Inflammation and proliferation – a causal event of host response to *Helicobacter pylori* infection

Vinod Vijay Subhash and Bow Ho

Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 117546, Singapore

*Helicobacter pylori* is a major aetiological agent in the development of various gastroduodenal diseases. Its persistence in gastric mucosa is determined by the interaction between various host, microbial and environmental factors. The bacterium colonizes the gastric epithelium and induces activation of various chemokine mediators, including NFκB, the master regulator of inflammation. *H. pylori* infection is also associated with an increase in expression of cell cycle regulators, thereby leading to mucosal cell hyper-proliferation. Thus, *H. pylori*-associated infections manifest activation of key host response events, which inadvertently could lead to the establishment of chronic infection and neoplastic progression. This article reviews and elaborates the current knowledge in *H. pylori*-induced activation of various host signalling pathways that could promote cancer development. Special focus is placed on the inflammatory and proliferative responses that could serve as suitable biomarkers of infection, since a sustained cell proliferation in an environment rich in inflammatory cells is characteristic in *H. pylori*-associated gastric malignancies. Here, the role of ERK and WNT signalling in *H. pylori*-induced activation of inflammatory and proliferative responses respectively is discussed in detail. An in depth analysis of the underlying signalling pathways and interacting partners causing alterations in these crucial host responses could contribute to the development of successful therapeutic strategies for the prevention, management and treatment of *H. pylori* infection.

**Abbreviations:** EGFR, epidermal growth factor receptor; ERK, extra-cellular signal-regulated kinase; MAPK, mitogen activated protein kinase; OMP, outer-membrane protein; PAI, pathogenicity island.

**Introduction**

*Helicobacter pylori* infects the human gastric mucosa and plays an important role in the pathogenesis of chronic gastritis, peptic ulcer disease and gastric cancer (Blaser, 1998; Kuipers et al., 1995). Studies have shown a strong association between *H. pylori* infection and the development of gastric adenocarcinoma and mucosa-associated gastric lymphoma (Parsonnet et al., 1991; Uemura et al., 2001). Although colonization with *H. pylori* occurs in a vast majority of the population, only a minority of individuals develop clinical manifestations of the infection. Thus, acquisition does not directly mean infection but is a condition that could enhance the relative risk of developing various clinical disorders of the upper gastrointestinal tract. For instance, the lifetime risk of developing peptic ulcer disease in *H. pylori* positive subjects is estimated to be 10–20% (Kuipers et al., 1995), which is three to fourfold higher than in non-infected individuals (Nomura et al., 1994). *H. pylori* manifests many mechanisms of pathogenicity, which include changes in host gene expression, infection-induced inflammation and cell proliferation, loss of epithelial cell polarity and cell elongation (Yamaoka, 2010). The primary disorder that accompanies *H. pylori* colonization is chronic acute gastritis. The intra-gastric severity of this chronic inflammatory process depends on a variety of factors, such as host genetics and immune response, diet, level of acid production and importantly, virulence characteristics of the infecting strain (Kusters et al., 1997). Studies on the differential pathogenic properties of *H. pylori* indicate that the increased pathogenicity correlates with the ability of the strain to induce morphological changes, infection-associated inflammation, vacuole formation and successive degeneration of in vitro cultured cells (Kusters et al., 2006). Some of the established virulence factors that are likely to play a crucial role in determining the outcome of *H. pylori* infection are as follows.

**cag Pathogenicity island (cag PAI)**

The cag PAI is a 40 kb DNA insertion element that contains 27 to 31 genes flanked by 31 bp direct repeats. It encodes one of the most intensely investigated *H. pylori* proteins, CagA, and its expression is strongly associated with peptic ulceration (Censini et al., 1996; Crabtree et al., 1991). Eighteen of the cag PAI-encoded proteins serve as building blocks of a type IV secretion system that functions in exporting bacterial proteins, like CagA, across the
bacterial membrane and into host gastric epithelial cells (Christie & Vogel, 2000). Due to its association with gastroduodenal diseases, the cag PAI is now a well characterized H. pylori virulence determinant, and CagA is frequently used as an indicator of the presence of the entire cag PAI (Wroblewski et al., 2010).

Cytotoxin-associated gene A (CagA)
The 132 kDa oncoprotein CagA is encoded by the gene cagA and is present in approximately 60–70% of H. pylori strains (Caleman Neto et al., 2014). It is translocated into the host cell through the type IV secretory system and is tyrosine phosphorylated at glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs (Noto & Peek, 2012). Once translocated into host cells, CagA induces actin cytoskeleton and cell morphological changes, termed a ‘hummingbird’ phenotype (Hatakeyama, 2008). This virulence factor has been studied extensively, especially its role in H. pylori-induced inflammation. Phospho-CagA activates a eukaryotic tyrosine phosphatase (SHP-2), leading to sustained activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). Additionally, ectopic expression of CagA in gastric epithelial cells induces NFκB nuclear translocation and IL-8 production, and was shown to be mediated through the ERK1/2 pathway (Backert & Naumann, 2010).

Vacuolating cytotoxin A (VacA)
VacA is a highly immunogenic 95 kDa protein that induces massive vacuolization in epithelial cells in vitro (Cover & Blaser, 1992). VacA is reported to play a role in gastric colonization of H. pylori (Oertli et al., 2013). Although all strains carry a functional vacA gene, there is considerable variation in vacuolating activities among strains (Yamaoka, 2009). The vacuolation process requires the presence of weak bases like ammonia (Cover et al., 1992). Many of the VacA-mediated effects arise either directly or indirectly from its membrane binding and pore formation. However, VacA is known to also enter the cytosol of host cell and interferes with cytoskeleton-dependent cell functions, induction of apoptosis, and immune modulation (Cover et al., 2003; Cover & Blanke, 2005; Hellmig et al., 2005).

Outer-membrane proteins (OMPs)
Adhesion of the bacterium to the gastric epithelium is critical for the establishment of a stable infection. At least 32 OMPs have been identified in H. pylori, the majority of which are involved in adhesion of the bacterium to the gastric epithelial cell surface (Tomb et al., 1997). Some of the widely studied OMPs of H. pylori include AlpA, OipA, BabA, BabB, SabA, SabB and HopZ, for which the fucosylated ABO blood group antigens and the related sialyl-Lewis x and sialyl-Lewis a antigens (sLe x and sLe a) have been identified as functional receptors (Yamaoka et al., 2006; Yamaoka, 2008). Association of peptic ulcer with increased expression of Lewis antigens in H. pylori has been reported in the Asian population (Zheng et al., 2000). The presence of some of these OMPs is associated with enhanced mucosal inflammation and colonization ability of H. pylori (Yamaoka et al., 2002) resulting in mucosal injury and increased risk of gastroduodenal diseases (Sugimoto et al., 2011).

Characteristics of H. pylori infection
The development of chronic gastritis associated with H. pylori is a multifactorial process and is characterized by gastric epithelial cell injury and infiltration of the mucosa with both acute and chronic inflammatory cells (Smoot et al., 1999; Yang et al., 2012a). The virulence factors produced by H. pylori inflict direct damage on gastric epithelial cells together with significant increases in inflammatory cytokine production, epithelial cell proliferation and apoptosis. In H. pylori-infected patients, gastric abnormalities are likely to be due to a combination of direct mucosal injury from bacterial virulence factors and indirect injury from the strong inflammatory response stimulated by infection. Interestingly, inflammation and proliferation show an invariable correlation in vivo and the increase in inflammation-induced epithelial cell injury was shown to be associated with a reflex increase in proliferation of uninjured cells (Tao et al., 2007). This appears to be a characteristic feature of H. pylori infection, as gastric mucosal cell proliferation is increased in H. pylori-associated chronic gastritis but not in chronic gastritis where the organism is absent (Lynch et al., 1995). Study by Munoz et al. (2007) also suggests proliferative activity as the epithelial regenerative response to cell damage caused by inflammatory mediators. The initiation of an active inflammatory response, concomitant with the failure to regulate proliferation and suppression of apoptosis, are the minimal requirements for a cell to become cancerous (Green & Evan, 2002; Hipfner & Cohen, 2004). Among the H. pylori virulence factors, the role of CagA in particular has been well-defined in contributing to disease severity, and was shown to stimulate proliferation, apoptosis induction and inflammation in infected cells (Backert & Selbach, 2008; Boonyanugomol et al., 2011; Smoot et al., 1999).

Inflammatory response
A key patho-physiological event in H. pylori infection is the initiation and continuance of an inflammatory response. Gastric inflammation is a characteristic outcome in patients infected with H. pylori and represents the host immune response to the organism. Histologically, H. pylori-associated chronic gastritis is characterized by surface epithelial degeneration and infiltration of the gastric mucosa by neutrophils, macrophages, T- and B-lymphocytes. Infiltration by these chronic inflammatory cells results in upregulation of numerous pro-inflammatory cytokines including IL-1β, IL6, IL-8, IL-10, IL-23, TNF-α and transforming growth factor beta (TGF-β) (Crabtree et al., 1991; Noach et al., 1994). H. pylori is also known to induce a strong T-helper (Th)17 immune response leading to upregulation of IL-17A (also...
known as IL-17), IL-17F, IL-21 and IL-22 (Sorelli-Lee et al., 2012; Zhuang et al., 2010). The persistence of Th17 has been shown to be a consequence of IL-1β levels, which remain elevated in the gastric mucosa. This might favour the proliferation of Th17 cells previously recruited into the gastric mucosa during active H. pylori infection (Figueiredo et al., 2014). However, these inflammatory and immune responses may not be effective in clearing the infection, but could leave the host prone to complications resulting from chronic inflammation (Naito & Yoshikawa, 2002). Qualitative or quantitative differences in H. pylori-induced gastric mucosal inflammation may play a pivotal role in determining the varied clinical outcomes of infection (Bodger & Crabtree, 1998).

**Mechanism of inflammation.** Gastric inflammation in H. pylori infection may occur through two different mechanisms. Firstly, the organism may interact with the surface epithelial cells, either producing direct cell damage or causing the liberation of epithelial derived pro-inflammatory mediators (chemokines). Secondly, H. pylori virulence factors (e.g. CagA, VacA) may gain access to the gastric mucosa, thereby stimulating specific and non-specific immune responses involving the liberation of a variety of cytokine messengers as illustrated in Table 1. The role of chemokines as mediators of gastric inflammation is well established. Many studies have shown that the gastric epithelium is an important source of chemokines. Interestingly, a contributory role of H. pylori virulence factors, especially CagA, has previously been reported in chemokine activation, especially IL-8, a potent neutrophil-activating chemotactic cytokine or chemokine (Crabtree et al., 1995; Eck et al., 2000; Watanabe et al., 1997). IL-8 is also upregulated by IL-17, which is induced in H. pylori-infected gastric mucosa (Sekkova et al., 2004). Furthermore, histological severity of H. pylori-induced gastritis (Ando et al., 1998) and gastric ulcers (Shimizu et al., 1999) correlates well with increased IL-8 levels in patient gastric mucosa. Similarly, *in vitro* co-culturing of H. pylori with gastric epithelial cells showed an increase in IL-8 secretion (Crabtree et al., 1995; Sharma et al., 1995). It was therefore no surprise that the whole genome profiling of H. pylori-infected gastric epithelial cells revealed IL-8 to be the most significantly upregulated gene (Eftang et al., 2012). IL-8 released by infected gastric epithelial cells is instrumental in regulating neutrophil infiltration of the gastric mucosa in H. pylori-associated gastritis (Nozawa et al., 2002). IL-8 is established as a chemotactic and activating peptide for T lymphocytes that also induces the production of reactive oxygen species (Naito & Yoshikawa, 2002). If defence mechanisms fail and chronic infection results, continued upregulation of IL-8, coupled with the activation of neutrophils and lymphocytes, could lead to mucosal damage and increased free radical formation, thereby instituting IL-8 as playing a pivotal role in the immunopathogenesis of H. pylori infection.

**Table 1. Mediators of inflammation in H. pylori gastritis**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Function</th>
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<tbody>
<tr>
<td>IL-1α/β</td>
<td>Pro-inflammatory (activation of leukocytes)</td>
</tr>
<tr>
<td>IL-6</td>
<td>Pro-inflammatory (B- and T-cell activation/ differentiation)</td>
</tr>
<tr>
<td>IL-7</td>
<td>T- and B-cell regulation</td>
</tr>
<tr>
<td>IL-8</td>
<td>Neutrophil recruitment and activation</td>
</tr>
<tr>
<td>IL-10</td>
<td>Immune downregulation</td>
</tr>
<tr>
<td>IL-12</td>
<td>Stimulation of Th1 response</td>
</tr>
<tr>
<td>IL-17</td>
<td>Neutrophil or mononuclear cell activation</td>
</tr>
<tr>
<td>IL-23</td>
<td>Promotes IL-17-secreting T cells</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Pro-inflammatory (especially cellular immunity)</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Neutrophil recruitment</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Pro-inflammatory (activation of leukocytes)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Pro-inflammatory, maturation factor</td>
</tr>
<tr>
<td>GRO-α</td>
<td>Neutrophil recruitment and activation</td>
</tr>
<tr>
<td>RANTES</td>
<td>Mononuclear cell recruitment and activation</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>Mononuclear cell recruitment and activation</td>
</tr>
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**Intracellular signalling in gastric inflammation.** The adherence of H. pylori to gastric epithelial cells induces tyrosine phosphorylation of host proteins, cytoskeletal reorganization and activation of several intracellular signalling events that have further downstream effects via activation of the transcription factor NFκB. In gastric and intestinal epithelial cells, NFκB has a central role in regulating genes that govern the onset of mucosal inflammatory responses following microbial infections (Lamb & Chen, 2010; Orlowski & Baldwin, 2002; Yang et al., 2012a). NFκB activation is effected through a series of phosphorylation and trans-activation events (Malinin et al., 1997; Nemoto et al., 1998) triggering a downstream signalling pathway that contributes to gastric inflammation in H. pylori-infected individuals (Bhattacharyya et al., 2002). Activation of NFκB leads to upregulation of expression of a variety of inflammatory mediators including IL-8 (De Luca & Iaquinto, 2004; Keates et al., 1999; Naito & Yoshikawa, 2002). This event is essential for the activation of innate and adaptive immune responses against pathogens (Lamb & Chen, 2013). Stimulation and activation of NFκB does not require protein synthesis, and therefore its effect is particularly important in immune, inflammatory and acute phase responses where rapid initiation of host response following exposure to pathogens is critical in facilitating survival (Naito & Yoshikawa, 2002). However, sustained and constitutive NFκB activation results in the progression of many diseases, including chronic inflammation, infectious diseases and cancer (Hayden & Ghosh, 2008). *H. pylori* strains harbouring the cag PAI (cag PAI<sup>+</sup>) can cause direct signalling in gastric epithelial cells to activate NFκB, leading to the release of IL-8 (Naumann, 2005). The role of bacterial virulence factors, especially CagA, in NFκB induction has also been identified previously (Papadakos et al., 2013).

In resting cells, NFκB activity is inhibited by its association with the IκB (IκBz, IκBβ) proteins in the cytoplasm (Verma et al., 1995). Activation of NFκB requires phosphorylation of two conserved serine residues within the
N-terminal domain of IκBα (Karin & Ben-Neriah, 2000). *H. pylori* infection results in the activation of specific intracellular signalling pathways with subsequent activation of the IκB kinase (IKK) complex. This complex comprises two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ), and can phosphorylate IκBα. Phosphorylation of IκBα is followed by its ubiquitination and proteosomal degradation, thereby resulting in NFκB nuclear translocation (Karin & Ben-Neriah, 2000; Maeda et al., 2000). Nuclear translocation of NFκB is followed by increased IL-8 mRNA and protein levels (Sharma et al., 1998) which, when secreted extracellularly, are critical in the initiation of an inflammatory response. Protein kinases are also required for optimal NFκB activation, by targeting functional domains of NFκB protein itself. More importantly, phosphorylation and activation of these key subunits play an important role in determining both the strength and duration of the NFκB-mediated transcriptional response (Karin & Ben-Neriah, 2000).

**Activation of mitogen activated protein kinases (MAPKs).** The MAPKs constitute an important group of serine and threonine signalling kinases that transduce a variety of extracellular stimuli through a cascade of protein phosphorylations leading to the activation of transcription factors. The MAPK signal transduction pathway plays a crucial role in many aspects of immune-mediated inflammatory responses. The involvement of MAPK pathways in NFκB activation has been well documented (Malinin et al., 1997; Nemoto et al., 1998). There are three principal MAPK family members: (i) p46 and p54 c-Jun N-terminal kinase (JNK), or stress-activated protein kinase, with multiple subisoforms, (ii) p38 MAPK, with a, b, c, and d isoforms, and (iii) p42 and p44 ERK (Bhattchararya et al., 2002). Previous studies have shown that exposure of gastric epithelial cell lines to *H. pylori* strains induced the production of IL-8 through the activation of MAPK signalling pathways (Keates et al., 1999; Meyer-ter-Vehn et al., 2000), identified marked stimulation of p38, ERK and JNK pathways in the event of *H. pylori* infection and showed that inhibition of these pathways showed differential attenuation of *H. pylori*-induced IL-8 secretion. Among the MAPKs, ERK was shown to play a prominent role in signal transmission for the efficient activation of *H. pylori*-induced NFκB activation, resulting in the production of IL-8 (Nozawa et al., 2002).

ERK exerts its multiple biological effects by phosphorylating membrane or cytoskeletal proteins and is activated upon phosphorylation by dual specificity kinases MEK1 and MEK2 (Roberts & Der, 2007). A study by Nozawa et al. (2002) indicated that the stimulation of the ERK signalling pathway by *H. pylori* may be directly responsible for NFκB activation and subsequent synthesis of IL-8. Moreover, when phosphorylated, ERK (pERK) translocates to the nucleus, which can result in the activation of other transcription factors, including activator protein-1, which when bound to the IL-8 promoter region would lead to maximal gene expression (Asim et al., 2010; Hoffmann et al., 2002). The role of CagA in ERK activation has been studied previously (Zhao et al., 2010). As shown in Fig. 1, CagA contributes to ERK phosphorylation and resultant IL-8 secretion, via phosphorylation-dependent and -independent pathways (Nguyen et al., 2008). Once translocated into the host cell, CagA binds to and activates the SHP-2 protein, which in turn, activates the ERK signalling cascade (Higashi et al., 2002, 2004). Furthermore, gene expression analysis of *in vitro* *H. pylori*-infected cells revealed a dominant role of the ERK/NFκB signalling cascade in regulating the host response, as it mediates the regulation of a large majority of the genes having crucial implications in carcinogenesis (Keates et al., 2007; Shibata et al., 2005).

Ras–Raf signalling pathway in ERK activation. The components of this pathway, Ras and Raf, are proto-oncogenes that contribute to ERK activation through complex protein–protein interactions (Kolch, 2000) (Fig. 1). Infection of gastric epithelial cells with *H. pylori* activates Ras proteins by inducing the exchange of GDP with GTP, which converts Ras into its active conformation. During this process, the archetypal Ras exchange factor, SOS (son of sevenless), is

![Fig. 1. Intracellular events in *H. pylori*-induced IL-8 secretion: *H. pylori* virulence factors are translocated into the host cells through a type IV secretion system. Among others, CagA is shown to play a key role in IL-8 secretion, through the activation of ERK signalling kinases. Both phosphorylated (CagA-P) and unphosphorylated CagA contribute to IL-8 secretion via SHP-2-mediated and Ras–Raf-mediated pathways, respectively.](http://mic.sgmjournals.org)
towed to the membrane by the growth-factor-receptor-bound protein 2 (GRB-2) adaptor protein, which recognizes tyrosine phosphate docking sites located on the receptors themselves or on receptor substrate proteins. Activated Ras binds to Raf with high affinity and induces its phosphorylation (Moodie & Wolfman, 1994). Phosphorylated Raf activates MEK1/2, which then leads to the activation of downstream ERKs (Kolch, 2000). *H. pylori* CagA was found to potentiate the activation of NFκB via the Ras→Raf→MEK→ERK pathway (Brandt et al., 2005). This process appears to involve phosphorylation-independent binding of CagA to GRB-2, the upstream effector of Ras (Mimuro et al., 2002).

EGFR in ERK activation. Epidermal growth factor receptors (EGFRs) are receptor linked tyrosine kinases, over-expression of which is prevalent in gastric cancer (Normanno et al., 2006). *H. pylori* infection triggers the ectodomain shedding of the EGFR ligand HB-EGF, which is required for EGFR activation through its phosphorylation at tyrosine residues (Fig. 2). Tyrosine kinase activity of the cytoplasmic domain of the EGFR also leads to its over-expression through an autocrine loop of EGFR transactivation (Keates et al., 2007). Phosphorylated EGFR (P-EGFR) binds to the GRB-2 docking protein and forms a complex with the guanine nucleotide exchange factor SOS (Zarich et al., 2006). This assembly promotes Ras activation. Activated Ras directly interacts with and activates Raf, which phosphorylates and activates MEK, which in turn phosphorylates and activates ERKs, leading to downstream activation of NFκB and IL-8 secretion (Avruch et al., 2001).

**Cell proliferation**

Another characteristic feature of *H. pylori*-induced gastric malignancies arises from its effects on cell proliferation.

Fig. 2. Schematic representation of the mechanism involved in EGFR-mediated ERK activation.

Aberrant regulation of proliferation together with the suppression of apoptosis is the minimal requirement for a cell to become cancerous (Green & Evan, 2002; Hipfner & Cohen, 2004). *H. pylori* infection of the gastric mucosa has been associated with an increase in gastric epithelial cell proliferation (Peek et al., 1999; Schneider et al., 2011), both in vitro and in vivo (Beki et al., 1996; Brenes et al., 1993; Fan et al., 1996). This effect in proliferation has an important pathogenic significance, because increased cell proliferation may elevate gastric mutation rates, and could well be a predisposing factor for gastric neoplasia. A study by Smoot et al. (1999) suggests that direct contact by *H. pylori* could inhibit gastric cell proliferation, supporting the theory that increased cell proliferation seen in vivo with *H. pylori*-associated gastritis is a reflex response to cell injury, and not directly caused by bacterial contact. Successful treatment of *H. pylori* infection was shown to reduce the hyper-proliferation rates of gastric epithelial cells to normal (Cahill et al., 1995; Lynch et al., 1995). Intriguingly, in vitro studies with *cagA* positive strains exhibited a pro-proliferative effect compared with the *cagA* negative strains (Smoot et al., 1999). This effect of CagA in cell proliferation, together with its contribution to inflammation, may explain in part the stronger association of *cagA* positive strains with gastric cancer (Cabral et al., 2007; Suzuki et al., 2009).

**Signalling events in cell cycle regulation and proliferation.** Several pathways have been proposed to explain the mechanisms underlying the alteration of cell proliferation during *H. pylori* infection. In mammalian cells, cellular proliferation is regulated in a cell cycle that is governed by the sequential formation and degradation of cyclins and cyclin-dependent kinases (Hirata et al., 2001). Among various cyclins, cyclin D1 regulates passage through the restriction point and entry into the S phase (Sherr, 1996). It was also found that over-expression of cyclin D1 shortens the G1 phase and increases the rate of cellular proliferation (Resnitzky & Reed, 1995; Robles et al., 1996). Various factors, such as the MAPK cascade (Lavoie et al., 1996) and the Wnt signalling pathway (Shtutman et al., 1999; Tetsu & McCormick, 1999), are known regulators of cyclin D1 expression (Hirata et al., 2001). Among these, activation of the Wnt pathway was shown to play a key role in cell proliferation (Zhang & Xue, 2008) and alteration in its signalling components has been described in about 30% of gastric cancer patients (Clements et al., 2002).

Wnt pathway in cell proliferation. The canonical Wnt signalling pathway plays a crucial role in regulating the proliferation and homeostasis of gastrointestinal epithelia. Aberrant activation of Wnt signalling was reported in *H. pylori*-induced gastritis, which could lead to enhanced proliferation, thereby leading to carcinogenesis (Yang et al., 2012b). A critical event in this process involves the nuclear translocation of β-catenin, where it forms heterodimers with lymphocyte enhancer factor (LEF) and T-cell factor (TCF) (Korinek et al., 1997). Association of β-catenin with LEF/TEF proteins could result in the transcriptional
upregulation of target genes that are involved in cellular processes like cell cycle control and proliferation (Brabletz et al., 1999; He et al., 1998; Lustig et al., 2002). Detection of β-catenin nuclear translocation provides an estimate for the frequency of Wnt activation in a large number of gastric cancers. Previous studies have shown increased nuclear β-catenin expression in between 27 and 31% of tumours (Tong et al., 2001; Woo et al., 2001). These findings are in tandem with the previous reports that showed Wnt activation in nearly one-third of patients with gastric cancers (Clements et al., 2002). Recent studies demonstrated that infection of epithelial cells with H. pylori stimulates transcriptional activity of β-catenin. H. pylori was shown to induce activation of WNT target genes including cyclin D1, c-myc and Axin2 (Murata-Kamiya et al., 2007; Neal et al., 2013). Also, in vivo studies have shown activation and nuclear accumulation of β-catenin in gastric epithelium of patients infected with H. pylori (Franco et al., 2005), suggesting the aberrant activation of β-catenin as a preceding factor for the development of gastric cancer. Increased β-catenin expression was reported in up to 50% of H. pylori-infected gastric adenocarcinoma specimens when compared with non-transformed gastric mucosa (Tsukashita et al., 2003).

Mutations that constitutively activate β-catenin signalling can lead to the development of cancer (Logan & Nusse, 2004). In non-stimulated cells, the protein level of free β-catenin is kept low by a so-called destruction complex (Fig. 3) consisting of adenomatous polyposis coli (APC), Axin, casein kinase 1α (CK1α) and glycogen synthase kinase 3β (GSK3β). GSK3β induces phosphorylation of β-catenin leading to its poly-ubiquitylation and subsequent degradation (Kimelman & Xu, 2006). Wnt ligands bind to their Frizzled family of serpentine receptors and to their coreceptors LDL-related protein (LRP) 5/6, which impairs β-catenin (Gnad et al., 2010). Binding of Wnt ligands to LRP6 activates the dishevelled homologue-1 (Dvl1) phosphoprotein. Dvl1 activation leads to a sequence of events in which the C terminus of LRP6 becomes phosphorylated by GSK3β and CK1γ, resulting in bringing Axin together with Dvl to the plasma membrane (Davidson et al., 2005; Zeng et al., 2005). As a consequence, the degradation complex is no longer functional and β-catenin translocates to and accumulates in the nucleus (Polk & Peek, 2010). Through sequential mutational analysis, Kurashima et al. (2008) showed that a functional CagA EPIYA-repeat region enhances β-catenin membranous translocalization. H. pylori CagA physically interacts with E-cadherin and disrupts E-cadherin and β-catenin complex formation, which also triggers cytoplasmic and nuclear accumulation of β-catenin. Together, these studies provide important insights into the mechanism of H. pylori, and in particular CagA, in the deregulation of the Wnt/β-catenin pathway and the promotion of gastric cancer. A recent study by Sokolova et al. (2008) demonstrated that infection of epithelial cells with H. pylori suppresses GSK3β activity via the phospho-inositol kinase 3 (PI3K)/AKT pathway. This would lead to β-catenin nuclear translocation,

![Fig. 3. Wnt pathway in activation of β-catenin: in the absence of Wnt activation, cytosolic β-catenin remains bound within a multi-protein inhibitory complex composed of GSK3β and APC tumour suppressor protein and Axin, where β-catenin is constitutively phosphorylated (P) by GSK3β, ubiquitylated and degraded. Binding of Wnt to Frizzled activates Dvl and Wnt coreceptors, low density lipoprotein receptor-related protein 5 (LRP5) and LRP6, which then interact with Axin and other members of the inhibitory complex, leading to GSK3β dephosphorylation. These events inhibit the degradation of β-catenin, leading to its nuclear accumulation and resulting in the transcriptional activation of target genes that influence carcinogenesis (Polk & Peek, 2010).](http://mic.sgmjournals.org)
and results in the upregulation of cyclin D1 in a CagA- and T4SS-independent manner.

MAPKs in cell proliferation. MAPK cascades have also been shown to play a role in regulation of cell proliferation in mammalian cells in a manner inextricable from other signal transduction systems, by sharing substrate and cross-cascade interaction (Chen et al., 2006). The activated ERKs translocate to the nucleus and transactivate various transcription factors changing gene expression to promote growth, differentiation or mitosis (Zhang & Liu, 2002). The interaction among the MAPK members (ERK, P38 and JNK), which may be important in controlling different signal cascades during bacterial infection, has not been determined in gastric epithelial cells. However, studies have demonstrated a cross-talk among MAPK members that may be important in modulating the downstream molecules, regulating cell cycle and proliferation (Cargnello & Roux, 2011). Thus, the interaction between bacterial infection and MAPK activation is likely to contribute to H. pylori-induced alterations in cell cycle control and proliferation of gastric epithelial cells (Ding et al., 2008).

Conclusions

H. pylori infection is characterized by a host environment rich with infiltration of inflammatory cells coupled with hyper-proliferation. These characteristics could have greater impacts in contributing to prolonged and enhanced survival of infected cells, hence favouring cancer progression. Targeting of signalling pathways and interacting partners causing alterations in inflammatory and proliferative responses could contribute to the development of successful therapeutic strategies for the prevention, management and treatment of H. pylori infection. Efforts are under way to develop specific inhibitors that can be used in molecularly targeted therapy to prevent NFκB activation without any effects on other signalling pathways, and be more active in infected/malignant cells than in normal cells (Baud & Karin, 2009). These could help in better management of H. pylori-infected individuals, as the persistence of chronic inflammation in gastric mucosa and elevated H. pylori antibodies after successful eradication therapy are common (Veijola et al., 2007). However, excessive and prolonged NFκB inhibition can be detrimental due to its important role in innate immunity, and hence should be transient and highly reversible to avoid long-term immunosuppression. Prospective studies should also explore altered host signalling and molecular markers in H. pylori-infected individuals, since the value of H. pylori genotypes as predictors of disease outcome is limited. Alterations in Wnt signalling leading to aberrant β-catenin expression at a precancerous stage were reported previously in H. pylori-infected patients (Chow et al., 2012). Since early aberrations in β-catenin expression could potentially be predictive of a severe disease outcome, future studies are required to validate the clinical utility of β-catenin expression as an early diagnostic marker for H. pylori-induced gastric malignancies. By the same token, studies aimed at identifying other potential virulence factors and their clinical prevalence could further the understanding of H. pylori pathogenicity.

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Edited by: L. Jodi