitotic spindles, cen-
trosomes, and basal bodies. During cell migration, the small Rho-GTPase protein Cdc42 controls the actin microfilaments in the cell migration front and binds PAR6. The Cdc42-PAR6 dimer recruits the microtubule-regulating dynein/dynactin complex, which directs the machinery of the secretory pathway to the migration front. Importantly, this facilitates the delivery of integrins and other proteins that mediate the interaction with the ECM to the sites of migration.

Although not many, some studies have addressed the role of *H. pylori* infection in microtubule regulation in the context of cell migration. Słomiński and colleagues\(^\text{(277)}\), for example, concluded that *H. pylori* lipopolysaccharide induced the secretion of MMP9 in a primary culture of murine gastric mucosal cells. In that study, the authors also found an accompanying increase of microtubule stabilization. Presumably, those changes are modulated by ghrelin and involve the activation of PKCδ and SFPK.

Focal adhesions provide the structural link between the stress fibers and the ECM. They need to be dynamically assembled and disassembled in order to allow cell migration. *H. pylori* impairs focal adhesion release during cell migration, which leads to the characteristic elongation of infected cells\(^\text{(68)}\). Paxillin, a multidomain protein that acts as an adaptor between the cytoplasmic tail of integrins and the actin cytoskeleton, has been designated as the convergent point of the epithelial growth factor receptor, FAK/Src, and PI3K/Akt signaling pathways in the context of *H. pylori* infection\(^\text{(78)}\). Paxillin phosphorylation is dependent on the presence of a functional Cag-PAI or OipA. The phosphorylated paxillin was localized along the elongations, suggesting a role in the formation of stress fibers\(^\text{(78)}\).

**PA system and *H. pylori***

The PA system comprises a few proteins that, by acting in sequence, lead to the conversion of zymogenic plasminogen into its active enzymatic form, plasmin. Extravascular activation of plasminogen is controlled by the urokinase-type plasminogen activator (upAR), its receptor (upAR), its inhibitor PAI-1, and α2-antiplasmin. Besides degrading major ECM proteins (e.g., fibrin, fibronectin, laminins, and vitronectin), the generated plasmin also releases latent growth and angiogenic factors sequestered in the matrix\(^\text{(79,80)}\). The expression of uPA, upAR, and PAI-1 under normal homeostatic conditions is almost undetectable; however, in cancer and other pathologies, their expressions increase significantly\(^\text{(81)}\). An important body of evidence correlates upAR expression in cancer lesions with invasive and metastatic disease. Accordingly, high levels of upAR in tissue and plasma are associated with poor patient survival in various types of cancer, including GC\(^\text{(82-84)}\). Most of these reports have focused on uPAR, since this receptor is crucial for the initiation of the sequential series of events that ultimately result in the activation of plasminogen.

As already mentioned, *H. pylori* has been linked to the induction of members of the PA system, primarily uPAR. Part of the experimental evidence supporting this phenomenon comes from *in vitro* studies, for example global gene-expression analyses ranking uPAR among the top up-regulated genes in AGS and TB4 cell lines, when co-cultured with *H. pylori*\(^\text{(85-87)}\). This has been confirmed by more specific *in vitro* studies, showing that in co-cultures, the bacterium rapidly induces uPAR expression in GC cell lines\(^\text{(88-91)}\). A few of these reports indicate that uPAR induction is predominantly linked to CagA-positive strains\(^\text{(87,89)}\). The potential connection between *H. pylori* and upAR induction has also been documented in non-neoplastic tissue adjacent to GC lesions\(^\text{(92)}\) and in gastric biopsies from healthy patients who are infected with the bacterium\(^\text{(93)}\). Interestingly, it has been reported that the expression of uPAR in neoplastic tissue may be correlated with the presence of *H. pylori* in adjacent non-neoplastic tissue\(^\text{(94)}\).

The link between *H. pylori* and uPAR has been systematically investigated in a mouse model of *H. pylori*-induced gastritis (Figure 3)\(^\text{(95)}\). In this model, uPAR expression is up-regulated very early in response to the infection and increases progressively during the course of infection, and this is reverted to its physiological baseline levels if *H. pylori* is eradicated by antimicrobial therapy\(^\text{(95)}\). Additional experiments in this model suggest that uPAR expression is directly induced by the bacterium (and Alpízar-Alpízar, unpublished results)\(^\text{(95)}\). It is not possible to rule out, however, that uPAR induction in murine gastric epithelium is a consequence of the inflammatory reaction against *H. pylori*.

A few signaling pathways and transcription factors have been proposed as potential inducers of uPAR in cancer; however, much less is known about the mechanisms of induction in response to *H. pylori*. Studies in cancer cell lines have found that the NF-κB can drive uPAR expression by direct binding to specific sequences within the regulatory region of the gene encoding uPAR\(^\text{(96)}\) or indirectly via HIF1α activation\(^\text{(97)}\). It is well known that *H. pylori* infection can lead to the activation of NF-κB\(^\text{(36,98)}\). Therefore, NF-κB is a likely transcriptional inducer of uPAR in epithelial cells of *H. pylori*-colonized mucosa, both in human and mouse. *In vitro* evidence supports this idea\(^\text{(98)}\), but no experimental data have been generated in vivo.

AP-1 is another transcriptional regulator that is activated by *H. pylori* infection\(^\text{(36)}\) and has been implicated in the induction of uPAR in cancer\(^\text{(99,100)}\). Thus, AP-1 may explain the potential connection between these two parameters\(^\text{(99)}\). Both NF-κB and AP-1 can be activated via the Ras–ERK MAPK signaling pathway\(^\text{(36)}\). This pathway is often manipulated by *H. pylori*\(^\text{(98)}\), which makes it an interesting study target to gain further insight about the mechanism of induction of uPAR in the gastric epithelium colonized with *H. pylori*.

**MMPs and *H. pylori***

The MMP family comprises more than 23 zinc-depen-
dent endopeptidases, subdivided into eight groups according to structural characteristics\textsuperscript{[42,102]}. MMPs are synthesized in the form of zymogens (pro-MMPs) by several cell types of the tumor microenvironment; and, when released to the extracellular space, they become activated by other proteases, including MMPs themselves and plasmin\textsuperscript{[42]}. Besides their role in invasion and metastasis, MMPs are involved in other aspects of tumor biology. The degradation of ECM constituents results in the liberation of sequestered growth, proliferative and angiogenic factors but also in the generation of ECM-derived peptides with similar biological properties to those factors. Some MMPs can cleave membrane-bound growth factor precursors, thus releasing their active form, for example TGF\textsubscript{β}\textsuperscript{[42]}. Elevated expression of several MMPs has been consistently correlated with poor cancer patient survival in several types of cancer, including GC. Of note, a few MMPs actually inhibit malignant transformation and tumor growth, including MMP8, MMP12, and MMP26\textsuperscript{[103-107]}.

The possible connection between \textit{H. pylori} infection and induction of MMPs (e.g., MMP2, MMP3 and MMP9) in gastric epithelial cells has been suggested; however, the most compelling evidence is probably for MMP7. MMP7 enhances tumor formation in rodents\textsuperscript{[108]}, and it is particularly interesting in the context of the gastric carcinogenesis because its expression is increased in human GC lesions\textsuperscript{[109,110]}. In human gastric cell lines co-cultured with \textit{H. pylori}, it was found that cag-PAI-positive strains augment the levels of MMP7 up to 7-fold compared to uninfected controls or to cells incubated with specific isogenic mutant strains\textsuperscript{[111]}. According to that report, the induction of MMP7 in the \textit{in vitro} system was dependent on the activation of ERK 1/2 and required an active interplay between viable bacteria and epithelial cells\textsuperscript{[111]}. That study also evaluated the expression of MMP7 in gastric biopsies of human patients and found that it was over-expressed in epithelial cells of gastritis-affected individuals infected with CagA-positive strains\textsuperscript{[111]}, which has also been previously documented by an independent report\textsuperscript{[112]}.

These observations served as the driving force for conducting subsequent investigations in MMP7 knockout mouse models. Such studies concluded that gastric inflammation and epithelial cellular turnover are substantially increased in MMP7-deficient mice infected with \textit{H. pylori}, compared to their wild-type counterparts\textsuperscript{[113,114]}. It is speculated that over-expression of MMP7 in response to \textit{H. pylori} colonization may be a mechanism to protect the gastric mucosa from damage and development of lesions that could ultimately result in GC\textsuperscript{[112,113]}. Nevertheless, it is also proposed that sus-
tained expression of MMP7 in the gastric epithelium could lead to malignant transformation[112]. In fact, a few studies suggest that MMP7 proteolytically cleaves specific pro-apoptotic molecules, such as Fas ligand, from tumors cells, thus promoting tumor survival[115,116].

CONCLUSION

H. pylori is a determining factor in the development of GC, due to the multiple ways in which it manipulates the host gastric epithelial cells. A key aspect of carcinogenesis is the acquisition of invasive capacities, and H. pylori could modulate several factors associated with invasion. A number of bacterial virulence factors may be of relevance in the manipulation of cellular and molecular programs that lead to increased invasive behavior; however, Cag-PAI stands out as a major orchestrator in hijacking these host cell pathways.

A number of key effectors of the EMT and cell polarity are deregulated in response to H. pylori infection. The two processes enhance cell motility and regulate the attachment of preneoplastic cells to the ECM and to other cells. This finally translates into an increased versatility of the cells to initiate the invasive process and adapt to the physiological changes suffered by the cell through the dedifferentiation induced by EMT. The acquisition of stem-like properties is a pivotal event that results from the activation of the EMT programs in response to H. pylori, since it confers the cells with augmented capability of survival and proliferation.

The induction of members of the PA system and MMPs by H. pylori could have important implications in the genesis of GC, given the wide array of aspects in which these molecules participate. Particularly interesting is the fact that this bacterium up-regulates the expression in non-neoplastic gastric mucosa of the uPAR, a protein that until now has been implicated in the expression in non-neoplastic gastric mucosa of the uPAR, a protein that until now has been implicated in the induction of MMPs and changes in cytoskeletal remodeling as independent processes, there are several points of interconnection among them (Figure 2). For instance, some factors implicated in the activation of MMPs and changes in cytoskeletal reorganization. Some of these proteinases, in the instance, some factors implicated in the activation of MMPs by H. pylori lead to the induction of MMPs and changes in cytoskeletal reorganization. Some of these proteinases, in the meantime, are capable of activating mediators of the EMT and cell polarity programs.

Therefore, the link between H. pylori and cell invasive properties is complex and an exciting open area of research where many aspects remain far from being clear. For instance, there is a need to gain further insight on how and under what circumstances H. pylori manipulates regulatory networks controlling the EMT and stem-cell programs. Also, it is important to unravel the cellular and molecular mechanisms underlying the induction of members of the PA system and MMPs in H. pylori-colonized gastric epithelium.

Elucidation of the key orchestrators governing these invasion-related programs is crucial to understanding the implications that these processes may have for the survival of the bacterium or in the pathological context. All this information could be of relevance for identifying individuals with an increased risk of GC, who may require H. pylori eradication therapy, especially in countries with limited resources and high prevalence of this bacterial infection. Finally, this may contribute to prediction of pre-neoplastic lesions that are more likely to progress in the pathogenic series of steps to malignancy, which may be of relevance to reducing GC burden.

REFERENCES

1 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med 2001; 345: 784-789 [PMID: 11556297 DOI: 10.1056/NEJMoa0100999]
2 Correa P, Houghton J. Carcinogenesis of Helicobacter pylori. Gastroenterology 2007; 133: 659-672 [PMID: 17681184 DOI: 10.1053/j.gastro.2007.06.026]
3 Cover TL. Helicobacter pylori Diversity and Gastric Cancer Risk. MBio 2016; 7: e01869-e01815 [PMID: 26814181 DOI: 10.1128/mBio.01869-15]
4 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]
5 Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell 2011; 147: 275-292 [PMID: 22000009 DOI: 10.1016/j.cell.2011.09.024]
6 Lambert AW, Pattabiraman DR, Weinberg RA. Emerging Biological Principles of Metastasis. Cell 2017; 168: 670-691 [PMID: 28178288 DOI: 10.1016/j.cell.2016.11.037]
7 Global Burden of Disease Cancer Collaboration., Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandonna R, Dandonna L, Fleming T, Forouzanfar MH, Hancock J, Hay RJ, Hunter-Merrill R, Huynh C, Hogood HD, Johnson CO, Jonas JB, Khuwando J, Kumar GA, Kurz M, Lan Q, Larson HJ, Liang X, Lim SS, Lopez AD, Macintyre MF, Marcecz L, Marquez N, Mokdad AH, Pinho C, Ponnuraj E, Salomon JA, Sanabrja JR, Sandar L, Sartorius B, Schwartz SM, Shackelford KA, Shibuya K, Stanaway J, Steiner C, Sun J, Takahashi K, Vollset SE, Vos T, Wagner JA, Wang H, Westerman R, Zeeh H, Zooker L, Abd-Allah F, Ahmed MB, Alabed S, Alnak NK, Alikhani SF, Alem G, Alemayohu MA, Ali R, Al-Raddadi R, Amare A, Amoako Y, Artaman A, Asayesh H, Atunafu N, Awasthi A, Saleem HB, Babacar B, Bedi N, Bensenor I, Berhane A, Bembade B, Betsu B, Binagwaho A, Boneva D, Campos-Novato I, Castaño-Orjuela C, Catala-Lopez F, Chiang P, Chiaueze C, Ciftingér A, Choi JY, Cowie B, Dantew S, das Neves J, Dey S, Dharmaratne S, Dhillon P, Ding E, Driscoll T, Ekwueme D, Endries AY, Faridvand F, Fernandes J, Fischer G, Ghiwot TT, Gebru A, Gopalani S, Hailu A, Horino M, Horita N, Hussein A, Huybrechts I, Inoue M, Isami F, Jakovljevic M, James S, Javanbakht M, Jeh JH, Kasaeeen A, Kedir MS, Khader KS, Khandg YH, Kim D, Kutz M, Lan Q, Le Nguyen Q, Nand D, Nolte S, Ogbo FA, Oladimeji KE, Oren E, Pa M, Park EK, Pereira DM, Plass D, Qorbani M, Radfar A, Rolland Y, A, Meles KG, Mendoza W, Mengiste DT, Meroeta TJ, Miller TR, Molina-Castro S et al. H. pylori alters cell invasive properties

WJGO | www.wjgnet.com 239 September 15, 2018 | Volume 10 | Issue 9 |
rafay a, rahman m, rana sm, sreide k, satpathy m, sawhney m, sepanlu sg, shahid ma, she j, shiue i, shore hr, shrimie mg, so s, sonjei s, stathopoulos v, stroupoulos k, sufyan mb, sykes bl, tabarés-seledros r, tadesse f, tedla ba, tessema ga, theilakis j, tran bx, ukojua kw, uzuobuike bsc, vlassov vv, weiderpass e, wubset terefe m, yehyo hg, yimham ih, yonemoto n, younis mz, yu c, zaidi z, zaki mes, zenebe zm, murray cjl, naghavi m, global, and regional, and national, cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. jama oncol 2015; 1: 23-32. doi: 10.1001/jamaoncol.2014.168.

hayakawa y, nishi n, sepulveda ar, bass aj, wang tc. oesophageal adenocarcinoma, gastric cancer: should we mind the gap? nat rev cancer 2016; 16: 305-318. doi: 10.1038/nrc.2016.24.

pounder re, ng d. the prevalence of helicobacter pylori infection in different countries. aliment pharmacol ther 1995; 9 suppl 2: 33-39. doi: 10.1111/j.1365-2036.1992.tb00973.x.

viziovic r, brunetti p, balducci m, sica a, dałe e, genazzani ar, sinigaglia m. helicobacter pylori infection in a high-risk population of costa rica. association of interleukin-1b and interleukin-1rn polymorphisms with gastric cancer in a high-risk population of costa rica. clin exp med 2005; 5: 169-176. doi: 10.1007/s10238-005-0082-3.

correa p. human gastric carcinogenesis: a multistep and multifactorial process—first american cancer society award lecture on cancer epidemiology and prevention. cancer res 1992; 52: 6735-6740. doi: 10.1158/0008-5472.ca-92-125.

yuasa y. control of gut differentiation and intestinal-type gastric carcinogenesis. nat rev cancer 2003; 3: 592-600. doi: 10.1038/nrc1141.

petrovich c, ford jm. genetic predisposition to gastric cancer. semin oncol 2016; 43: 554-559. doi: 10.1053/j.seminoncol.2016.05.006.

hansford s, kaurah p, li-chang h, woo m, senj z, pinheiro h, schrader ka, schaeffer df, shumansky k, zogopoulos g, santos ta, claro i, carvalho j, nelson c, padilla s, lum a, talhauk a, baker-lange k, richardson s, lewis i, linndor nm, pellenn e, macmillan a, fernandez b, keller g, Lynch h, shah sp, guiford p, gallinger s, corso g, roiffany e, caldas c, oliveira c, pharoah pd, huntsman dg. hereditary diffuse gastric cancer syndrome: cdh1 mutations and beyond. jama oncol 2015; 1: 23-32. doi: 26182300. doi: 10.1001/jamaoncol.2014.168.

molina-castro s, soerjomataram i. global patterns of cardia and non-cardia gastric cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions. jama oncol. 2016; 2: 6735-6740. doi: 10.1001/jamaoncol.2016.5688.

sanchez-garcia r, lescoere a, tanner b, tojeti z, ebutu o, mbanza p, kou TRACKING 2015. 2016; 16: 305-318. doi: 10.1038/nrc.2016.24.

pounder re, ng d. the prevalence of helicobacter pylori infection in different countries. aliment pharmacol ther 1995; 9 suppl 2: 33-39. doi: 10.1111/j.1365-2036.1992.tb00973.x.

wen y, marcus ea, matrubutham u, gleeson ma, scott dr, sachs g. acid-adaptive genes of helicobacter pylori. infect immun 2003; 71: 5921-5939. doi: 10.1128/IAI.71.10.5921-5939.2003.

dunn be, campbell gp, perez-perez gi, blaser mj. purification and characterization of urease from helicobacter pylori. j biol chem 1990; 265: 9464-9469. doi: 10.1074/jbc.265.15.9464-9469.2003.

eaton ka, brooks cl, morgan dr, krukova s. essential role of urease in pathogenesis of gastritis induced by helicobacter pylori in gnotobiotic piglets. infect immun 1991; 59: 2470-2475. doi: 10.1128/IAI.59.9.2470-2475.1991.

tsuda m, karita m, mizote t, morshed mg, okita k, nakazawa t. essential role of helicobacter pylori urease in gastric colonization: definite proof using a urease-negative mutant constructed by gene replacement. eu j gastroenterol hepatol 2004; 6 suppl 1: s49-s52. doi: 10.1111/j.1365-3040.2004.tb00014.x.

scott dr, marcus ea, wen y, oh j, sachs g. gene expression in vivo shows that helicobacter pylori colonizes an acidic niche on the gastric surface. proc natl acad sci usa 2007; 104: 7225-7230. doi: 10.1073/pnas.0702300104.

yamaoka y. mechanisms of disease: helicobacter pylori virulence factors. nat rev gastroenterol hepatol 2010; 7: 629-641. doi: 10.1038/nrgastro.2010.154.

polk db, peek rm jr. helicobacter pylori: gastric cancer and beyond. nat rev cancer 2010; 10: 403-414. doi: 10.1038/nrc2857.

rhead jl, letley dp, mohammadi m, hussein n, mahagehgi ma, eshagh hosseini m, atherton jc. a new helicobacter pylori vacuolating cytotoxin determinant, the intermediate region, is essential for vacuolating cytotoxin and helicobacter pylori gene expression. j pathol 2016; 238: 430-437. doi: 10.1002/path.4822.

miller a, oertl m, arnold ic. helicobacter pylori urease; results of immunochemical studies on an improved purification method. j biol chem 1994; 269: 18053-18060. doi: 10.1038/16186.

naumann m, sokolova o, tegmeyer n, baccert s. helicobacter pylori: a paradigm pathogen for subverting host cell signal transmission. trends microbiol 2017; 25: 316-328. doi: 10.1016/j.tim.2016.12.004.

basso d, zambron cf, letley dp, stranges a, marchet a, rhead jl, schiavon s, guariso g, cerotti m, nitti d, rugge m, piebani m, atherton jc. clinical relevance of helicobacter pylori cag a and vac a gene polymorphisms. gastroenterology 2008; 135: 91-99. doi: 10.1053/j.gastro.2008.03.041.

kalluri r, weinberg ra. the basics of epithelial-mesenchymal transition. j clin invest 2009; 119: 1420-1428. doi: 10.1172/jci39104.

lamouille s, xu j, derynick r. molecular mechanisms of epithelial-mesenchymal transition. nat rev mol cell biol 2014; 15: 178-196. doi: 10.1038/nrm3758.

halouci r, mccaffrey l. rewiring cell polarity signaling in cancer. oncogene 2015; 34: 939-950. doi: 10.1038/onc.2014.59.
Dano K, Ramer J, Nielsen BS, Bjørn S, Pyke C, Rygaard J, Lund LR. Cancer invasion and tissue remodeling—cooperation of protease systems and cell types. *APMIS* 1999; 107: 120-127 [PMID: 10198285 DOI: 10.1111/j.1699-0643.1999.tb01534.x]

Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; 141: 52-67 [PMID: 20371345 DOI: 10.1016/j.cell.2010.03.015]

Strynke N, Goossens S, De Smedt E, Vandamme N, Bers G. Epithelial-to-Mesenchymal Transition: Epigenetic Reprogramming Driving Cellular Plasticity. *Trends Genet* 2017; 33: 943-959 [PMID: 28919019 DOI: 10.1016/j.tig.2017.08.004]

Bonnans C, Jou W, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014; 15: 786-801 [PMID: 25415508 DOI: 10.1038/nrm3904]

Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol* 2011; 12: 23-36 [PMID: 20027185 DOI: 10.1038/nrm2821]

Yeh TS, Wu CW, Hsu KW, Liao WJ, Yang MC, Li AF, Wang AM, Kuo ML, Chi CW. The activated Notch1 signal pathway is associated with gastric cancer progression through cyclooxygenase-2. *Cancer Res* 2009; 69: 5039-5048 [PMID: 19491270 DOI: 10.1118/0005-8547.CAN-08-4021]

Montgros PS, Rustgi AK. The role of the miR-200 family in epithelial-mesenchymal transition. *Cancer Biol Ther* 2010; 10: 219-222 [PMID: 20592490 DOI: 10.4161/cbt.10.3.12548]

Sun D, Mealdon KR, Davies JA, Bernfield M, Hay ED. Simultaneous loss of expression of syndecan-1 and E-cadherin in the embryonic palate during epithelial-mesenchymal transformation. *J Cell Biol* 1998; 142: 733-736 [PMID: 9712558]

Rodriguez FJ, Lewis-Tuffin LJ, Anastasiadis PZ. E-cadherin’s dark side: possible role in tumor progression. *Biochim Biophys Acta* 2012; 1826: 23-31 [PMID: 22440943 DOI: 10.1016/j.bbabio.2012.03.002]

Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S, Naksathri H. NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. *Oncogene* 2007; 26: 711-724 [PMID: 16682183 DOI: 10.1038/sj.oto.1602080]

Peinado H, Olmeda D, Cano A, Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007; 7: 415-428 [PMID: 17508028 DOI: 10.1038/nrc2131]

Lin Y, Dong C, Zhou BP. Epigenetic regulation of EMT: the Snail story. *Curr Pharm Des* 2014; 20: 1698-1705 [PMID: 23888971 DOI: 10.2174/13816121139990512]

Theys J, Jutten B, Habets R, Paesmans K, Groot AJ, Lambin P, Wouters BG, Lammers GJ, Voort M. E-cadherin loss associated with EMT promotes radioresistance in human tumor cells. *Radiother Oncol* 2011; 99: 392-397 [PMID: 21680037 DOI: 10.1016/j.radonc.2011.05.044]

Laurie L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphorylated Ino1 3 kinase/AKT pathways. *Oncogene* 2005; 24: 7443-7454 [PMID: 16282891 DOI: 10.1038/sj.onc.1209091]

Ye X, Weinberg RA. Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression. *Trends Cell Biol* 2015; 25: 675-686 [PMID: 26437589 DOI: 10.1016/j.tcb.2015.07.012]

Scheel C, Weinberg RA. Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. *Semin Cancer Biol* 2012; 22: 396-403 [PMID: 22554795 DOI: 10.1016/j.semcancer.2012.04.001]

Yang L, Ping FY, Yu X, Qian F, Guo ZJ, Qian C, Cui YH, Bian XW. Gastric cancer stem-like cells possess higher capability of invasion and metastasis in association with a mesenchymal transition phenotype. *Cancer Lett* 2011; 310: 46-52 [PMID: 21782323 DOI: 10.1016/j.canlet.2011.06.003]

Ryu HS, Park DJ, Kim HH, Kim WH, Lee HS. Combination of epithelial-mesenchymal transition and cancer stem cell-like phenotypes has independent prognostic value in gastric cancer. *Hum Pathol* 2012; 43: 520-528 [PMID: 22018628 DOI: 10.1016/j.humpath.2011.07.003]
Performance of the Helicobacter pylori CagA Oncoprotein. *Toxins* (Basel) 2017; 9: pii: E136 [PMID: 28406453 DOI: 10.3390/toxins9040136]

Le Roux-Goglin E, Varon C, Spaul P, Ascencio C, Mégraud F, Genot E. Helicobacter infection induces podosome assembly in primary hepatocytes in vitro. *Eur J Cell Biol* 2012; 91: 161-170 [PMID: 22306377 DOI: 10.1016/0014-4827(87)92033-8]

Varon C, Mocan I, Mihăi B, Pérè-Védrenne C, Aboubacar A, Moraté C, Oleastro M, Doignon F, Laharie D, Mégraud F, Ménard A. Helicobacter pullorum cytochalasin distending toxin targets vinculin and cortactin and triggers formation of lamellipodia in intestinal epithelial cells. *J Infect Dis* 2014; 209: 588-599 [PMID: 24470577 DOI: 10.1093/infdis/jiu153]

Slomiany BL, Slomiany A. Helicobacter pylori-induced changes in microtubule dynamics conferred by α-tubulin phosphorylation on Ser/Tyr mediates gastric mucosal secretion of matrix metalloproteinase-9 (MMP-9) and its modulation by ghrelin. *Inflammopharmacology* 2016; 24: 197-205 [PMID: 27613723 DOI: 10.1007/s10787-016-0278-z]

Tabassam FH, Graham DY, Yamaoka Y. Paxillin is a novel cellular target for converging Helicobacter pylori-induced cellular signaling. *Am J Physiol Gastrointest Liver Physiol* 2011; 301: G601-G611 [PMID: 21757638 DOI: 10.1152/ajpgi.00375.2010]

Lamarre J, Vasudev J, Gonias SL. Plasmin cleaves betaglycan and releases a 60 kDa transforming growth factor-beta complex from the cell surface. *Biochem J* 1994; 302 (Pt 1): 199-205 [PMID: 8068006 DOI: 10.1042/bj3020199]

Lijnen HR. Matrix metalloproteinases and cellular fibrinolytic activity. *Biochemistry (Moscow)* 2002; 67: 92-98 [PMID: 11841344 DOI: 10.1023/A:1019083323232]

Kriegbaum MC, Persson M, Haldager L, Alpízar-Alpízar W, Malespín-Bendaña W, Une C, Ramírez JA, Mueller A, Tompkins LS, Falkow S, Merrell DS, Mizokami Y, Takahashi K, Matsuoka T, Matsuzaki M, Tao H, Carloni E, Sepulveda J, Graham DY, Mizokami Y, Takahashi K, Nakajima K, Ohtsubo K, Kim MH, Jang HJ, Park JS, Chung IJ, Shin BA, Molina-Castro S. Signaling in human gastric carcinoma cells. *Mol Biol Cell* 2002; 13: 174-182 [PMID: 12411577 DOI: 10.1016/j.bjhep.2005.06.002]

Iwamoto J, Mizokami Y, Takahashi K, Matsuoka T, Mitsuzaki Y. The effects of cyclooxygenase-2-prostaglandinE2 pathway on Helicobacter pylori-induced urokinase-type plasminogen activator system in the gastric cancer cells. *Helicobacter* 2008; 13: 174-182 [PMID: 18466392 DOI: 10.1111/j.1532-5378.2008.00597.x]

Alpízar-Alpízar W, Nielsen BS, Sierra R, Illemann M, Ramírez JA, Arias A, Durán S, Skarstein A, Ovrebo K, Lund LR, Laerum OD. Urokinase plasminogen activator receptor is expressed in invasive cells in gastric carcinomas from high- and low-risk countries. *Int J Cancer* 2010; 126: 405-415 [PMID: 19609941 DOI: 10.1002/ijc.24755]

Kenny S, Duval C, Sammut SJ, Steele I, Pritchard DM, Atherton JC, Argent RH, Dimaline R, Dockray GJ, Varo A. Increased expression of the urokinase plasminogen activator system by Helicobacter pylori in gastric epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2008; 295: G431-G441 [PMID: 18595856 DOI: 10.1152/ajpgi.00283.2008]

Beyer BC, Heiss MM, Simon E, Gruetzner KU, Babić R, Jauch KW, Schildberg FW, Allgayer H. Urokinase system expression in gastric carcinoma: prognostic impact in an independent patient series and first evidence of predictive value in preoperative biopsy and intestinal metaplasia specimens. *Cancer* 2006; 106: 1026-1035 [PMID: 16435385 DOI: 10.1002/cncr.21682]

Alpízar-Alpízar W, Skindersoe ME, Rasmussen L, Kriegbaum MC, Christensen JI, Laerum OD, Lund IK, Krogfelt KA, Andersen LP, Ploug M. Helicobacter pylori colonization drives urokinase receptor expression in murine gastric epithelium during early pathogenesis. In: Mégraud F, Malfurétherin P, editors. *Abstracts of the European Helicobacter Study Group XVIIIth International Workshop on Helicobacter and Related Bacteria in Chronic Digestive Inflammation and Gastric Cancer.* Helicobacter 2013; 18 Suppl S1: 77-154 [DOI: 10.1111/hel.12079]

Wang Y, Dang J, Wang H, Allgayer H, Murrell GA, Boyd D. Identification of a novel nuclear factor-kappaB sequence involved in expression of urokinase-type plasminogen activator receptor gene. *J Biol Chem* 2000; 275: 3248-3254 [PMID: 10824110 DOI: 10.1074/jbc.275.42.3248]

Rius J, Gunna M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature* 2008; 453: 807-811 [PMID: 18432192 DOI: 10.1038/nature06905]

Riecke C, Papendieck A, Sokolova O, Naumann M, Helicobacter pylori-induced tyrosine phosphorylation of IKKβ contributes to NF-κB activation. *Biochim Biophys Acta* 2011; 1812 Suppl 1: S5-S15 [PMID: 21294676 DOI: 10.1016/j.bbadis.2011.02.081]

Baek MK, Kim MH, Jung HJ, Park JS, Chung IJ, Shin BA, Ahn BW, Jung YD. EGF stimulates uPAR expression and cell invasiveness through ERK, AP-1, and NF-kappaB signaling in human gastric carcinoma cells. *Onco Rep* 2008; 20: 1569-1575 [PMID: 19020743]

Lengyel E, Wang H, Stepp E, Juarez J, Wang Y, Doe W, Pfarr CM, Boyd D. Requirement of an upstream AP-1 motif for the constitutive and phorbol ester-inducible expression of the urokinase-type plasminogen activator receptor gene. *J Biol Chem* 1996; 271: 23176-23184 [PMID: 8798512 DOI: 10.1074/jbc.271.38.23176]

Lengyel E, Gum R, Stepp E, Juarez J, Wang H, Boyd D. Regulation of urokinase-type plasminogen activator expression by an ERK1-
dependent signaling pathway in a squamous cell carcinoma cell line. J Cell Biochem 1996; 61: 430-443 [PMID: 8761947 DOI: 10.1002/(SICI)1097-4644(19960601)61:3<430::AID-JCB10>3.0.CO;2-N]

102 Egeland M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002; 2: 161-174 [PMID: 11990853 DOI: 10.1038/ng1249]

103 Balbín M, Fueyo A, Tester AM, Pendás AM, Pitiot AS, Astudillo A, Overall CM, Shapiro SD, López-Otín C. Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. Nat Genet 2003; 35: 252-257 [PMID: 14517555 DOI: 10.1038/ng1249]

104 Gutiérrez-Fernández A, Fueyo A, Folgueras AR, Garabaya C, Pennington CJ, Pilgrim S, Edwards DR, Jones JL, Span PN, Sweep FC, López-Otín C. Matrix metalloproteinase-8 functions as a metastasis suppressor through modulation of tumor cell adhesion and invasion. Cancer Res 2008; 68: 2755-2763 [PMID: 18413742 DOI: 10.1158/0008-5472.CAN-07-5154]

105 Houghton AM, Grisolano JL, Baumann ML, Kobayashi DK, Hautamaki RD, Nehring LC, Cornelius LA, Shapiro SD. Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. Cancer Res 2006; 66: 6149-6155 [PMID: 16778188 DOI: 10.1158/0008-5472.CAN-04-0297]

106 López-Otín C, Matrisian LM. Emerging roles of proteases in tumour suppression. Nat Rev Cancer 2007; 7: 800-808 [PMID: 17851543 DOI: 10.1038/nrc2228]

107 López-Otín C, Palavalli LH, Samuels Y. Protective roles of matrix metalloproteinases: from mouse models to human cancer. Cell Cycle 2009; 8: 3657-3662 [PMID: 19884170 DOI: 10.4161/cc.8.22.9956]

108 Rudolph-Owen LA, Chan R, Muller WJ, Matrisian LM. The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. Cancer Res 1998; 58: 5500-5506 [PMID: 9850086]

109 Honda M, Mori M, Ueo H, Sugimachi K, Akiyoshi T. Matrix metalloproteinase-7 expression in gastric carcinoma. Gut 1996; 39: 444-448 [PMID: 8949652 DOI: 10.1136/gut.39.3.444]

110 Yamashita K, Azumano I, Mai M, Okada Y. Expression and tissue localization of matrix metalloproteinase 7 (matrilysin) in human gastric carcinomas. Implications for vessel invasion and metastasis. Int J Cancer 1998; 79: 187-194 [PMID: 9583735 DOI: 10.1002/(SICI)1097-4215(19980417)79:2<187::AID-IJC15>3.0.CO;2-7]

111 Crawford HC, Krishna US, Israel DA, Matrisian LM, Washington MK, Peek RM Jr. Helicobacter pylori strain-selective induction of matrix metalloproteinase-7 in vitro and within gastric mucosa. Gastroenterology 2003; 125: 1125-1136 [PMID: 14517796 DOI: 10.1016/S0016-5085(03)01206-X]

112 Wroblewski LE, Noble PJ, Pagliocca A, Pritchard DM, Hart CA, Campbell F, Dodson AR, Dockray GJ, Varro A. Stimulation of MMP-7 (matrilysin) by Helicobacter pylori in human gastric epithelial cells: role in epithelial cell migration. J Cell Sci 2003; 116: 3017-3026 [PMID: 12808021 DOI: 10.1242/jcs.005158]

113 Ogden SR, Noto JM, Allen SS, Patel DA, Romero-Gallo J, Washington MK, Fingleton B, Israel DA, Lewis ND, Wilson KT, Chaturvedi R, Zhao Z, Shyr Y, Peek RM Jr. Matrix metalloproteinase-7 and premalignant host responses in Helicobacter pylori-infected mice. Cancer Res 2010; 70: 30-35 [PMID: 20048070 DOI: 10.1158/0008-5472.CAN-09-2899]

114 Krakowiak MS, Noto JM, Pizaruelo MB, Hardbower DM, Romero-Gallo J, Delgado A, Chaturvedi R, Correa P, Wilson KT, Peek RM Jr. Matrix metalloproteinase-7 restrains Helicobacter pylori-induced gastric inflammation and premalignant lesions in the stomach by altering macrophage polarization. Oncogene 2015; 34: 1865-1871 [PMID: 24837365 DOI: 10.1038/onc.2014.135]

115 Mitsiades N, Yu WH, Poulaki V, Tsokos M, Stamnenkovic I. Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. Cancer Res 2001; 61: 577-581 [PMID: 11212252]

116 Vargo-Gogola T, Fingleton B, Crawford HC, Matrisian LM. Matrilysin (matrix metalloproteinase-7) selects for apoptosis-resistant mammary cells in vivo. Cancer Res 2002; 62: 5559-5563 [PMID: 12359768]
