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Helicobacter pylori: gastric cancer and beyond

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Abstract

Helicobacter pylori is the dominant species of the human gastric microbiome, and colonization causes a persistent inflammatory response. *H. pylori*-induced gastritis is the strongest singular risk factor for cancers of the stomach; however, only a small proportion of infected individuals develop malignancy. Carcinogenic risk is modified by strain-specific bacterial components, host responses and/or specific host–microbe interactions. Delineation of bacterial and host mediators that augment gastric cancer risk has profound ramifications for both physicians and biomedical researchers as such findings will not only focus the prevention approaches that target *H. pylori*-infected human populations at increased risk for stomach cancer but will also provide mechanistic insights into inflammatory carcinomas that develop beyond the gastric niche.

Gastric adenocarcinoma is the second leading cause of cancer-related death in the world. Approximately 700,000 people succumb to this malignancy each year and 5-year survival rates in the United States are <15%[1]. Two histologically distinct variants of gastric adeno-carcinoma have been described, each with different pathophysiological features. Diffuse-type gastric adeno-carcinoma more commonly affects younger people and consists of individually infiltrating neoplastic cells that do not form glandular structures. The more prevalent form of gastric adenocarcinoma, intestinal-type adeno-carcinoma, progresses through a series of histological steps that are initiated by the transition from normal mucosa to chronic superficial gastritis, which then leads to atrophic gastritis and intestinal metaplasia, and finally to dysplasia and adenocarcinoma[2]. *Helicobacter pylori* is a microbial species that specifically colonizes gastric epithelium and it is the most common bacterial infection worldwide. Everyone infected by this organism develops coexisting gastritis, which typically persists for decades, coupling

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Competing interests statement
The authors delcare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene *CDX1* | *IL1B* | IL2RA | keratin 19 | *Rag2*
UniProtKB: http://www.uniprot.org α_5 | α_V | β_1 | β_5 | β-catenin | AAG | ABL | ADAM17 | APC | AREG | BRAF | caspase 3 | CD4 | CSK | E-cadherin | EGFR | ERBB4 | FAK | FAP | fibronectin | GRB2 | GSK3β | HBEGF | IL-2 | IL-6 | IL-8 | integrin β2 | JAMA | Le^b | Le^x | Le^y | NOD1 | MET | MIP2 | MMP1 | PAR1B | PTPRZ1 | RAC1 | RANTES | RAP1A | SHP2 | SRC | TGFα | TLR4 | TNFα | WNT1 | WNT2 | WNT5A | ZO1
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H. pylori and its human host into a dynamic and prolonged equilibrium. However, there are biological costs incurred by such long-term relationships.

H. pylori infection is the strongest known risk factor for malignancies that arise within the stomach, and epidemio-logical studies have determined that the attributable risk for gastric cancer conferred by *H. pylori* is approximately 75%[3]. Although *H. pylori* significantly increases the risk of developing both diffuse-type and intestinal-type gastric adenocarcinoma, chronic inflammation is not required for the development of diffuse-type cancers, suggesting that mechanisms underpinning the ability of *H. pylori* to induce malignancy are different for these cancer subtypes. Eradication of *H. pylori* significantly decreases the risk of developing cancer in infected individuals without pre-malignant lesions[4], reinforcing the tenet that this organism influences early stages in gastric carcinogenesis. However, only a small proportion of colonized people ever develop neoplasia, and disease risk involves well-choreographed interactions between pathogen and host, which are in turn dependent on strain-specific bacterial factors and/or host genotypic traits. These observations, in conjunction with recent evidence that the carriage of certain strains is inversely related to oesophageal adenocarcinoma and atopic diseases[1,5] (BOX 1), underscore the importance and timeliness of reviewing mechanisms that regulate the biological interactions of *H. pylori* with its hosts and that promote carcinogenesis.

Chronic superficial gastritis

An early step in the histological cascade proceeding from normal gastric mucosa to intestinal-type gastric cancer. Characterized by the infiltration of the gastric lamina propria with mononuclear and polymorphonuclear inflammatory cells.

Atrophic gastritis

An intermediate histological step in the progression to intestinal-type gastric adenocarcinoma. Characterized by variable gland loss and the encroachment of inflammatory cells into the glandular zones.

H. pylori constituents that mediate oncogenesis

H. pylori strains are extremely diverse, freely recombining as panmictic populations. Genetic variability is generated through intra-genomic diversification (for example, point mutations, recombination and slipped-strand mis-pairing) as well as inter-genomic recombination[6]. The use of broad-range 16S ribosomal RNA (rRNA) PCR coupled with high-throughput sequencing has demonstrated that *H. pylori* does not exist simply as a monoculture within the human stomach but is instead a resident of a distinct gastric microbial ecosystem[7]. Although *H. pylori* is the dominant species, the presence of other microorganisms provides a genetic repository, which facilitates the generation of new traits that may influence gastric carcinogenesis.

At a glance

- Infection with *Helicobacter pylori* is the strongest known risk factor for gastric adenocarcinoma, but only a minority of colonized individuals develop cancer of the stomach.
- *H. pylori* strains exhibit extensive genetic diversity and strain-specific proteins augment the risk for malignancy.

- β-catenin signalling has an important role in conjunction with other oncogenic pathways in the regulation of host responses to *H. pylori* that have carcinogenic potential.
- Transactivation of epidermal growth factor receptor may help us understand the epithelial signalling pathways that mediate *H. pylori*-induced carcinogenesis.
- Chronic inflammation can induce aberrant β-catenin activation in the context of *H. pylori* infection.
- A mechanistic understanding of *H. pylori* activation of oncogenic signalling may lead to key insights into malignancies that arise from inflammatory foci in other organ systems.

The H. pylori vacuolating cytotoxin

The *H. pylori* gene *vacA* encodes a secreted protein (VacA) that was initially identified on the basis of its ability to induce vacuolation in cultured epithelial cells. VacA-induced vacuoles are hybrid compartments of late endosomal origin that depend on the presence of several cellular factors, such as v-ATPase and the GTPases RAB7, RAC1 and dynamin. However, VacA also exerts other effects on host cells, and *vacA* is a specific locus linked with gastric malignancy. All strains contain *vacA*, but there is marked variation in *vacA* sequences among strains with the regions of greatest diversity localized to the 5′ signal terminus (allele types s1a, s1b, s1c and s2), the mid-region (allele types m1 and m2) and the intermediate region (allele types i1 and i2)[8] (FIG. 1a). Each *vacA* gene contains a single signal, mid-region and intermediate region allele, and *vacA* sequence diversity corresponds to variations in vacuolating activity.

VacA is secreted and undergoes proteolysis to yield two fragments, p33 and p55 (REF. [9]), which are VacA functional domains (FIG. 1b). The p33 domain contains a hydrophobic sequence that is involved in pore formation, whereas the p55 fragment contains cell-binding domains. Full-length VacA binds multiple epithelial cell-surface components, including the transmembrane protein receptor-type tyrosine protein phosphatase-ζ (PTPRZ1)[10], fibronectin[11], epidermal growth factor receptor (EGFR)[12], various lipids[13] and sphingomyelin[14], as well as CD18 (integrin β2) on T cells[15].

VacA not only induces vacuolation but also stimulates apoptosis in gastric epithelial cells[16] (FIG. 1c). Transient expression of p33 or full-length VacA induces cyto-chrome *c* release from mitochondria, leading to the activation of caspase 3, and VacA proteins that contain an s1 signal allele induce higher levels of apoptosis than VacA proteins that contain an s2 allele or VacA mutants lacking the hydrophobic amino terminus region[9]. VacA also exerts effects on the host immune response that permit long-term colonization with an inherent increased risk of transformation. VacA binding to integrin β2 blocks antigen-dependent proliferation of transformed T cells by interfering with interleukin-2 (IL-2)-mediated signalling through the inhibition of Ca^{2+} mobilization and downregulation of the Ca^{2+}-dependent phosphatase calcineurin[17] (FIG. 1c). This in turn inhibits the activation of the transcription factor nuclear factor of activated T cells (NFAT) and its target genes *IL2* and the high-affinity IL-2 receptor-α (*IL2RA*). VacA exerts effects on primary human $CD4^+$ T cells that are different from its effects on transformed T cell lines by suppressing IL-2-induced cell cycle progression and proliferation in an NFAT-independent manner[18]. Collectively, these observations suggest that VacA inhibits the expansion of T cells that are activated by bacterial antigens, thereby allowing *H. pylori* to evade the adaptive immune response.

Most of the evidence linking VacA production to gastric cancer has been derived from epidemiological investigations. *H. pylori* strains that express forms of VacA that are active *in vitro* are associated with a higher risk of gastric cancer than the strains that express inactive forms of VacA[8,19–22]. This relationship is consistent with studies that have examined the distribution of *vacA* genotypes throughout the world. In regions in which the background rate of distal gastric cancer is high, such as Colombia and japan, most *H. pylori* strains contain *vacA* s1 and m1 alleles[23]. By contrast, animal studies have yielded mixed results, as some investigations indicate that VacA enhances the ability of *H. pylori* to colonize and induce damage in the stomach, although others have not demonstrated such an effect[10,24,25]. However, there are limitations to using animal model systems for evaluating the effects of VacA; for example, human T cells are susceptible to the effects of VacA but murine T cells are not[15,26]. Similar to VacA, strains that express the outer membrane protein BabA (as discussed below) are associated with a higher risk of gastric cancer than the strains that lack this factor[19]. On the basis of data from epidemiological studies alone, however, it is difficult to determine which of these bacterial factors is most closely linked to adverse disease outcomes, as these virulence constituents tend to cluster together in *H. pylori* strains[19].

Attributable risk

The risk for a particular condition or disease that is defined by differences in the rates of that condition or disease between an exposed group and an unexposed group.

Panmictic population

A microbial population that is not clonal but is characterized by extensive recombination and genetic diversity.

Adaptive immune response

Also known as specific or acquired immunity. It is mediated by antigen-specific lymphocytes and antibodies, is highly antigen-specific and includes the development of immunological memory.

Lewis histo-blood-group antigen

A fucosylated antigen that is expressed on erythrocytes as well as in other body compartments, including the gastric epithelium.

Outer membrane proteins

Although most *H. pylori* reside within the semi-permeable mucous gel layer of the stomach blanketing the apical surface of the gastric epithelium, approximately 20% bind to gastric epithelial cells. Genome analysis from completely sequenced *H. pylori* strains has revealed that an unusually high proportion of identified open reading frames encode proteins that reside in the outer, as well as the inner, membrane of the bacterium (known as outer membrane proteins (OMPs))[27–30]. Consistent with genomic studies, *H. pylori* strains express multiple paralogous OMPs, several of which bind to defined receptors on gastric epithelial cells, and strains differ in both expression and binding properties of certain OMPs (FIG. 2).

BabA, a member of a family of highly conserved OMPs, and encoded by the strain-specific gene *babA2*, is an adhesin that binds the Lewis histo-blood-group antigen Le^b (also known as MUC5AC) on gastric epithelial cells[19,31,32]; *H. pylori babA2*$^+$ strains are associated with an increased risk of gastric cancer[19]. SabA is an *H. pylori* adhesin that binds the sialyl-lewis^x (Le^x; also known as FUT4) antigen, which is an established tumour antigen and a marker of gastric dysplasia that is upregulated by chronic gastric inflammation[33]. Exploitation of host lewis antigens is further evidenced by data demonstrating that the O-antigen of *H. pylori*

lipopolysaccharide (LPS) contains various human lewis antigens, including Le^x, Le^y (also known as FUT3), Le^a and Le^b; and the inactivation of Le^x- and Le^y-encoding genes prevents *H. pylori* from colonizing mice[34]. Approximately 85% of *H. pylori* clinical isolates express Le^x and Le^y, and although both can be detected on individual strains one antigen usually predominates[35]. *H. pylori* lewis antigens can undergo phase variation *in vitro*[35,36], and *in vivo* studies using Rhesus monkeys or mice have demonstrated that the Lewis antigen expression pattern of colonizing bacteria is directly altered in response to the expression pattern of their cognate host[37,38]. In Le^b-expressing transgenic or wild-type control mice challenged with an *H. pylori* strain that expressed Le^x and Le^y, only bacterial populations recovered from Le^b-positive mice expressed Le^b, and this was mediated by a putative galactosyltransferase gene (β-*(1,3)galT*)[38]. This suggests that Lewis antigens facilitate molecular mimicry and allow *H. pylori* to escape host immune defenses by preventing the formation of antibodies against shared bacterial and host epitopes.

OipA is another differentially expressed OMP that has been linked to disease outcome[39]. Expression of OipA is regulated by slipped strand mispairing within a CT-rich dinucleotide repeat region located in the 5′ terminus of the gene. Several reports have demonstrated that OipA co-regulates the expression of proinflammatory cytokines, including IL-8, IL-6 and RANTES (also known as CCL5), as well as other effector proteins that may have a role in pathogenesis, such as matrix metalloproteinase 1 (MMP1; also known as interstitial collagenase)[40–44]. However, other studies have not demonstrated an effect of OipA on cytokine production[45–48], which may be due to different *H. pylori* strains possessing in-frame versus out-of-frame *oipA* sequences or to differences in experimental conditions. Interestingly, OipA has now been shown to mediate the adherence of *H. pylori* to gastric epithelial cells and trigger β-catenin activation[48,49]. Collectively, these observations underscore the pivotal role of direct contact between *H. pylori* and epithelial cells in the induction of chronic inflammation and injury.

Phase variation

The alteration of bacterial surface proteins (for example, outer membrane proteins, flagella and lipopolysaccharide) to evade the host immune system.

Pilus

Projection from the bacterial cell surface that allows bacteria to attach to other cells to facilitate the transfer of proteins or genetic material.

Box 1 | Reciprocity between *H. pylori* and oesophageal and allergic diseases

The decline in *Helicobacter pylori* acquisition during the past century in the United States has been mirrored by an expected decrease in distal gastric cancer, but these changes have been opposed by a rapidly increasing incidence of gastroesophageal reflux disease (GERD) and its sequelae, Barrett's oesophagus and oesophageal adenocarcinoma[1]. This reciprocal effect is almost entirely attributable to cag^+ strains, and the location of inflammation in the stomach seems to be crucial. Patients with gastritis that primarily affects the distal stomach, with sparing of the acid-secreting gastric body, have increased gastric acidity, and acid secretion rates are attenuated in infected subjects with gastritis that affects the body of the stomach, which houses acid-producing parietal cells. By inhibiting parietal cell function and/or accelerating the development of gastric atrophy, in which parietal cells are lost, more severe gastric body inflammation that is induced by cag^+ strains may blunt the levels of acid secretion required for the development of GERD and its sequelae.

In addition to oesophageal diseases, there have also been increases in the prevalence of allergic diseases in industrialized nations[5]. Significant inverse relationships are present between the prevalence of *H. pylori* and asthma, atopy, allergic rhinitis and eczema[5], and these reciprocal relationships are most pronounced in young people infected with *cag*$^+$ strains. Low acid conditions may partially explain these inverse relationships as a substantial proportion of asthma cases in adults are due to GERD. However, *H. pylori* also aberrantly activates T regulatory cells[137], which in turn may dampen immunomodulatory activities against environmental allergens.

Therefore, the interactions of *H. pylori cag*$^+$ strains with their hosts confer opposing risks for important diseases, underscoring the clinical relevance of identifying specific strains with which individuals are colonized so that their risks for different pathological outcomes can be more correctly identified.

The H. pylori type IV cag secretion system

Another *H. pylori* strain-specific determinant that influences pathogenesis is the *cag* pathogenicity island, and *cag*$^+$ strains significantly augment the risk for distal gastric cancer compared with *cag*$^-$ strains1. Genes within the *cag* island encode proteins that form a prototypic type IV bacterial secretion system (T4SS) that exports microbial proteins. The product of the terminal gene in the island (CagA) is translocated into and phosphorylated within host epithelial cells following bacterial attachment[50] (FIG. 2). Transgenic expression of CagA in mice leads to gastric epithelial cell proliferation and carcinoma development, and CagA attenuates apoptosis *in vitro* and *in vivo* — implicating this molecule as a bacterial oncoprotein[51,52]. Recent evidence suggests that the *H. pylori* genome might also contain another T4SS, although the relationships between non-*cag* T4SS and disease remain to be clearly established. For example, duodenal ulcer-promoting gene A (*dupA*) is an *H. pylori* gene that has homology to *virB4* (a component of bacterial T4SS required for energy-dependent delivery of substrates from the bacterial cytoplasm to host cells), suggesting that it may function as an ATPase within an as yet undefined T4SS[53]. Some studies have suggested a positive association between the presence of *dupA* and duodenal ulceration, but a negative association with gastric cancer[53–55]; however, other investigations have shown no associations between the presence of *dupA* and disease[56–60].

Integrin receptors on host cells represent a portal of entry for CagA injection[61], and CagL (FIG. 2), a T4SS-pilus-localized protein, has an important role. CagL bridges the T4SS to integrin $\alpha_5\beta_1$ on target cells and activates the host cell kinases focal adhesion kinase (FAK) and SRC to ensure that CagA is directly phosphorylated at its site of injection. In addition to integrin $\alpha_5\beta_1$ (REF. [61]), CagL can also bind integrin $\alpha_v\beta_5$ and fibronectin, although the downstream consequences of binding to these receptors remain undefined. Recently, additional *cag* proteins (CagA, CagI and CagY) have been shown to bind integrin β_1 and induce conformational changes of integrin heterodimers, which permits CagA translocation[62]. Integrins are not found at the apical membrane but are present at the basolateral membrane (FIG. 2), indicating that *H. pylori* may inject effector proteins into target cells only at specific sites, such as the basolateral surface of polarized epithelial cells. This is consistent with recent observations that viable *H. pylori* are present within paracellular spaces and the gastric lamina propria[63,64], in addition to occupying an apical niche.

Following its injection into epithelial cells, CagA undergoes targeted tyrosine phosphorylation by SRC and ABL kinases at motifs containing the amino acid sequence EPIYA, which are located within the 3′ terminus of CagA[65,66,67] (BOX 2). Intracellular phospho-CagA activates a cellular phosphatase (SHP2; also known as PTPN11), leading to morphological aberrations that mirror the changes that are induced by growth factor stimulation[68,69]. Specifically,

transfection studies have demonstrated that phospho-CagA–SHP2 interactions contribute to cytoskeletal rearrangements and cell elongation by stimulating the RAP1A–BRAF–ERK signalling pathway[68].

Lamina propria

A constituent of the moist linings of mucous membranes, which line different tubes of the body, including the gastrointestinal tract.

Polymorphic mosaic gene

A gene that exists as different alleles owing to defined regions that vary in sequence.

H. pylori tightly regulates the activity of SRC and ABL in a specific and time-dependent manner. SRC is activated during the initial stages of infection and is then rapidly inactivated, but ABL is continuously activated by *H. pylori* with enhanced activities at later time points, supporting a model of successive phosphorylation of CagA by SRC and ABL[67]. Phospho-CagA can also inhibit SRC through the recruitment of c-src tyrosine kinase (CSK), a negative regulator of SRC[70]. As SRC is the primary kinase activated by CagA, inhibition of SRC by phospho-CagA generates a negative feedback loop that carefully controls the amount of intracellular phospho-CagA. However, non-phosphorylated CagA also exerts effects in the cell that might lower the threshold for carcinogenesis. The cell adhesion protein E-cadherin, the hepatocyte growth factor receptor MET, the phospholipase Cγ (PLCγ), the adaptor protein growth factor receptor-bound protein 2 (GRB2) and the kinase PAR1β (also known as MARK2) all interact with non-phosphorylated CagA[71–74], which leads to pro-inflammatory and mitogenic responses, the disruption of cell–cell junctions and the loss of cell polarity. Non-phosphorylated CagA associates with the epithelial tight junction scaffolding protein ZO1 and the transmembrane protein junctional adhesion molecule A (JAMA; also known as F11R), leading to nascent but incomplete assembly of tight junctions at sites of bacterial attachment that are distant from sites of cell–cell contact[75]. CagA was recently shown to directly bind PAR1B (BOX 2; FIG. 2), a central regulator of cell polarity, and inhibit its kinase activity. This interaction dysregulates mitotic spindle formation in addition to promoting loss of cell polarity[74,76,77]. These events are dependent on conserved 16 amino acid repeat motifs embedded within the 3′ terminus of CagA, which have been termed CagA multimerization (Cm)[78], conserved repeat responsible for phosphorylation-independent activity (CRPIA)[79] or MARK2 kinase inhibitor (MKI)[80] motifs. These motifs bind PAR1B and mediate the dimerization of CagA, which confers stronger SHP2 binding; the number of motifs can also vary between strains. A recent co-crystallography analysis of CagA bound to PAR1B demonstrated that the initial 14 amino acids of this motif occupy the substrate-binding site of PAR1B, leading to the inhibition of kinase function[80].

Cag delivery of peptidoglycan

Another consequence of *cag* island-mediated host cell contact is the production of chemokines. Although the induction of inflammatory cytokines is dependent on the host signalling molecules nuclear factor-κb (NF-κB) and MAPK[81–83], the specific bacterial effector that mediates chemokine production is not as clearly defined. In certain *H. pylori* strains, CagA can induce IL-8 expression through NF-κB activation[84–86]; however, the ability of CagA to mediate IL-8 expression is not universal across all *cag*-bearing strains[81,82,87]. In addition to CagA, the *cag* secretion system can also deliver components of *H. pylori* peptidoglycan into host cells through outer membrane vesicles[88], where they are sensed by an intracytoplasmic pathogen-recognition molecule nucleotide-binding oligomerization domain-containing protein 1 (NOD1)[89]. NOD1 activation by *H. pylori* peptidoglycan stimulates NF-κB, p38 and ERK, culminating in the expression of the cytokines MIP2 (also known as CXCL2) and IL-8

(REFS [89,90]). NOD1 activation by *H. pylori* peptidoglycan also initiates the production of type I interferon (IFN) through a signalling pathway that has been previously associated with viral infections[91] (FIG. 2).

The delivery of peptidoglycan components into host cells induces additional epithelial responses with carcinogenic potential, such as the activation of PI3K and cell migration. The *H. pylori* gene *slt* encodes a soluble lytic transglycosylase that is required for peptidoglycan turnover and release[89], thereby regulating the amount of peptidoglycan translocated into host cells. Inactivation of *slt* has been shown to inhibit *H. pylori*-induced PI3K signalling and cell migration[92]. The protein encoded by the *H. pylori* gene *HP0310* deacetylates *N*-acetylglucosamine peptidoglycan residues and is required for normal peptidoglycan synthesis[93]. Loss of *HP0310*, which leads to decreased peptidoglycan production, reciprocally augments the delivery of the other major *cag* secretion system substrate, CagA, into host cells. This suggests that functional interactions occur between *H. pylori* translocated effectors[94]. These findings indicate that contact between *cag*$^+$ strains and host cells activates multiple signalling pathways that regulate oncogenic cellular responses, which may heighten the risk for transformation, particularly over prolonged periods of colonization.

Box 2 | Modifiable motifs within the *H. pylori* CagA protein

CagA phosphorylation sites consist of the amino acid motif EPIYA, which are embedded within the carboxyl terminus of CagA (see the figure). Four distinct EPIYA sites have been described, termed A, B, C and D, each of which is flanked by different sequences. EPIYA-A and EPIYA-B motifs are present in strains throughout the world. By contrast, EPIYA-C is found predominantly in strains from Western countries, and EPIYA-D is found in strains from East Asian countries. *H. pylori* CagA proteins may contain varying numbers of EPIYA motifs. *H. pylori* strains possessing more than three EPIYA-C motifs are more frequently associated with gastric atrophy, intestinal metaplasia and gastric cancer[138,139]. *In vitro*, EPIYA-D motifs exhibit a higher affinity for binding SHP2 than EPIYA-C motifs[140], which may partially explain the increased risk of gastric carcinoma among *H. pylori*-infected people residing in East Asia. CagA EPIYA repeats are flanked by repetitive DNA sequences that are involved in recombination, which probably explains the variability in motif number among CagA variants, as well as strain-specific differences in pathogenicity, exerted by *H. pylori* strains harbouring *cagA* and *cag* pathogenicity island. In addition to EPIYA motifs, there are also PAR1B-interacting amino acid repeat motifs within the C terminus of CagA, which can vary in number.

Western-type CagA proteins contain the phosphorylation motifs EPIYA-A, EPIYA-B and EPIYA-C (pink boxes). Conserved 16 amino acid repeat motifs (FPLKRHDKVDDLSKVG; green boxes) embedded within the 3′ terminus of CagA bind PAR1B and mediate the dimerization of CagA.

β-catenin in *H. pylori* carcinogenesis

A specific host molecule that may influence carcinogenic responses in conjunction with *H. pylori* is β-catenin, a ubiquitously expressed protein that has distinct functions within host cells. Membrane-bound β-catenin is a component of adherens junctions that link cadherin receptors to the actin cytoskeleton, and cytoplasmic β-catenin is a downstream component of the Wnt signal transduction pathway (FIG. 3a). In the absence of Wnt ligand, the inhibitory complex that is composed of axin, adenomatous polyposis coli (APC) and glycogen synthase kinase-3β (GSK3β) induces the degradation of β-catenin and maintains low steady-state levels of free β-catenin either in the cytosol or the nucleus. After binding of Wnt to its receptor

Frizzled, Dishevelled is activated, which prevents GSK3β from phosphorylating β-catenin, thus allowing β-catenin to translocate to the nucleus and activate the transcription of target genes that are involved in carcinogenesis.

Increased β-catenin expression or *APC* mutations are present in up to 50% of gastric adenocarcinoma specimens when compared with non-transformed gastric mucosa[95], and the nuclear accumulation of β-catenin is increased in gastric adenomas and foci of dysplasia[96], suggesting that aberrant activation of β-catenin precedes the development of gastric cancer. *H. pylori* increases the expression of β-catenin target genes in colonized mucosa and during co-culture with gastric epithelial cells *in vitro*; therefore, it is likely that the activation of β-catenin signalling is a central component in the regulation of pre-malignant epithelial responses to *H. pylori*.

H. pylori isogenic mutant studies have revealed that the translocation of CagA into gastric epithelial cells induces the nuclear accumulation and functional activation of β-catenin, events that are recapitulated in colonized rodent and human tissue[97,98]. Using a CagA-inducible gastric epithelial model system, Murata-Kamiya *et al.*[73] demonstrated that intracellular CagA interacts with E-cadherin, disrupts the formation of E-cadherin–β-catenin complexes and induces nuclear accumulation of β-catenin, all of which are independent of CagA phosphorylation (FIG. 3b). Consequences of CagA-dependent β-catenin activation include the upregulation of target genes that influence gastric cancer, such as caudal type homeobox 1 (*CDX1*), which encodes an intestinal cell-specific transcription factor that is required for the development of intestinal metaplasia[73]. Concordant with the requisite motifs that regulate PAR1B inhibition by non-phosphorylated CagA, the specific molecular determinants that mediate the trans-location of β-catenin from the membrane to the nucleus have now been identified as CagA CM motifs (BOX 2).

Recently, additional pathways, including those that are mediated by the transactivation of EGFR (discussed below), have been demonstrated to regulate β-catenin activation in response to *H. pylori*. Activation of PI3K and AKT leads to the phosphorylation and inactivation of GSK3β, permitting β-catenin to accumulate in the cytosol and the nucleus. Suzuki *et al.*[79] have shown that CagA CM motifs interact with MET, leading to the sustained induction of PI3K–AKT signalling in response to *H. pylori* and the subsequent activation of β-catenin *in vitro* and *in vivo* (FIG. 3b).

Studies focused on PI3K and AKT have revealed that other *H. pylori* constituents may also influence β-catenin activation. Infection of Madin-Darby canine kidney (MDCK) epithelial cells with *H. pylori* leads to AKT-dependent β-catenin activation through GSK3β phosphorylation, although activation occurs independently of CagA in this system[99]. Using a different model cell system, Nakayama *et al.*[100] reported that VacA can activate PI3K-dependent β-catenin activation, and OipA has also been implicated in aberrant nuclear localization of β-catenin, although the specific mechanism underpinning this observation has not yet been delineated[49]. Therefore, multiple *H. pylori* cancer-associated determinants seem to influence β-catenin activation, which is consistent with previous reports investigating mechanisms that regulate β-catenin activation by other bacteria. Co-culture of intestinal epithelial cells with nonpathogenic *Salmonella typhimurium* leads to the activation of β-catenin signalling by the AvrA-mediated blockade of β-catenin ubiquitylation[101,102]. *Bacteroides fragilis* toxin induces nuclear accumulation of β-catenin in human intestinal cells[103], and bacterial LPS can stimulate the nuclear localization of β-catenin in myeloid cells through toll-like receptor 4 (TLR4)-dependent activation of PI3K, which subsequently inhibits GSK3β, thereby increasing steady-state levels of free β-catenin[104]. Owing to its prolonged colonization period in the stomach, however, it is difficult to discern which microbial factors elaborated by *H. pylori* exert dominant effects.

Transactivation of EGFR by *H. pylori*

EGFR is an important target for the treatment of several malignancies other than gastrointestinal cancers, and the phosphorylation and activation of EGFR increases the transcriptional activity of β-catenin by the inactivation of GSK3β. *H. pylori* infection, gastric epithelial hyperplasia and gastric atrophy are strongly linked to the dysregulation of EGFR and/or cognate ligands, such as heparin-binding EGF-like growth factor (HBEGF) in human, animal and cell culture models[105–108]. The *in vitro* transactivation of EGFR by *H. pylori* is dependent on genes in the *cag* pathogenicity island and secreted proteins as well as host factors such as TLR4 and NOD1 (REFS [109,110]).

EGFR can be activated by direct interaction with ligands, which initiate dimerization and increased kinase activity (FIG. 4). Cytokines, such as tumour necrosis factor-α (TNFα), as well as cell adhesion molecules and G protein-coupled receptors (GPCRs) transactivate EGFR in gastric epithelial cells[111,112]. EGFR transactivation by these elements is mediated through metalloproteinase-dependent cleavage of EGFR (Erbb family) ligands[111] in a manner similar to *H. pylori*-induced EGFR transactivation[83,105]; the required metalloproteinases are likely to be members of the a disintegrin and metalloproteinase (ADAM) family. Many membrane-bound proteins undergo proteolytic release from the membrane by ADAM family proteases. Given a requirement for metalloproteinase activity in *H. pylori*-initiated HBEGF release, ADAM17, a multi-domain type I transmembrane protein that contains an extracellular zinc-dependent protease domain, is an ideal candidate enzyme for the regulation of this pathway[83,105]. ADAM17 was the first ADAM to have a defined physiological substrate, the precursor transmembrane form of TNFα. Inhibitors of ADAM17 block the release of soluble TNFα, several members of the EGF ligand family and the ERBB4 ectodomain. Although ADAM17 is ubiquitously expressed in the gastrointestinal tract and is a target of drug development for inflammatory conditions, the disorganized and inflamed nature of the gastrointestinal tract that develops in ADAM17-deficient mice suggests that this metalloproteinase may also have an important role in gut epithelial homeostasis, perhaps through the regulation of EGFR ligands. Furthermore, the processing and availability of at least three EGFR ligands, HBEGF, transforming growth factor-α (TGFα) and amphiregulin (AREG), requires ADAM17 expression[113,114]. Therefore, a better understanding of the function of ADAM17 during *H. pylori*-induced gastric epithelial injury could provide insights into its potential role in gastric carcinogenesis.

H. pylori specifically amplifies EGFR signalling by both activating EGFR and decreasing EGFR degradation by blocking endocytosis[115]. In turn, the transactivation of EGFR by this pathogen mediates several cellular responses with pre-malignant potential (FIG. 4). Alterations in apoptosis have been implicated in the pathogenesis of *H. pylori*-induced injury before the development of gastric cancer. The ability of *H. pylori* to induce apoptosis in gastric epithelial cells has been well demonstrated *in vitro*[16]. However, chronically infected humans and mongolian gerbils harbouring cag^+ strains exhibit increased gastric epithelial cell proliferation without a concordant increase in apoptosis[116,117], which may contribute to the augmented risk for gastric cancer that is associated with cag^+ strains. *H. pylori* has been shown to induce anti-apoptotic pathways in gastric epithelial cells through *cag*-mediated EGFR transactivation[118] (FIG. 4). Altered cell polarity and migration are phenotypic responses to *H. pylori* infection and, although they may acutely promote gastric mucosal repair, long-term stimulation of these responses has been linked to transformation and tumorigenesis[74,92]. *H. pylori*-mediated transactivation of EGFR has also been shown to regulate epithelial cell migration through the *cag*-dependent activation of PI3K and AKT[92] (FIG. 4). As the biological responses to EGFR activation include increased proliferation, reduced apoptosis, the disruption of cell polarity and enhanced migration, transactivation of EGFR by *H. pylori* is an attractive target for studying early events that may precede transformation.

Although the preponderance of evidence suggests that EGFR overexpression and activation are associated with tumorigenesis, recent studies raise questions about targeting EGFR for the treatment of gastric cancer[119,120]. Gastric cancer cells are resistant to inhibition of EGFR in the absence of concurrent MEK inhibition *in vitro*[119], and surveys of human gastric cancer specimens for evidence of overexpression or mutations of EGFR have found both events to be rare[120]. Furthermore, when viewed within the context of the cytoprotective role of EGFR during infection with *H. pylori*[121], future investigations using transgenic rodent models that are then confirmed by human studies will be crucial for defining the true link between EGFR transactivation, protection from gastritis and the potential for enhanced gastric cancer progression.

Inflammation, oncogenesis and *H. pylori*

Although *H. pylori* proteins and induced epithelial responses clearly influence disease risk, they are not absolute determinants of carcinogenesis, and the chronic inflammation that develops in response to this organism undoubtedly contributes to transformation. Studies in mice infected with the related mouse-adapted *Helicobacter* species, *H. felis*, have demonstrated that bone marrow-derived cells (BMDCs) home to and engraft in sites of chronic gastric inflammation — particularly within foci in which tissue injury induces excessive apoptosis and overwhelms the population of endogenous tissue stem cells[122]. Within the inflamed stomach, BMDCS degenerate into adenocarcinoma, suggesting that gastric epithelial carcinomas can originate from bone marrow-derived sources[122]. Polymorphisms in the human *IL1B* gene promoter that are associated with increased expression of IL-1β (a pro-inflammatory cytokine with potent acid-suppressive properties) heighten the risk for atrophic gastritis and gastric adenocarcinoma[123]. These relationships are present only among *H. pylori*-colonized people, emphasizing the importance of host–environment interactions and inflammation in the progression to gastric cancer. Recently, transgenic mice overexpressing human IL-1β in parietal cells were shown to develop spontaneous gastritis and dysplasia after 1 year of age, and they progressed to carcinoma when infected with *H. felis*[124]. These findings were linked to the activation of myeloid-derived suppressor cells (MDSCs) through an NF-κB-dependent, but lymphocyte-independent, mechanism. Previous studies have demonstrated that gastric carcinogenesis in mice is dependent on the presence of $CD4^+$ T cells. However, in this model of IL-1β overexpression, the marked infiltration of MDSCs into the gastric mucosa occurred early, accompanied by only a sparse infiltration of T cells. Furthermore, when IL-1β-transgenic mice were crossed on a T cell-deficient background (recombination activating gene 2 (*Rag2*)$^{-/-}$), gastritis and dysplasia still developed[124], suggesting that IL-1β induces gastric injury in an MDSC-dependent but T cell-independent manner.

In addition to IL-1β, high-expression polymorphisms in TNFα (a pro-inflammatory cytokine) also increase the risk of gastric cancer[123]. Oguma *et al.*[125] recently identified a link between the expression of TNFα and aberrant β-catenin signalling in gastric cancer. They used transgenic mice that overexpress WNT1, which was under the control of the keratin 19 promoter (*K19-Wnt1*) in this model, in gastrointestinal epithelial cells; these mice develop gastric dysplasia. Within dysplastic foci, nuclear β-catenin was present in gastric epithelial cells that were in close juxtaposition to infiltrating macrophages, prompting *in vitro* experiments to determine whether secreted macrophage products could activate epithelial β-catenin signalling[125]. Conditioned media from activated macrophages induced β-catenin signalling in gastric epithelial cells, which was attenuated by the inhibition of TNFα. TNFα was then shown to induce phosphorylation of AKT and subsequently GSK3β, liberating β-catenin to translocate to the nucleus (FIG. 3b). Recapitulation of these *in vitro* events was accomplished by infecting *K19-Wnt1* mice with *H. felis*, which resulted in macrophage infiltration and the accumulation of β-catenin in proliferating gastric epithelial cells[125]. *H. felis* infection also led to a loss of parietal cells, which are required for epithelial cell

differentiation in gastric glandular units. These results invoke a model (FIG. 3b) in which microbial-induced gastritis promotes epithelial hyperproliferation and aberrant differentiation through Wnt-mediated pathways, thereby coupling inflammation and β-catenin signalling in the gastric carcinogenesis cascade.

Parietal cell

A secretory cell that produces acid and is present within the gastric corpus.

Myeloid-derived suppressor cell (MDSC)

A heterogeneous and plastic cell. When isolated from normal bone marrow, it does not exhibit immunosuppresive effects. However, when exposed to the tumour microenvironment, it inhibits both $CD4^+$ and $CD8^+$ T cells.

Foveolar hyperplasia

Excessive proliferation of epithelial cells within foveolae, small pits from which gastric glands form that result in elongation and tortuosity of the glandular lumen.

Perspectives

Studies that focus on the specific interactions between *H. pylori* and its host can provide models for general patterns that may be extended to other malignancies that arise from inflammatory foci within the hepatobiliary and gastrointestinal tract. The vast majority of hepatocellular carcinomas are attributable to chronic Hepatitis B and Hepatitis C infections, and cholangiocarcinoma of the biliary tract is strongly linked to chronic inflammation that is induced by parasites[126,127]. Chronic oesophagitis, pancreatitis and ulcerative colitis each confer a significantly increased risk for the development of adenocarcinoma within their respective anatomic sites[126]. Focusing on colorectal neoplasia, commonalities between inflammation-induced gastric cancer and carcinomas that arise in the context of ulcerative colitis have been reported. DNA damage resulting from inflammation-associated reactive oxygen and nitrogen species (RONS) has a key role not only in dextran sulphate sodium (DSS)-induced colon tumours but also in the development of pre-malignant lesions in *H. pylori*-infected gastric mucosa. Specifically, the loss of a key DNA repair enzyme, alkyladenine DNA glycosylase (AAG; also known as MPG) that repairs RONS-induced DNA damage, augmented the severity of adenomas and adenocarcinomas in the colons as well as atrophy and foveolar hyperplasia in the stomachs of mice challenged with DSS or *H. pylori*, respectively[128].

Another key effector influencing both gastric and intestinal carcinomas is β-catenin. In conjunction with the strong evidence implicating *H. pylori*-induced β-catenin activation in the pathogenesis of gastric cancer, numerous studies have demonstrated aberrant β-catenin signalling in carcinomas of the lower intestinal tract. In human colorectal carcinoma specimens, inactivating mutations of APC or axin are present in 70–75% of cases[129]. Germline *APC* mutations are responsible for familial adenomatous polyposis (FAP), an autosomal dominant disorder that is characterized by the formation of hundreds to thousands of adenomatous polyps that carpet the colon, which initiate an inexorable progression to carcinoma in untreated cases. In regions of the world in which *H. pylori* prevalence rates are high (for example, Japan), the incidence of gastric cancer in patients with FAP ranges from 39% to 50%, and coexisting *H. pylori* infection increases the risk of gastric adenomas in these subjects compared with patients without FAP[130,131].

Wnt-mediated pathways are crucial for stem cell renewal, and evidence indicates that *H. pylori* may intimately interact with this cell population. In transgenic mice that overexpress Le^b, *H. pylori* directly adhere to gastric epithelial cells[132,133]. The genetic ablation of parietal

cells in Le^b-expressing transgenic mice permits the gastric epithelial progenitor (GEP) stem cell population to expand, which is accompanied by an expansion of *H. pylori* colonization and inflammation within glandular epithelium[134,135]. *H. pylori* has the capacity to directly interact with GEP cells[29,136], and the delineation of the GEP transcriptome has identified several pathways that are over-represented in this lineage and which are of particular biological importance for carcinogenesis, including Wnt–β-catenin[29]. Aberrant activation of Wnt signalling in stem cells is a fundamental principle that underpins carcinogenesis in several organ systems, and within the gastrointestinal tract macrophages have been demonstrated to produce the Wnt ligands WNT2 and WNT5A in human colorectal carcinoma specimens. Therefore, mechanisms through which *H. pylori* aberrantly activates oncogenic signalling pathways may be applied more broadly to cancers affecting the entire gastrointestinal tract and facilitate a deeper understanding of how chronic inflammation leads to malignant degeneration in other organ systems.

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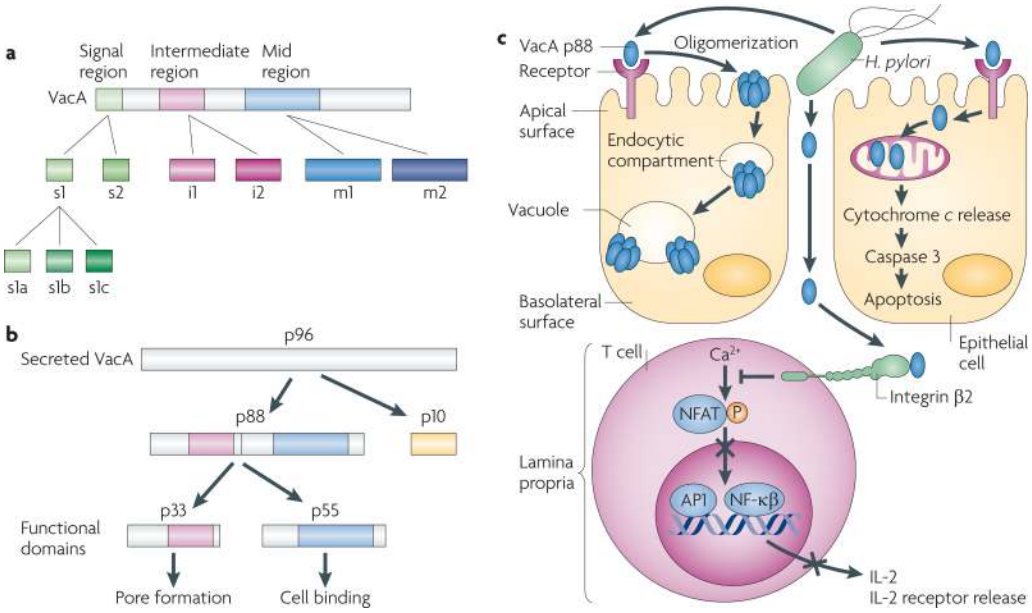

Figure 1. *Helicobacter pylori* VacA structure and functional effects

a | *vacA* is a polymorphic mosaic gene that arose through homologous recombination. Regions of sequence diversity are localized to the signal (s), intermediate (i) and mid (m) region. The s1 signal region is fully active, but the s2 region encodes a protein with a different signal peptide cleavage site, resulting in a short amino-terminal extension that inhibits vacuolation. The mid region encodes a cell-binding site, but the m2 allele is attenuated in its ability to induce vacuolation. The function of the i region is undefined. **b** | VacA is secreted as a 96 kDa protein, which is rapidly cleaved into a 10 kDa passenger domain (p10) and an 88 kDa mature protein (p88). The p88 fragment contains two domains, designated p33 and p55, which are VacA functional domains. **c** | The secreted monomeric form of VacA p88 binds to epithelial cells nonspecifically and through specific receptor binding. Following binding, VacA monomers form oligomers, which are then internalized by a pinocytic-like mechanism and form anion-selective channels in endosomal membranes; vacuoles arise owing to the swelling of endosomal compartments. The biological consequences of vacuolation are currently undefined, but VacA also induces other effects, such as apoptosis, partly by forming pores in mitochondrial membranes, allowing cytochrome *c* release. VacA has also been identified in the lamina propria, and probably enters by traversing epithelial paracellular spaces, where it can interact with integrin β2 on T cells and inhibit the transcription factor nuclear factor of activated T cells (NFAT), leading to the inhibition of interleukin-2 (IL-2) secretion and blockade of T cell activation and proliferation. AP1, activator protein 1; NF-κB, nuclear factor-κB; P, phosphorylation.

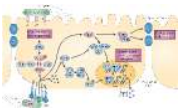

Figure 2. Interactions between pathogenic *H. pylori* and gastric epithelial cells

Several adhesins such as BabA, SabA and OipA mediate binding of *Helicobacter pylori* to gastric epithelial cells, probably through the apical surface. *H. pylori* can also bind to $\alpha_5\beta_1$ integrins, which are located on the basolateral surface of epithelial cells. After adherence, *H. pylori* can translocate effector molecules such as CagA and peptidoglycan (PGN) into the host cell. PGN is sensed by the intracellular receptor nucleotide-binding oligomerization domain-containing protein 1 (NOD1), which activates nuclear factor-κB (NF-κB), p38, ERK and IRF7 to induce the release of pro-inflammatory cytokines. Translocated CagA is rapidly phosphorylated (P) by SRC and ABL kinases, leading to cytoskeletal rearrangements. Unphosphorylated CagA can trigger several different signalling cascades, including the activation of NF-κB and the disruption of cell–cell junctions, which may contribute to the loss of epithelial barrier function. Injection of CagA seems to be dependent on basolateral integrin $\alpha5\beta_1$. AJ, adherens junction; CSK, c-src tyrosine kinase; IFN, interferon; IKKε, IκB kinase-ε; IRF7, interferon regulatory factor 7; RICK, receptor-interacting serine-threonine kinase 2; TBK1, TANK-binding kinase 1; TJ, tight junction.

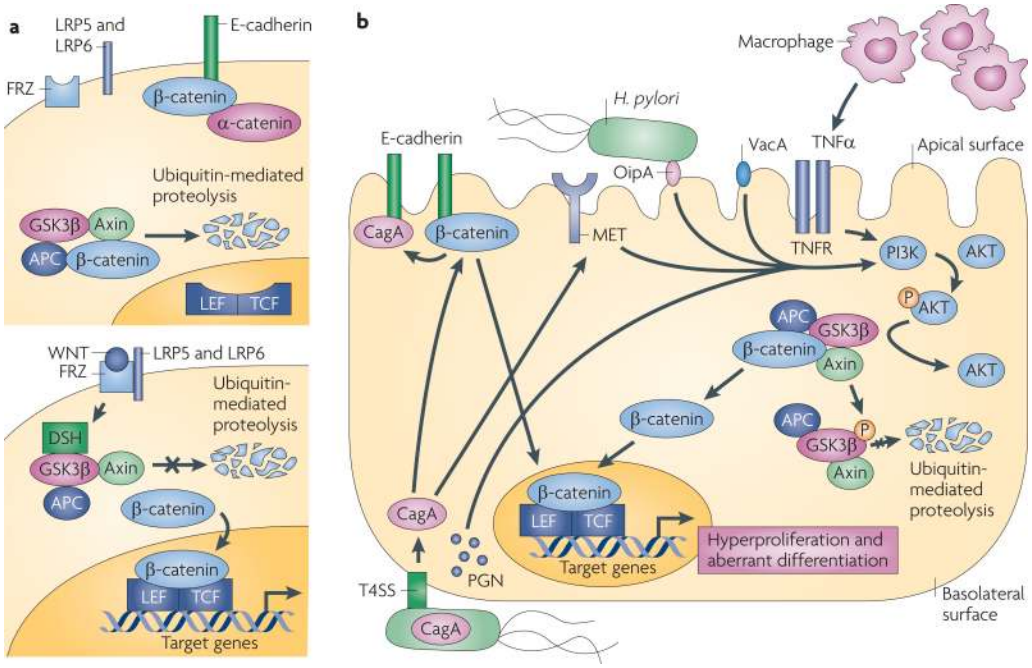

Figure 3. Aberrant activation of β-catenin by *Helicobacter pylori*

a | Membrane-bound β-catenin links cadherin receptors to the actin cytoskeleton, and in non-transformed epithelial cells β-catenin is primarily localized to E-cadherin complexes. Cytoplasmic β-catenin is a downstream component of the Wnt pathway; in the absence of Wnt (upper panel), cytosolic β-catenin remains bound within a multi-protein inhibitory complex comprised of glycogen synthase kinase-3β (GSK3β), the adenomatous polyposis coli (APC) tumour suppressor protein and axin[141]. Under unstimulated conditions, β-catenin is constitutively phosphorylated (P) by GSK3β, ubiquitylated and degraded[141]. Binding of Wnt to its receptor, Frizzled (FRZ; lower panel), activates dishevelled (DSH) and Wnt co-receptors, low density lipoprotein receptor-related protein 5 (LRP5) and LRP6, which then interact with axin and other members of the inhibitory complex, leading to the inhibition of the kinase activity of GSK3β[141]. These events inhibit the degradation of β-catenin, leading to its nuclear accumulation and formation of heterodimers with the transcription factor lymphocyte enhancer factor/T cell factor (LEF/TCF), resulting in the transcriptional activation of target genes that influence carcinogenesis. **b |** Injection of CagA results in the dispersal of β-catenin from β-catenin–E-cadherin complexes at the cell membrane, allowing β-catenin to accumulate in the cytosol and nucleus. CagA, potentially by binding MET or other *H. pylori* constituents such as OipA, VacA and peptidoglycan (PGN) as well as tumour necrosis factor-α (TNFα), which is produced by infiltrating macrophages, can activate PI3K, leading to the phosphorylation and inactivation of GSK3β. This liberates β-catenin to translocate to the nucleus and upregulate genes, leading to increased proliferation and aberrant differentiation; TNFR, TNF receptor.

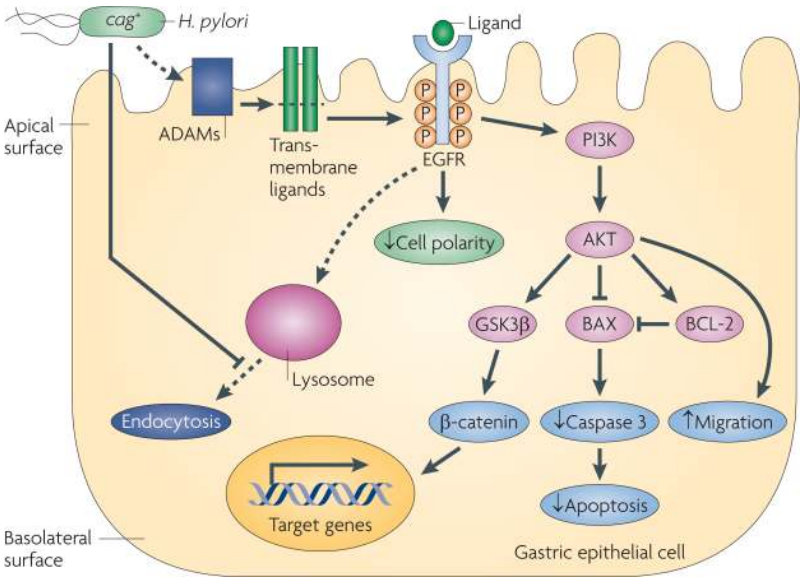

Figure 4. Transactivation of EGFR by *H. pylori* and induced cellular consequences with carcinogenic potential

Helicobacter pylori transactivates epidermal growth factor receptor (EGFR) through cleavage, which is dependent on the a disintegrin and metalloproteinase (ADAM) family proteinases, of EGFR ligands, such as heparin-binding EGF-like growth factor (HBEGF) in gastric epithelial cells. One downstream target of EGFR transactivation is PI3K–AKT, which leads to AKT-dependent cell migration, inhibition of apoptosis and β-catenin activation. BAX, BCL-2-associated X protein; GSK3β, glycogen synthase kinase-3β; P, phosphorylation.