**Helicobacter pylori**-induced epithelial cell signalling in gastric carcinogenesis

Michael Naumann¹ and Jean E. Crabtree²

¹Experimental Internal Medicine, Medical Faculty, Otto-von-Guericke-University, 39120 Magdeburg, Germany
²Molecular Medicine Unit, St. James’s University Hospital, Leeds, UK LS9 7TF

*Helicobacter pylori* represents a highly successful human microbial pathogen that infects the stomach of more than half of the world’s population. *H. pylori* induces gastric inflammation, and the diseases that can follow such infection include chronic gastritis, peptic ulcers and, more rarely, gastric cancer. The reasons why a minority of patients with *H. pylori* develops gastric cancer could be related to differences in host susceptibility, environmental factors and the genetic diversity of the organism. This review examines the features of *H. pylori*-induced epithelial cell signalling in gastric diseases. Clinical studies and animal models, and also evidence for *H. pylori* strain-related differences in gastric epithelial cell proliferation in vivo are discussed. In addition, the mechanisms by which *H. pylori* triggers hyperproliferative processes and takes direct command of epithelial cell signalling, including activation of tyrosine kinase receptors, cell–cell interactions and cell motility are reviewed.

The bacterium *Helicobacter pylori* has a major aetiopathological role in human gastric carcinogenesis and has been classified as a class I carcinogen. *H. pylori* is acquired primarily in childhood, and in the majority of cases infection and associated chronic gastritis is lifelong. There is a marked diversity in the clinical outcome of *H. pylori* infection with only a small percentage of infected subjects developing gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma [1]. The risk of gastric adenocarcinoma is greatest in infected subjects with non-ulcer dyspepsia or gastric ulceration who have developed severe gastric atrophy and intestinal metaplasia [2]. Bacterial virulence factors, such as the cag pathogenicity island (PAI) type IV secretion system [1] and genetic polymorphisms in proinflammatory and immunoregulatory cytokines [3], have been linked to an increased risk of developing gastric atrophy or intestinal-type gastric cancer. A key feature of the associated risk in developing gastric cancer with *H. pylori* infection is gastric epithelial hyperproliferation. The cellular and molecular signalling pathways used during *H. pylori* infection to promote epithelial hyperproliferation and transformation are being investigated.

*H. pylori* is an excellent model system to use as a tool to investigate bacterial-induced epithelial cell signalling pathways of relevance to neoplasia. Bacterial virulence factors (Box 1) have been identified that mediate key phenotypic and genotypic changes in gastric epithelial cells. In this review the evidence for *H. pylori*-induced epithelial cell proliferation in human infection and in animal models is described, and the potential role of both bacterial virulence factors and inflammation in the hyperproliferative response is discussed. The molecular basis by which *H. pylori* triggers cell signalling cascades, and promotes inflammation and epithelial cell proliferative and motogenic responses is also described.

Epithelial cell proliferation in *H. pylori*-induced gastric diseases

**Clinical studies**

There is increasing evidence that several chronic infections increase the risk of carcinogenesis. A key factor is probably infection-related alterations in epithelial cell homeostasis. *H. pylori* is associated with increased gastric epithelial cell proliferation in children [4] and adults [5,6]. Lifelong increased cell turnover is considered an important risk factor for increased mutational changes and the development of gastric cancer. Epithelial proliferation has been positively correlated with the degree of histological inflammation in the antrum and corpus mucosa of *H. pylori*-infected patients [5–7]. However, stepwise multiple regression analysis has indicated that the only independent predictor of epithelial cell proliferation in infected patients is the density of *H. pylori* colonization [7]. The enhanced epithelial proliferation observed with infection is probably promoted as a consequence of the inflammatory response and also by a route independent of inflammation. Eradication of *H. pylori* infection results in a significant decrease in gastric epithelial cell proliferation [8].

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**Box 1. *H. pylori* virulence factors associated with gastric cancer**

VacA: an 87 kD protein (140 kD precursor protein) that assembles into oligomeric structures.

CagA: a 128 kD protein translocated by the type IV secretion system. It becomes phosphorylated within epithelial cells and contributes to *H. pylori*-induced cell scattering.

FliA: a 19 kD putative flavodoxin protein as a potential marker for neoplasia.

BabA2: a 78 kD adhesin that binds to LewisB on gastric epithelial cells.
In addition, attenuation of epithelial cell proliferation has also been observed in those patients where H. pylori eradication has failed, probably because of decreased bacterial density or inflammation [9]. To date, only one study has failed to demonstrate a reduction in gastric epithelial cell proliferation after eradication of H. pylori infection [10]. Divergent results might be attributed to varying patient populations, labelling techniques or treatment with pharmacological agents, such as proton-pump inhibitors.

Animal models
Animal models of gastric Helicobacter infection have been useful in delineating the potential role of the pathogen and associated inflammation in gastric epithelial cell proliferative responses. Experimental infection with H. pylori or related gastric Helicobacter species, such as H. felis, increases gastric epithelial cell proliferation in animals, including Mongolian gerbils [11–13] (Figure 1) and mice [14]. Gastric epithelial cell proliferation has been extensively examined in murine H. felis and H. pylori models. H. felis infection in mice causes severe inflammation and marked epithelial hyperplasia [14]. The extent of epithelial proliferation and apoptosis is dependent on the mouse strain [14]. In C57BL/6 mice H. felis induces extensive epithelial hyperplasia in the corpus mucosa, with parietal and chief cells being replaced with mucous-secreting cells [14,15]. H. felis induces greater gastric epithelial cell proliferative responses in these mice than H. pylori [13], and interestingly, there are marked gender differences in proliferative responses [15]. Significant increases in epithelial proliferation and apoptosis are only observed in H. felis-infected C57BL/6 female mice, possibly reflecting sex differences in the immune response and cytokine production [15]. By contrast, in transgenic hypergastrinaemic (INS-GAS, insulin-gastrin) mice on a FVB/N background, H. pylori infection results in gastric cancer in males only [16]. Gastrin, which is elevated in H. pylori infection, is known to promote gastric epithelial hyperproliferation [17]. H. pylori-induced epithelial cell proliferation has been correlated with elevated serum gastrin in Mongolian gerbils [11]. In INS-GAS transgenic mice, H. felis and H. pylori infection accelerates the development of gastric adenocarcinoma [16,18]. However, the effects of H. felis or H. pylori infection on gastric epithelial cell proliferation in hypergastrinaemic mice remain to be examined.

Experimental infection using gastric Helicobacter species in transgenic mice that have specific gene deletions has also been a useful approach to identify potential host factors that promote or regulate epithelial hyperproliferation. In immune-deficient mice, epithelial proliferative responses to infection with gastric Helicobacter species are reduced, emphasizing the importance of mucosal inflammation in the murine model. RAG$^{-/-}$ (recombinase-activating gene-deficient) mice [19], those with severe combined immune deficiency disorder (SCID) [20] and also mice deficient in interferon regulatory factor [21] or gamma interferon [20], do not develop gastritis and associated epithelial hyperproliferative responses following gastric Helicobacter infection. By contrast, interleukin (IL)-10$^{-/-}$ mice [22] and those that lack functional transforming growth factor (TGF)-β type II receptor [23] develop severe hyperplastic gastritis during infection.
indicating the importance of anti-inflammatory and immuno-regulatory cytokines in bacterial-induced epithelial hyperproliferation.

**Influence of H. pylori strains on epithelial proliferation**

*H. pylori* is a genomically diverse pathogen and several bacterial virulence factors, including the *cag* PAI, *VacA* and adhesins such as BabA2, are considered to have a key role in disease pathogenesis [1,24] (Box 1). Only strains containing the *cag* PAI trigger signalling cascades in gastric epithelial cells, resulting in nuclear factor kappa B (NF-κB) activation [25] and multiple associated changes in epithelial gene expression [26] (Table 1). In human gastric epithelial cells, the upregulation of IL-8 and other neutrophil chemotactic C-X-C chemokines, such as GRO-α (growth-regulated oncogene protein-alpha), is considered crucial in the association between infection with *cag* PAI-positive strains, neutrophilic responses and more severe gastroduodenal disease [27]. The profile of gene expression in gastric epithelial cells, as determined by cDNA array analysis, is markedly different after culture with wild-type *cag* PAI-positive and negative strains [26], with many genes involved in cell cycle control and apoptosis being differentially expressed [26]. By contrast, a recent study demonstrated that isogenic *cag* PAI-negative mutants failed to elicit significant changes in epithelial gene expression [28]. This implies that in *cag* PAI-positive strains, the *cag* PAI is the major mediator of changes in gene expression but that wild-type *cag* PAI-negative strains induce changes in epithelial gene expression by other pathways.

It is currently unclear whether gastric epithelial cell proliferation differs in patients that are infected with *cag* PAI-positive or negative strains. In one study, gastric epithelial cell proliferation was greater in patients infected with *cagA*-positive strains than *cagA*-negative strains [5], whereas, another study in patients with non-ulcer dyspepsia did not confirm these observations [6]. The presence or absence of bacterial adhesins could account for earlier discrepant results. A recent study based in China, where 98% of patients were infected with *cagA*-positive *H. pylori* strains, found increased epithelial cell proliferation in patients infected with strains expressing the blood group antigen-binding adhesin BabA2 [29]. There have also been divergent clinical studies regarding the effects of the *cag* PAI on apoptosis in human gastric epithelial cells [5,6]. In addition to the varying patient populations studied, other factors, such as the use of non-steroidal anti-inflammatory agents that decrease apoptosis in the gastric epithelium [8], might account for divergent results.

Infection of animal models with genetically defined *H. pylori* strains is an alternative approach to use when investigating the importance of the *cag* PAI on gastric epithelial proliferation and apoptosis. In the gerbil, *cag* PAI strains of *H. pylori* induce gastritis with greater severity [12,30] and increased epithelial proliferation and apoptosis [12], compared with strains that lack a functional *cag* PAI. In the early stages of infection, the epithelial hyperproliferative responses in the gerbil are confined to the antrum, with subsequent progression to the corpus [12,13], therefore mirroring the pathology and proliferative responses observed during human infection [6]. As a result, the gerbil is a useful model to use when analysing the role of *H. pylori* virulence factors on gastric epithelial proliferation and apoptosis. Further studies in the gerbil model using isogenic mutants of other virulence factors will be important to delineate their importance in the epithelial hyperproliferative response.

**H. pylori affects apoptosis and cell cycle control**

Apoptosis and cell cycle control are processes required for the regulation of cellular homeostasis. The disturbed equilibrium of apoptosis and cell proliferation associated with *H. pylori* infection could lead to an overall increase in cellular turnover and persistence of mutated cells, which will favour the development of neoplasia. Apoptosis is often difficult to observe in vivo because the dying cells are rapidly phagocytosed by tissue macrophages. This phagocytosis is different from that seen in inflammation, when activated macrophages are recruited from outside the immediate area of cell death. From a biological viewpoint, the chronic imbalance between apoptosis and cell proliferation is the first step of gastric carcinogenesis, as in all tumours. The cell cycle, the programme for cell growth and division (proliferation), consists of four phases that are known as G₁, G₀, S, G₂, and M. The important protein families used during this cycle include the cyclins, the cyclin-dependent kinases (Cdks), the Cdk inhibitors and the tumour-suppressor genes (in particular, Rb and p53) [31].

The *H. pylori* toxin VacA induces gastric epithelial cell apoptosis, suggesting that differences in levels of gastric mucosal apoptosis among infected persons might result from strain-dependent variations in VacA structure [32]. In addition, Shibayama et al. [33] have shown that γ-glutamyl transpeptidase from *H. pylori* membrane preparations induces apoptosis. At the molecular level,

Table 1. Type IV secretion system dependent and independent signalling a,b

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<tr>
<th>Type IV secretion system-dependent</th>
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<td>Kinase</td>
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<td>GT Pases</td>
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<td>Transcription factors</td>
<td>NF-κB, AP-1</td>
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<td>Genes</td>
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aAbbreviations: AP-1, activator protein 1; BP1 and 2, beta protein 1 and 2; Cdc42, cell division cycle protein 42; CREB, cAMP responsive element-binding protein; ERK, extracellular signal-regulated kinase; IKK, IkappaB kinase; JNK, c-Jun N-terminal kinase; MEK, MAP kinase kinase; NF-κB, nuclear factor kappa; USF1 and 2, upstream stimulatory factor 1 and 2.

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in gastric epithelial cells in vitro in a time-dependent manner. *H. pylori* induces caspases 3, 7 and 8, and also anti-apoptotic proteins c-IAP1 and c-IAP2. Whereas, the proteins Bax, Bak and Bel-X_L are not changed [34]. In recent studies, activation of the peroxisome proliferator-activated receptor γ (PPARγ) suppressed NF-κB-mediated apoptosis in vitro [35]; this inhibition was independent of cag PAI status. When studying colonic T84 epithelial cells, LeNegrate et al. [36] showed that *H. pylori* triggers apoptosis through a Fas-dependent pathway, which depends on the expression of the cag PAI. In another study, apoptosis of gastric epithelial cells was mediated by elevated levels of Smad5 as a result of cag PAI-dependent *H. pylori* infection [37]. From the limited clinical data available, the effects of bacterial virulence factors, such as the cag PAI, on gastric epithelial apoptosis in vivo is unresolved.

Exposure of epithelial cells to *H. pylori* alters cell cycle control both in vitro and in vivo. Mucosal expression of cyclin D1, the tumour-suppressor p53 and the cell cycle inhibitor p21 was significantly higher in *H. pylori*-infected patients with intestinal metaplasia [38], but p53 mutations and p21 expression were not analysed in detail. A clear effect of *H. pylori* on cell cycle progression has been described in infected patients with intestinal metaplasia that overexpress cyclin D2 and show reduced expression of the cell cycle inhibitor p27 [39]. Cdx2 (caudal-related homeobox 2) plays an important role in differentiation and maintenance of intestinal epithelial cells [40]. The role of *H. pylori* virulence factors and the molecular mechanisms involved in contributing to malignant transformation in the gastric mucosa remain to be clarified. However, there is marked variability in the expression of cell cycle regulatory proteins, such as p27, cyclin D1, cyclin E—Cdk2 activity and cyclin B1, that are induced by *H. pylori* in different gastric epithelial cell lines [41,42], making extrapolation of in vitro studies to events in vivo difficult.

**Epithelial cell signalling under the direct control of *H. pylori***

The ability of a cell to respond to its extracellular environment involves a complex and highly organized series of events referred to as cellular signalling. These signalling processes regulate fundamental cellular responses and their abrogation can lead to the development of various human diseases, such as cancer. Despite numerous data, the genetic basis of gastric cancer remains unknown. None of the gene errors identified to date in gastric cancer are totally specific or unique, which indicates that gastric cancer is not a monomorphic entity. Multiple molecular and genetic alterations have been identified in gastric cancer, including activated oncogenes, growth factors and growth-factor receptors. In the following, the current knowledge of molecular signalling processes and the cell biology in *H. pylori*-induced epithelial cell responses will be reviewed.

**Epithelial cell signalling and inflammation**

Recently, the concept that inflammation is a crucial component of tumour progression has expanded. Cancer could arise from sites of infection, chronic irritation and inflammation. The tumour microenvironment, which largely accumulates inflammatory cells, is an indispensable participant in the process of cancer development.

The physical contact between *H. pylori* and gastric epithelial cells leads to the activation of signal transduction pathways, directing the induction of immediate early response transcription factors NF-κB and activator protein 1 (AP-1) [43]. These transcription factors contribute to the activation of proinflammatory C-X-C chemokines, which in vivo attract neutrophils towards the colonized epithelium, and other innate defences [25,27]. Therefore, gastric epithelial cells actively participate in inflammation and mucosal immunity during initial and persistent *H. pylori* infection. *H. pylori* stimulates the transcription factor NF-κB, which involves the activity of the kinases IKKα (I kappaB kinase alpha) and IKKβ (Table 1). These kinases are active within a complex with the essential modulator IKKγ [44]. AP-1 activation involves c-Jun N-terminal kinase (JNK) activity [45]. The bacterial effector injected by the cag PAI type IV secretion system is peptidoglycan. This is recognized by the intracellular nucleotide-binding oligomerization domain protein (Nod1) receptor molecule [46], which directly activates NF-κB. Nod1 belongs to a family that includes multiple members with NOD and leucine-rich repeats and recognizes peptidoglycan derived primarily from Gram-negative bacteria [46]. Besides stimulating innate defences in gastric epithelial cells, *H. pylori* induces arachidonic acid synthesis, which is required for the production of prostaglandins. The release of arachidonic acid involves activation of cytosolic phospholipase A2 (PLA2), pertussis toxin-sensitive heterotrimeric Gαq and Gαo proteins and the p38 kinase [47]. Activation of p38 in epithelial cells was only observed following culture with *H. pylori* strains that carry a functional cag PAI [47,48]. Therefore, certain signalling cascades that lead to the activation of the IKK complex, JNK kinase and p38 kinase, are only activated by *H. pylori* strains carrying the active cag PAI (Table 1). Whereas, the cag PAI-encoded CagA protein, which is translocated into the gastric epithelial cell via the type IV secretion system [43], is dispensable.

**Proliferation-associated signalling cascades**

Cell growth and differentiation in response to extracellular stimuli is mediated through various intracellular signal transduction pathways. The mitogen-activated protein kinase (MAPK) pathway is a major player in this kinase-signalling cascade from growth factors to the cell nucleus. The pathway involves kinases at two levels: MAP kinases, also known as extracellular signal-regulated kinases (ERKs); and MAP kinase kinases, also known as MEKS or MAP–ERK kinases. MEK is activated by the phosphorylation of two serine residues by upstream kinases, such as members of the raf gene family. When activated, MEK catalyzes the phosphorylation of threonine and tyrosine residues of ERK. The activated ERK then phosphorylates and activates transcription factors in the nucleus, such as the ternary complex factor (TCF) Elk-1, which regulate early genes including c-myc, fos and jun. Because MEK and ERK enzymes are known to be essential for normal cell proliferation and differentiation,
deregulation (overexpression, hyperactivity or gene mutation) of the MAPK signal transduction pathway might lead to proliferative diseases, such as cancer [31]. Therefore, cancer can be considered as a disease of communication at the molecular level.

*H. pylori* activates ERK1 and 2 and MEK1 and 2, in AGS gastric epithelial cells, in a *cag* PAI-independent manner [48,49] (Table 1). With regards to the intensity of activation of these MAP-kinases, the *cag* PAI–positive strains can induce moderately stronger ERK and MEK phosphorylation than *cag* PAI-negative strains [48]. Supernatants from *H. pylori* cultures also induce, using unknown secreted factors, activation of ERK and MEK kinases leading to regulation of the histidine decarboxylase (HDC) promoter (Table 1). HDC is the key enzyme involved in histamine biosynthesis and converts L-histidine into histamine in enterochromaffin-like cells of the corpus mucosa. Histamine stimulates the parietal cells that are responsible for acid secretion in the corpus of the stomach. The signalling cascade that leads to activation of the HDC promoter in *H. pylori*-infected gastric epithelial cells comprises activation of the kinase B-Raf and MEK regulated by the Ras-like GTPase Rap1. The activity of Rap1 is triggered by the accumulation of cyclic-adenosine-monophosphate (cAMP) and is under the control of the heterotrimeric G-protein Gαs [50]. Another target-gene induced by *H. pylori* using ERK and MEK kinases is cyclooxygenase-2 (COX-2) [51]. (Table 1) Enhanced transcription of COX-2 in *H. pylori*-infected gastric epithelial cells is regulated through a proximal CRE-Ebox (cAMP responsive element-Ebox) enhancer element by activation of the upstream stimulatory factor 1 and 2 (USF-1 and 2) and CREB (cAMP responsive element-binding protein) transcription factors [51]. Furthermore, *H. pylori* induces upstream activators of ERK and MEK in Ras-dependent and Ras-independent pathways [52] (Table 1).

Activation of ERK and MEK kinases appears to be through tyrosine kinase receptors [EGFR (epidermal growth-factor receptor), Her2–Neu and c-Met], which are activated in a *cag* PAI-dependent manner [53,54]. In contrast to Wallach et al. [53], the report by Keates et al. [52] describes EGFR activation in a *cag* PAI-dependent manner. Therefore, ERK and MEK kinases are regulated during *H. pylori* infection using secreted factors, and also in an epithelial contact-dependent manner in which *cag* PAI-positive strains exert the ability to induce higher levels of ERK and MEK phosphorylation.

**Activation of tyrosine kinase receptors**

Tyrosine kinase receptors control a diverse array of cellular responses including growth, proliferation, differentiation and migration. Tyrosine phosphorylation of target proteins is a reversible, dynamic process controlled by the activities of the kinases, including the tyrosine kinase receptors and the competing actions of the protein tyrosine phosphatases. For some of the tyrosine kinase receptors, an important role in carcinogenesis has been described [55].

Tyrosine kinase receptors have an important role in gastric carcinogenesis [55]. Recent studies have demonstrated that *H. pylori* activates EGFR, HER2–Neu (ErbB-2) and c-Met in gastric epithelial cells [52–54] (Figure 2). The EGFR activation is dependent on extracellular transmembrane metalloprotease cleavage of pro-heparin binding epidermal growth factor (proHB-EGF) and signalling by mature HB-EGF [53]. The upregulation of HB-EGF gene transcription by *H. pylori* requires metalloprotease, EGFR and MEK1 activities [53], indicating the involvement of the ‘triple membrane-passing signal’ (TMPS) for EGFR transactivation [56] (Figure 3). Disruption of epithelial tight junctions by the interaction of translocated CagA with the scaffolding protein ZO-1 [57] and VacA-mediated phosphorylation of G protein-coupled receptor kinase-interactor 1 (Git1) [24] probably promotes binding of EGF ligands to the EGFR located on basolateral membranes of the epithelial cells (Figure 4).

Overexpression of key elements of the TMPS cascade in those patients with gastric cancer or atrophic gastritis suggests that the EGFR autocrine–paracrine signalling pathway induced by *H. pylori* is of pathophysiological relevance. *H. pylori* infection in humans is associated with increased gastric mucosal levels of EGF protein and EGFR transcripts [58]. The proteases that are involved in the processing of transmembrane proHB-EGF remain to be identified. *H. pylori* increases matrix metalloprotease-3 (MMP-3), MMP-7 and MMP-9 in gastric carcinoma cell lines [59–61], and the bacterium itself has also been reported to have MMP-3 activity [59]. *H. pylori* also upregulates several ADAM (a disintegrin and metalloprotease) genes in cultured gastric epithelial cells [26,62]. ADAM proteins are cell-surface glycoproteins responsible for ectodomain shedding of membrane proteins, including growth factors, cytokines and their receptors, and ligands involved in apoptosis. Expression of ADAM10 and ADAM17 is increased in the gastric mucosa of *H. pylori*-infected patients [62]. Additionally, expression of several ADAM genes is strongly increased in gastric cancer [62].

![Figure 2. *Helicobacter pylori* activates receptor tyrosine kinases. *H. pylori* induces the activity of receptor tyrosine kinases, such as EGFR (epidermal growth-factor receptor), Her2–Neu (ErbB-2) and c-Met in epithelial cells by unknown bacterial effectors [52–54]. The *H. pylori* effector protein CagA targets the c-Met receptor intracellularly [54]. Activation of, and alterations in, tyrosine kinase receptor signaling result in proliferation-associated processes and the motogenic response. Preferentially, the c-Met receptor promotes epithelial cell survival and cell scattering, where it stimulates the dissociation and dispersal of epithelial cells and the transition to a fibroblastic morphology, which occur during crucial phases of development and tumour progression.](http://www.trends.com)
raising the possibility that overexpression might promote amplification of TMPS signalling cascades and dysregulated EGFR transactivation.

Cell–cell and cell–matrix interactions and the motogenic response

Decreased cell–cell or cell–matrix interactions are common in gastric cancer and might be related to the tendency to produce metastasis [63]. In polarized epithelial cells *H. pylori* affects the scaffolding protein ZO-1 and the tight junctional adhesion protein (JAM) in a CagA-dependent manner, and disrupts junction-mediated epithelial barrier functions [57] (Figure 4). Among the many types of adhesion molecules, E-cadherin serves as a prime mediator of cell–cell adhesion within the zonula adherens junctions. Downregulation of E-cadherin in antral biopsies of *H. pylori*-infected patients has been described [64]. The cytoplasmic domains of E-cadherin interact with catenins (α and β), and alterations in this system have been ascribed an important role in tumour initiation and progression [65].

Loss of E-cadherin expression in epithelial cells is associated with the acquisition of the mesenchymal phenotype. Epithelial-mesenchymal transition (EMT) occurs during crucial phases of development and tumour progression. The factor involved in this transition is represented by the hepatocyte growth factor or scatter factor (HGF or SF). HGF activates the c-Met receptor and promotes epithelial cell growth and survival and also cell scattering, where it stimulates the dissociation and dispersal of colonies of epithelial cells and the acquisition of a fibroblastic morphology [55]. Interestingly, in gastric epithelial cells *H. pylori* activates c-Met in a *cag* PAI-independent manner [54]. However, the translocated *H. pylori* effector protein CagA targets the c-Met receptor intracellularly and enhances the cell scattering (motogenic response), which suggests that dysregulation of growth-factor receptor signalling could play a role in motility and invasiveness of cells [54]. Cell motility induced by *H. pylori* in epithelial cells needs actin-reorganization with ruffle-like structures, which involves the activity of the Rho-GTPases Rac1 and Cdc42. Activation of these GTPases depends on the functional type IV secretion system, but appears independent of *H. pylori* CagA protein expression [66]. Therefore, c-Met activation, its modulation by translocated CagA, and transient polarization of the *H. pylori*-infected AGS cells by the activity of Rho-GTPases contribute to a forceful motogenic response.

The active c-Met receptor recruits, intracellularly, adaptor proteins to form an intricate signalling complex [67]. Growth factor treatment can induce Gab1 tyrosine phosphorylation and its direct association with several signal transducers, including Grb2, PI3-K, PLCγ and SHP-2 [68]. The interaction of the tyrosine phosphatase SHP-2 with translocated CagA of *H. pylori* has recently been described [69]. Furthermore, the C-terminal region of Src kinase (Csk) interacts with CagA [70], and it has been...
speculated that this interaction attenuates CagA phosphorylation. However, CagA fails to co-immunoprecipitate with Gab1 or Grb-2 [54,70]. In contrast to these reports, direct physical interaction between CagA and Grb2 in vitro has been described [71]. Therefore, CagA directly interacts with signal-transducing proteins and might play a role as an adaptor protein in growth-factor receptor signalling.

Conclusions
A characteristic of H. pylori infection in humans is gastritis, which persists for decades without causing serious damage in most cases. Therefore, the clinical complications of H. pylori infection, such as peptic ulcer disease and gastric cancer, appear to represent an imbalance in gastric homeostasis. It is tempting to speculate that H. pylori, together with environmental factors, contributes to the host responses by negatively regulating intracellular epithelial signalling pathways. Furthermore, the clinically apparent diseases probably result from the cumulative effect of multiple interactions between H. pylori and its host.

Cancer is a multistep process, which involves alterations of several different factors including tumour-suppressor genes, dominant oncogenes and receptor tyrosine kinases. The induction of the motogenic response by H. pylori in epithelial cells represents an example of how a human microbial pathogen can activate growth-factor receptor tyrosine kinases, and modify signal transduction in the cell using translocated bacterial proteins. It will be essential to deepen our understanding of receptor crosstalk in H. pylori-infected epithelium and its contribution to EGFR, Her2–Neu and c-Met activation. The study of signalling pathways that regulate EGFR, Her2–Neu and c-Met expression and activity in H. pylori infection may identify promising therapeutic targets for suppression of transformation, and offer novel potential targets for the treatment and/or prevention of malignancies (Box 2). To downregulate the expression of anti-apoptotic genes is another potential therapeutic approach. In addition, it will be important in the future (Box 2) to characterize the functions of matrix metalloproteases (MMPs) and metalloprotease-disintegrins (ADAMs), and to identify the proteases involved in EGFR transactivation. These few examples show that the understanding of the pivotal role of H. pylori in modifying the cellular microenvironment is a high requirement to define prognostic markers and develop therapeutic strategies to avoid development of gastric tumours.

Box 2. Future questions
Do animal models help to understand the pathogenesis of gastric cancer in humans?
What are the key steps in the signalling cascades that lead to gastric carcinogenesis?
What is the therapeutic potential of inhibiting key signalling pathways on gastric carcinogenesis?
Does the mucosal immune response to key virulence factors interfere with signalling?

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