

Epithelial Cell Signaling in *Helicobacter pylori* Infection

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Abstract: The human microbial pathogen *Helicobacter pylori* colonises the stomach of more than half of the world's population. The microorganism can induce chronic gastritis, peptic ulceration and more rarely, gastric adenocarcinoma. Highly virulent *H. pylori* strains carry a *cag* pathogenicity island (*cag* PAI), which encodes proteins involved in a specialised type IV secretion system (T4SS). *H. pylori* induces T4SS-dependent and -independent processes by which *H. pylori* takes direct command of gastric epithelial cell signaling. The *H. pylori* effector protein cytotoxin associated gene A (CagA), which is translocated via the T4SS into epithelial cells, contributes to the modulation of cellular functions. In addition, *H. pylori* transactivates the EGFR, a process involving inter-receptor cross talk and extracellular ADAM metalloproteinase cleavage of membrane bound EGFR ligands. The multiple signal transduction pathways activated during *H. pylori* infection lead to a complex series of events promoting inappropriate inflammatory responses, epithelial hyperproliferation, epithelial survival and transformation. The *H. pylori* induced epithelial cellular changes, as well as chemopreventative therapeutic strategies, will be introduced in this review.

Key Words: *Helicobacter pylori*, CagA, epithelial proliferation, NF- κ B, EGFR, ADAM metalloproteinases, c-Met.

INTRODUCTION

The discovery over twenty years ago of the Gram negative bacterium *Helicobacter pylori*, which specifically colonises the epithelial surface of the human gastric mucosa, heralded a major re-evaluation of the aetiology of gastroduodenal disease. It is now firmly accepted that *H. pylori* infection is a major risk factor for peptic ulcer disease [1], gastric cancer [2-4] and gastric MALT lymphoma [5,6]. Infection with *H. pylori* is chronic and almost invariably results in long term chronic gastritis. The association between chronic inflammation and risk of neoplasia has long been recognised [7]. Inflammation is likely to be an important component contributing to the increased risk of gastric carcinogenesis with *H. pylori* infection.

H. pylori was the first bacterial pathogen to be classified as a Class I carcinogen. More recently other chronic bacterial infections such as *Salmonella typhi* have also been linked to increased risk of neoplasia [8]. These bacterial infections, in addition to promoting chronic inflammatory responses, directly interfere with the regulation of epithelial cell homeostasis which is considered important in the promotion of neoplasia [9]. *H. pylori* infection of the human gastric mucosa is associated with hyperproliferation of gastric epithelial cells [10,11]. The molecular and cellular mechanisms by which *H. pylori* perturbs epithelial homeostasis are being actively investigated.

H. pylori, in addition to inducing hyperproliferation of non specialised gastric epithelial cells, promotes marked functional changes in specialised gastric epithelial cells regulating acid homeostasis such as enterochromaffin-like (ECL) cells, parietal cells, gastrin producing G cells and

somatostatin producing D cells [12,13]. *H. pylori* induced changes in the function of specialised gastric epithelial cells is largely mediated indirectly via cytokines released by bacterial associated inflammatory responses [14-17]. Cytokine modulation of endocrine and exocrine function in the gastric mucosa impacts on the colonisation patterns of *H. pylori*, the distribution of gastritis and the pathogenesis of *H. pylori* induced atrophy. IL-1 β is a potent acid suppressant [16]. Genetic polymorphisms in IL-1 β linked to increased production are associated with increased risk of gastric atrophy as well as increased risk of gastric cancer in *H. pylori* infected subjects [18,19]. *H. pylori* is therefore either directly, or indirectly via inflammatory mediators, inducing cellular signaling responses in non specialised epithelial cells and specialised epithelial endocrine and exocrine cells within the gastric mucosa.

H. PYLORI VIRULENCE FACTORS INVOLVED IN BACTERIAL-EPITHELIAL INTERACTIONS

Only a minority of infected subjects will develop peptic ulceration and fewer still gastric adenocarcinoma or gastric MALT lymphoma with *H. pylori* infection. Longitudinal studies in Japan indicate the risk of developing gastric cancer is greatest in infected subjects who have non-ulcer dyspepsia or gastric ulceration with severe gastric atrophy and intestinal metaplasia [20]. *H. pylori* is an extremely genomically diverse pathogen [21]. There is now increasing evidence that certain virulence factors, which are strain specific, impact on clinical outcome. Infections with *H. pylori* strains carrying three virulence factors, the Type IV secretory system (T4SS) encoded by the *cag* pathogenicity island (*cag* PAI) [22,23], the vacuolating cytotoxin A (VacA) s1m1 alleles [24,25], and the blood group antigen-binding adhesin A2 (BabA2), which binds Lewis B on gastric epithelial cells [26,27], have been linked to increased risk of more severe gastritis, gastric atrophy and/or gastric cancer in differing populations [reviewed 3,4,24, 28]. The effects of

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these virulence factors on gastric epithelial cells have been the focus of considerable research. Collectively, these *H. pylori* virulence factors have important roles in promoting bacterial-epithelial interactions (BabA2), disrupting epithelial tight junctions and barrier function (CagA, VacA), promoting innate epithelial defences (*cag* PAI) and activating epithelial cell signaling pathways (*cag* PAI, CagA, VacA). The specific epithelial cell signaling responses induced by the T4SS *cag* PAI, CagA and VacA are considered below.

H. PYLORI INFECTION PERTURBS GASTRIC EPITHELIAL HOMEOSTASIS: IN VIVO EVIDENCE

There is considerable clinical evidence that *H. pylori* infection significantly increases gastric epithelial cell proliferation [10,11,29]. Therapeutic eradication of *H. pylori* infection results in a significant decrease in proliferation [30,31]. Enhanced epithelial proliferation has been related to the extent of gastric inflammation [10,11,32]. However, multiple regression analysis of histopathological data indicates that the only independent predictor of epithelial cell proliferation in infected patients is the density of *H. pylori* colonisation [32], suggesting both inflammation and bacterial factors contribute to epithelial proliferative responses.

The potential contribution of the bacterium and specific virulence factors, and associated inflammation, in promoting gastric epithelial cell hyperproliferation has been investigated in rodent models. Infection with *H. pylori*, or the related gastric *Helicobacter* species *H. felis*, increases gastric epithelial cell proliferation in Mongolian gerbils [33–36] and mice [37,38], respectively. The extent of epithelial proliferation and apoptosis in response to *H. felis* infection is markedly influenced by the strain of mouse, with C57BL/6 showing strong proliferative responses [37]. Interestingly in mice, *H. felis* induces much greater gastric epithelial proliferative responses than *H. pylori* [35]. *H. pylori* also induces less inflammation in the murine model than *H. felis* [35], and as a consequence *H. felis* has been used more extensively in the murine model to delineate the potential role of host factors in the hyperproliferative response. The lack of gastric epithelial hyperproliferation with gastric *Helicobacter* infection in immune-deficient mice RAG^{-/-} mice [39] and mice deficient in interferon regulatory factor [40] or gamma interferon [41], suggests that in the murine model inflammation may be the key factor inducing epithelial hyperproliferative responses. Mice lacking the anti-inflammatory cytokine IL-10 develop severe hyperplastic gastritis with *H. felis* infection, reinforcing the importance of mucosal inflammation in regulation of epithelial proliferation [42].

Infection with *cag* PAI positive *H. pylori* strains induces enhanced inflammatory responses compared to *cag* PAI negative strains both clinically [43–45], and in the gerbil model [34,46]. Disassociation of the contribution of inflammation and bacterial factors in epithelial responses is thus difficult. Clinical studies on the effects of *cag* PAI status on gastric epithelial cell proliferation have been equivocal [10,11,47], and factors such as the BabA2 adhesin [27,48] could account for variable results. In a Chinese population increased epithelial cell proliferation was observed in *cag* PAI positive patients infected with strains that expressed the

adhesin BabA2 [48]. In the gerbil, a *cag* PAI positive *H. pylori* strain (B128) induced increased gastric epithelial proliferation and apoptosis compared with a wild type strain lacking a functional *cag* PAI [34]. In further studies an isogenic mutant (Δ *cagY*) of B128, with a defective T4SS, induced similar epithelial proliferation in the gastric antrum of gerbils as the parental strain, suggesting antral epithelial proliferation may be independent of the functional T4SS [49]. However, differences were observed in epithelial proliferative responses in the corpus, which were attributed to enhanced colonisation of the parental strain in the corpus mucosa [49]. Future studies in the gerbil model with isogenic mutants of other virulence factor genes will be important to ascertain their importance in stimulating epithelial hyperproliferation.

The imbalance between apoptosis and cell proliferation associated with *H. pylori* infection could lead to an overall increase in cellular turnover and persistence of mutated cells which would favour the development of intraepithelial neoplasia. The cell cycle controls cell growth and cell division, and important protein families within the cell cycle are the cyclins, the cyclin-dependent kinases (CDKs), the CDK inhibitors, and the tumour-suppressor genes (e.g. p53) [50]. *In vitro* studies have demonstrated that cyclin D1 expression induced in *H. pylori*-infected epithelial cells is partly dependent on the *cag* PAI [51]. Cyclin D1 regulates passage through the G1 phase, and cyclin D1 overexpression shortens the G1 phase and increases the rate of cellular proliferation. Mucosal expression of cyclin D1 and the tumour-suppressor p53 were significantly higher in *H. pylori*-infected patients with intestinal metaplasia [52]. However, there is marked variability in the expression of cell cycle regulatory proteins such as p27, cyclin D1, cyclin E/CDK2 and cyclin B1 induced by *H. pylori* in different gastric epithelial cell lines [51,53]. The role of *H. pylori* *cag* PAI⁺ strains in the molecular mechanisms contributing to the intraepithelial neoplasia remains to be clarified. Studying colonic T84 epithelial cells LeNegrato *et al.* [54] showed that *H. pylori* triggered apoptosis via a Fas-dependent pathway, which depended on the expression of the *cag* PAI. Reduced expression of the pro-apoptotic factor Bax and overexpression of Bcl2 has been observed in individuals with intestinal metaplasia in the antrum [55]. Decreased expression of the *Bak* and *Bax* genes (pro-apoptotic), but increased expression of the *Bcl2* gene, is also evident in individuals with gastric cancer [56]. Although apoptosis is increased in patients with *cag* PAI⁺ strains [11], the effects of the *cag* PAI on gastric epithelial apoptosis *in vivo* is unresolved due to the limited clinical data.

H. PYLORI TYPE IV SECRETION SYSTEM

A T4SS was first described in the plant pathogen *Agrobacterium tumefaciens* where it delivers DNA and proteins into plant cells. Other microorganisms including *Bordetella pertussis*, *Brucella suis*, *Bartonella henlae*, as well as *H. pylori*, have also been recognized to express a T4SS. Evolutionary T4SSs are related to bacterial conjugation systems [57]. The *H. pylori* T4SS is encoded by the *cag* PAI within a 37 kb locus inserted in the glutamate racemase gene, which has been acquired by horizontal transfer from an

unknown organism. During evolution, IS605, a mobile sequence encoding two transposases, entered the *H. pylori* genome and interrupted or deleted parts of the *cag* PAI in some strains [22]. The *H. pylori* T4SS is capable of exporting the virulence factor CagA across the membrane into gastric epithelial cells. The VirB-VirD system of *A. tumefaciens* has served as a prototype for T4SSs. The proteins of the T4SS can be grouped according to their functions and/or localisations. The *H. pylori* proteins Hp0524 (VirD4), Hp0525 (VirB11) and Hp0544 (VirB4) belong to the cytoplasm- or inner-membrane-associated ATPases. The T4SS represents a filamentous surface organelle protruding in a needle-like structure, which is either covered focally, or completely, by the Hp0527 (VirB10) protein. Together with Hp0527, the *H. pylori* proteins Hp0528 (VirB9) and Hp0530 (VirB8) form the core complex in the periplasm and/or membrane. The Hp0546 (VirB2) protein establishes the pilus for the physical contact between *H. pylori* and the target cells [58]. The number and distribution of the needle-like structure increases during infection of the epithelial cells [59,60].

TYPE IV SECRETION SYSTEM-DEPENDENT EPITHELIAL CELL SIGNALING

H. pylori triggers *cag* PAI-dependent signaling cascades that lead to the induction of multiple changes in gastric epithelial gene expression and the release of proinflammatory cytokines/chemokines [61]. Nuclear factor kappa B (NF- κ B), an immediate early response transcription factor, is the key regulator of inflammatory processes. NF- κ B becomes induced by physical contact between *H. pylori* and gastric epithelial cells [62]. The activation of NF- κ B and the upregulation of IL-8 and other neutrophil chemotactic C-X-C chemokines such as GRO- α in human gastric epithelial cells are considered critical for the association of the infection with *cag* PAI⁺ strains with neutrophilic responses and more severe gastroduodenal diseases [63]. For the development of neoplasia in gastric diseases provoked by anti-apoptotic survival signals, epithelial NF- κ B activation may be of considerable importance. While the exact molecular mechanism is unknown, it has been shown in a recent study that the I κ B kinase β (IKK β) acts through suppression of the mitochondrial apoptosis pathway by induction of the NF- κ B target gene *Bcl-x_L* [64].

Only *H. pylori* strains containing the *cag* PAI direct signaling cascades in gastric epithelial cells resulting in the activation of the IKK complex [65], C-terminal Jun-kinase (JNK) [66,67], p38 kinase [66,68], and the transcription factors NF- κ B [69-72] as well as activator protein 1 (AP-1) [67]. Activation of JNK involves p21-activated kinase (PAK1), an unknown mitogen activated protein (MAP) kinase kinase kinase (MKKK) and MAP kinase kinase 4 (MKK4) [67]. Activation of the IKK complex (composed of IKK α , IKK β , and the essential modulator IKK γ) involves PAK1 and NF- κ B inducing kinase (NIK) [65]. The IKK complex phosphorylates I κ B molecules leading to their ubiquitylation and degradation and nuclear translocation of NF- κ B [73]. The translocation of the *cag* PAI-encoded CagA protein into gastric epithelial cells via the T4SS is dispensable in these signaling processes [3,4] Fig. (1A).

Recent studies have demonstrated that the peptidoglycan-derived muropeptides (meso-diaminopimelate-containing N-glucosamine-N-acetylmuramic acid tripeptide (GM-tripeptide)) are injected into epithelial cells by the *H. pylori* T4SS. These muropeptides are recognised by the intracellular nucleotide-binding oligomerisation domain protein 1 (NOD1) receptor molecule, which then activates NF- κ B [74]. The complete mechanism leading to NF- κ B activation in response to *H. pylori* is not clear yet. NOD1 belongs to a family of intracellular receptors that has been variously termed e.g. NACHT-LRR proteins (NLRs) [75]. The NLRs NOD1, NOD2 and Nalp3 have been shown to respond to microbial peptidoglycan (PGN) in the cytoplasm of eukaryotic cells [75]. Viala *et al.* [74] analysed an *H. pylori* mutant deficient in lytic transglycosylase activity, which is involved in bacterial muropeptide release. These deficient bacteria induced significantly lower NF- κ B activation in epithelial cells. Interestingly, the lytic transglycosylase was found to be induced in human gastric biopsies [76], suggesting that *H. pylori* generates peptidoglycan degradation products in an active manner. PGN is not recognized by toll-like receptor 2 (TLR2) and this assigns unique sensing specificities to the NLRs [77]. The domain organization of NLRs is characterized by a centrally located NACHT-(domain present in NAIP, CIITA, HET-E, TP-1) domain with homology to the oligomerization module found in AAA+ ATPases [78]. C-terminal to the NACHT domain is a series of leucine-rich repeats (LRRs), likely to be involved in the sensing of the microbial elicitor. Finally, the N-terminus is characterized by effector domains: CARD, PYRIN or BIR [79].

PUTATIVE ROLE OF TOLL-LIKE RECEPTORS IN *H. PYLORI*-INDUCED EPITHELIAL CELL SIGNALING

As *H. pylori* colonises the epithelium, surface-expressed toll-like receptor 4 (TLR4) could be involved in NF- κ B activation. Bäckhed *et al.* [80] did not observe expression of epithelial TLR4, which recognises the lipopolysaccharide (LPS) of Gram-negative bacteria, whilst studying TLR4 mRNA expression in human primary epithelial cells of the gastric antrum in response to *H. pylori* infection. In another report [81], TLR4 mRNA expression was observed in epithelial cells of antral biopsy specimens from *H. pylori* negative patients, but only little TLR4 protein was identified by immunohistochemical staining. TLR4 protein expression was mainly present in lamina propria mononuclear cells. In contrast, expression of TLR4 protein in *H. pylori*-infected patients was detected clearly in both epithelial and infiltrating mononuclear cells. The staining of TLR4 in epithelial cells was located in the cytoplasm as well as at the apical surface. In addition, the mRNA of MD-2, a small secreted protein which is required for TLR4-mediated LPS recognition, was specifically upregulated in the gastric epithelium of *H. pylori* infected patients [81]. A role of TLR4 as a surface receptor for LPS in intestinal epithelia is not clear yet. Data from epithelial cells of the murine small intestine have shown that TLR4 is predominantly localised in the Golgi apparatus while LPS becomes internalised and co-localises with intracellular TLR4 [82].

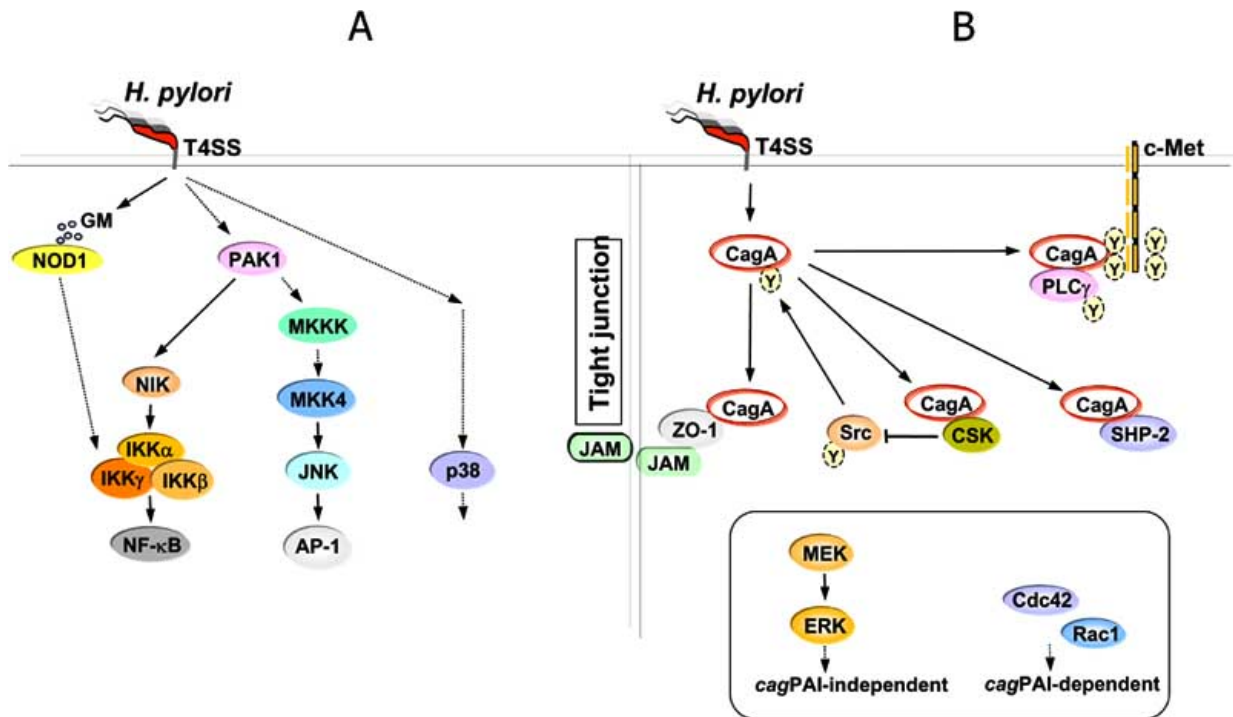


Fig. (1). Type IV secretion system-dependent epithelial signaling.

A. Muropeptides (GM) translocated by the T4SS of *H. pylori* are recognized by the intracellular receptor molecule NOD1, which directs activation of the transcription factor NF- κ B. In addition, *H. pylori*-induces the kinases PAK1, NIK and the IKK complex leading to the phosphorylation of I κ B molecules and nuclear translocation of active NF- κ B. Activation of the transcription factor AP-1 is triggered by PAK1 which activates an unknown MAP 3 kinase (MKKK), MKK4 and JNK. In addition, p38 kinase is strictly induced by *H. pylori* strains carrying a T4SS. The solid arrows indicate direct activation of downstream targets, and the dotted arrows indicate indirect activation through an unknown component.

B. The T4SS of *H. pylori* translocates CagA, an effector protein which becomes tyrosine phosphorylated by Src kinases. CagA may disrupts epithelial tight junction in a process which comprises co-localisation of CagA with JAM molecules and the scaffolding protein ZO-1. Further, CagA directly binds to the cytoplasmic domain of the phosphorylated and active c-Met receptor and enhances the motogenic response (cell scattering). In addition, CagA recruits PLC γ to the c-Met receptor. *H. pylori* *cag* PAI negative strains activate extracellularly the c-Met receptor, but the *cag* PAI is required to induce cell scattering, and CagA is required to enhance cell scattering. The cell scattering involves Cdc42 and Rac1 which are activated in a *cag* PAI-dependent manner as well as the activity of MEK and ERK which are *cag* PAI-independently activated. The phosphatase SHP-2 associates with phosphorylated CagA, and in an autoregulatory loop the interaction between CagA and the kinase CSK stimulates phosphorylation and inactivation of Src kinases leading to less phosphorylation of CagA.

The role of other relevant TLRs (TLR2 recognising lipoproteins and peptidoglycan, TLR5 recognising flagellins) in *H. pylori*-induced NF- κ B activation was studied in human embryonal kidney 293 (HEK293) epithelial cells by overexpressing the TLR receptors [83]. These data are not convincing since TLR2 was supposed to recognise the intact peptidoglycan macromolecule and could only detect Gram-positive peptidoglycan because the outer membrane shields the Gram-negative peptidoglycan [84]. Thus, only the intracellular NODs clearly represent peptidoglycan sensors in epithelial cells and these recognise degradation products, which are hydrolysed by *H. pylori* prior to translocation via the *cag* PAI into epithelial cells. The fact that all *H. pylori* strains are flagellated, but not all are able to induce NF- κ B activation, argues against the idea of a possible function of *H. pylori* flagellin in signaling via TLR5 in gastric epithelial cells. In addition, disruption of *H. pylori* *flaA*, which encodes the major 53-kD Flagellin A, has no effect on *H. pylori*-induced IL-8 secretion from gastric epithelial cells [85].

***H. PYLORI* EFFECTOR MOLECULE CagA**

The *H. pylori* *cag* PAI-encoded T4SS is the major virulence determinant and mediates the translocation of the effector protein CagA (Hp0547) into gastric epithelial cells. The *cagA* gene is localised at one end of the *cag* PAI and a systematic mutagenesis of the PAI revealed that 18 out of 27 genes are essential for CagA translocation into host cells and 14 genes are essential for *cag* PAI-dependent induction of IL-8 [86]. CagA is a 120-145 kDa protein, which once it is injected into gastric epithelial cells by the T4SS CagA, undergoes tyrosine phosphorylation presumably by kinases of the Src-family [87]. The translocated and phosphorylated CagA co-localise with the Hp0532 protein, a protein of the secretion organelle base, indicating that CagA is recruited to the host cell membrane at the site of bacterial attachment [60]. CagA is a major bacterial virulence factor involved in host cell modulation. The presence of a phosphorylation motif in the *H. pylori* translocated effector protein CagA affected the grades of gastric inflammation and atrophy in

patients [88]. After injection CagA becomes tyrosine phosphorylated at the EPIYA motif, a 5-amino-acid sequence (Glu-Pro-Ile-Tyr-Alu) that is present in the carboxyterminal variable region of the molecule [89]. Four EPIYA sites (EPIYA-A, -B, -C and -D) have been described which differ in their flanking sequences. The CagA proteins of *H. pylori* strains which circulate in Europe, North-America and Australia (Western CagA) possess EPIYA-A and B sites followed by 1-3 EPIYA-C sites. CagA proteins from Japan, Korea and China (East-Asian CagA) possess EPIYA-A, -B and D sites [28]. An association between infection with CagA positive *H. pylori* strains and, the risk of peptic ulcer disease, and the development of atrophic gastritis and gastric carcinoma has been described [4,43, 90-96]. The grades of inflammation as well as the activity of gastritis and atrophy were significantly higher in patients infected by *H. pylori* carrying the East-Asian CagA than in patients infected with *H. pylori* carrying Western CagA [88].

CagA directs a variety of cellular activities in gastric epithelial cells inducing multiple effects on host signal transduction. *H. pylori* disrupts the junction-mediated epithelial barrier functions in a *cag* PAI-dependent manner in polarised epithelial Marbin-Darby canine kidney (MDCK) cells. This event involves colocalisation of unphosphorylated CagA with the scaffolding protein ZO-1 and the tight junctional adhesion protein (JAM) Fig. (1B) [97]. Disruption of epithelial tight junctions by translocated CagA probably allows the *H. pylori*-induced activation of epidermal growth factor receptors (EGFR) and Her2/Neu [98-100], which are located on basolateral membranes of the epithelial cells. Fundamental mechanisms like cell growth and differentiation are regulated in response to extracellular stimuli and alterations in cellular signaling can promote the development of various human diseases. Molecular changes which affect the cell-cell contact of epithelial cells are associated with the epithelial-mesenchymal transition (EMT) which occurs during critical phases of development and tumour progression. The hepatocyte growth factor (HGF) activates the c-Met receptor and promotes EMT by stimulation of the dissociation and dispersal of colonies of epithelial cells [101]. T4SS-dependent signaling and the binding of the translocated CagA to the cytoplasmic part of the c-Met receptor are required for cell scattering (motogenic response) [100,102]. *H. pylori* PAI negative strains activate the c-Met receptor, but do not induce cell motility in AGS cells. Cell scattering induced in epithelial cells by *H. pylori* requires actin-reorganisation with ruffle-like structures, which involves the activity of the Rho-GTPases Rac1 and Cdc42. Activation of these GTPases depends on the functional T4SS, but appears to be independent of *H. pylori* CagA protein expression [103]. Furthermore, the epithelial cell scattering needs *H. pylori*-induced extracellular signal regulated kinase 1/2 (ERK1/2) and mitogen-activated protein kinase/extracellular signal regulated kinase 1/2 (MEK1/2) activity. *H. pylori* activates ERK1/2 and MEK1/2 in a *cag* PAI-independent manner [66,104], but the *cag* PAI⁺ strains can induce moderately stronger ERK/MEK phosphorylation than *cag* PAI negative strains [66]. Thus, the modulation of the c-Met receptor signaling by translocated CagA, and PAI-dependent activity of Rho-GTPases contribute to a forceful motogenic response.

The c-Met receptor induces Grb2-associated binder 1 (Gab1) tyrosine phosphorylation and its direct association with several signal transducers including growth factor receptor bound 2 (Grb2), phosphatidylinositol 3-OH kinase (PI3-K), phospholipase C γ (PLC γ) and Src homology 2 (SH2)-domain containing protein tyrosine phosphatase (PTP) called SHP-2 [105]. CagA associates with PLC γ but not with Gab1 [100], or Grb2 [100,106]. CagA interacts with and stimulates the phosphatase SHP-2, which requires tyrosine phosphorylation [107]. In addition to SHP-2 the tyrosine-phosphorylated CagA binds the carboxy-terminal Src kinase (CSK) [108]. CSK is a kinase that negatively regulates Src-family kinases by specific phosphorylation of their inhibitory tyrosine residue. The interaction of CagA with CSK stimulates the kinase and thereby inhibits Src-family kinase activity [28] Fig. (1B). Furthermore, β -catenin translocation seems to be CagA-dependent by an unknown mechanism [109]. Thus, CagA interacts with different signal transducing proteins and might play a role as an adapter protein in growth factor receptor signaling.

H. PYLORI EFFECTOR MOLECULE VacA

Another virulence factor, VacA, is synthesized as a 140 kDa pro-toxin which undergoes cleavage to yield a mature secreted 90 kDa toxin [110]. Further cleavage of the cytotoxin leads to a N-terminal 37 kDa fragment and a 58 kDa fragment, which are non-covalently associated. The 58 kDa B-subunit binds to the tyrosine phosphatase receptor PTP- β on epithelial cells and the induced signaling leads to the phosphorylation of the G protein-coupled receptor kinase-interactor 1 (Git1) and induces ulcerogenesis in mice [111]. The A-subunit of VacA is internalised, which results in vacuolation in epithelial cells [112]. Alignment of the *vacA* nucleotide sequences of *H. pylori* strains has revealed two distinct families namely s1 (s1a; s1b) and s2 with one of two different *vacA* alleles in the middle region (m1 and m2). There is a high level of vacuolation activity of the *vacA* s1/m1 genotype while the *vacA* s1/m2 genotype correlates with low activity [113]. No vacuolisation was observed in strains with the *vacA* s2/m2 genotype. Further, it has been shown that *H. pylori* strains with the s1a genotype are responsible for more severe inflammatory diseases [114]. However, recent studies show both m1 and m2 VacA recognize the tyrosine phosphatase receptor PTP- α and PTP- β (also known as Ptrz) [115], suggesting m-region allelic variation in VacA is not responsible for variation in receptor recognition on epithelial cells. Physiologically VacA can induce apoptosis in epithelial cells via a mitochondria-dependent pathway involving caspase-3 [116]. VacA channel-forming activity is important for cytochrome c release, and this release is inhibited by channel inhibitors [117].

H. PYLORI STIMULATED EGF RECEPTOR TRANSACTIVATION

EGFR transactivation is being increasingly implicated in hyperproliferative diseases and cancer [118]. A variety of known carcinogens such as tobacco smoke [119] and asbestos [120] induce hyperproliferative epithelial responses via the EGFR. It is of considerable interest that the class I carcinogen *H. pylori* similarly transactivates the EGFR in

gastric epithelial cells [98,99]. *H. pylori* EGFR transactivation involves a triple membrane passing signal cascade initially described by Prenzel *et al.* [121]. Activation of the EGFR by *H. pylori* is dependent on extracellular transmembrane metalloproteinase cleavage of pro-heparin binding epidermal growth factor (proHB-EGF) and signaling via mature HB-EGF [99] Fig. (2). The specific G-protein-coupled receptor (GPCR) involved in the inter-receptor cross-talk resulting in EGFR transactivation by *H. pylori* is unknown. EGFR activation by *H. pylori* could also be mediated via NADPH oxidase dependent generation of reactive oxygen species in a similar manner to tobacco smoke induced EGFR transactivation in lung epithelial cells [119]. EGFR transactivation in gastric epithelial cells is stimulated by both *cag* PAI positive and *cag* PAI negative strains [99], consistent with the observations that *H. pylori* activates ERK1/2 and MEK1/2 in a *cag* PAI-independent manner [66,104]. *H. pylori* γ -glutamyltranspeptidase has been identified as a potential upregulator of HB-EGF in gastric epithelial cells [122]. In this latter study, p38 kinase and PI3-K inhibitors, but not MAP kinase kinase inhibitors, blocked *H. pylori* γ -glutamyltranspeptidase-induced HB-EGF upregulation. In contrast, MEK inhibitors completely blocked *H. pylori* induced upregulation of HB-EGF in the study of Wallasch *et al.* [99] indicating *H. pylori* induces an autocrine signaling loop in gastric epithelial cells via the EGFR.

The ADAM (a disintegrin and metalloprotease) family of zinc-dependent metalloproteinases have a critical role in the ectodomain shedding of EGFR ligands [123]. HB-EGF is essential for *H. pylori* induced EGFR transactivation in gastric epithelial cells [99], but the specific ADAM protein involved in HB-EGF cleavage in response to *H. pylori* infection remains to be fully determined. Preliminary reports suggest that ADAM 17 may be important in mediating *H. pylori* induced EGFR transactivation [124]. *H. pylori* upregulates expression of several ADAM genes in gastric epithelial cells, including ADAM 10 and ADAM 17 [125]. *H. pylori*-induced upregulation of ADAM 10 and ADAM 17 is independent of the *cag* PAI [125]. *In vivo* transcripts for ADAM 10 and ADAM 17 are also increased in the gastric mucosa in patients with *H. pylori* infection, but there is no increase in ADAM 15 or ADAM 20 transcripts [61,125]. ADAM 17 has been implicated in HB-EGF shedding following GPCR agonist signaling in some experimental systems [126,127] and ADAM 9 [128], ADAM 10 [129] and ADAM 12 [130] in others. In gastric epithelial cells, in addition to *H. pylori*, other distinct-ligand dependent pathways involving IL-8 [131], prostaglandin E₂ (PGE₂) [132], gastrin (in a paracrine manner) [133], as well as potentially oxidative pathways involving tobacco smoke [119], may also contribute to EGFR transactivation Fig. (2). Interestingly, smoking is also associated with elevated IL-8 in the gastric mucosa of *H. pylori* positive subjects [134]. IL-8 stimulated shedding of EGFR ligands and EGFR transactivation in gastric epithelial cells is mediated by ADAM 10 [131]. Prostaglandin E₂ transactivation of the EGFR is also metalloproteinase dependent [132]. There is increasing evidence that within the same cellular system, different ADAMs metalloproteinases depending on the nature of the stimulus, may be involved in processing of the same, or different EGF ligands, which collectively contribute

to EGF transactivation [118] Fig. (2). EGF ligand specificity may depend on the enzyme specificity of the ADAM metalloproteinase activated by the specific ligand [135].

In gastric tumour tissue expression of ADAM 10, ADAM 15, ADAM 17 and ADAM 20 transcripts is markedly increased relative to both histologically normal and *H. pylori* infected gastric mucosa [125]. The potential physiological role of the ADAMs metalloproteases in promoting autocrine signaling loops and *H. pylori* induced gastric malignancy requires further investigation. Importantly, increases in epithelial ADAM metalloproteinases in *H. pylori* infection are mirrored by increased gastric mucosal levels of epidermal growth factor (EGF) and EGF receptor (EGFR) transcripts [136]. Gastric expression of EGF-related peptides such as heparin-binding EGF (HB-EGF) and amphiregulin [61,137] are also increased in patients with *H. pylori* infection and/or gastric cancer. Overexpression of HB-EGF in human gastric cancer [137] and hypergastrinaemic transgenic mice, which develop gastric cancer [138], suggests EGFR signaling has an important functional role in progression to gastric neoplasia.

THERAPEUTIC POTENTIAL OF SIGNALING INHIBITORS IN *H. PYLORI* INDUCED GASTRIC PATHOLOGY

It is clear from *in vitro* studies that *H. pylori* induces multiple signaling pathways in gastric epithelial cells. The pathogen represents an excellent model system to dissect out the importance of specific signaling pathways in infection-related carcinogenesis. Animal models are being used extensively to dissect the importance of such signaling pathways. Several transgenic murine models develop gastric cancer independently of *H. pylori* infection (e.g. Cox-2/microsomal prostaglandin E synthase over-expressing mice [139], IL-6 receptor subunit gp 130^{757F} mice which have increased STAT3 activity [140], INS-GAS mice which overexpress gastrin [138], as well as mice genetically deficient in Smad4 [141], RUNX3 [142] and Cdx2 [143]. In some transgenic models, such as the INS-GAS mice, gastric *H. felis* infection synergistically accelerates the progression to atrophy and gastric cancer [138], providing a useful tool for testing therapeutic interactions. Both a CCK2 gastrin receptor antagonist, and histamine H₂-receptor antagonist, inhibit *H. felis*-induced cancer in INS-GAS mice, implicating the gastrin-histamine axis in epithelial hyperproliferation and carcinogenesis in this model [144]. The draw back of the murine model is that *cag* PAI positive *H. pylori* strains do not readily colonise mice [145], so the therapeutic potential of inhibiting *cag* PAI related epithelial signaling pathways can not be addressed. In addition, *H. pylori* induces less gastric inflammation and epithelial proliferation than *H. felis* [35].

An alternative model is the Mongolian gerbil where *H. pylori* induces enhanced inflammation relative to mice [35]. Additionally, the pathology of *H. pylori* infection closely mimics that in humans commencing with an antral predominant gastritis, which progresses with time to pan gastritis [33,36,146]. Importantly, *H. pylori* infection induces gastric cancer in the gerbil model [4,109,146,147]. Infection

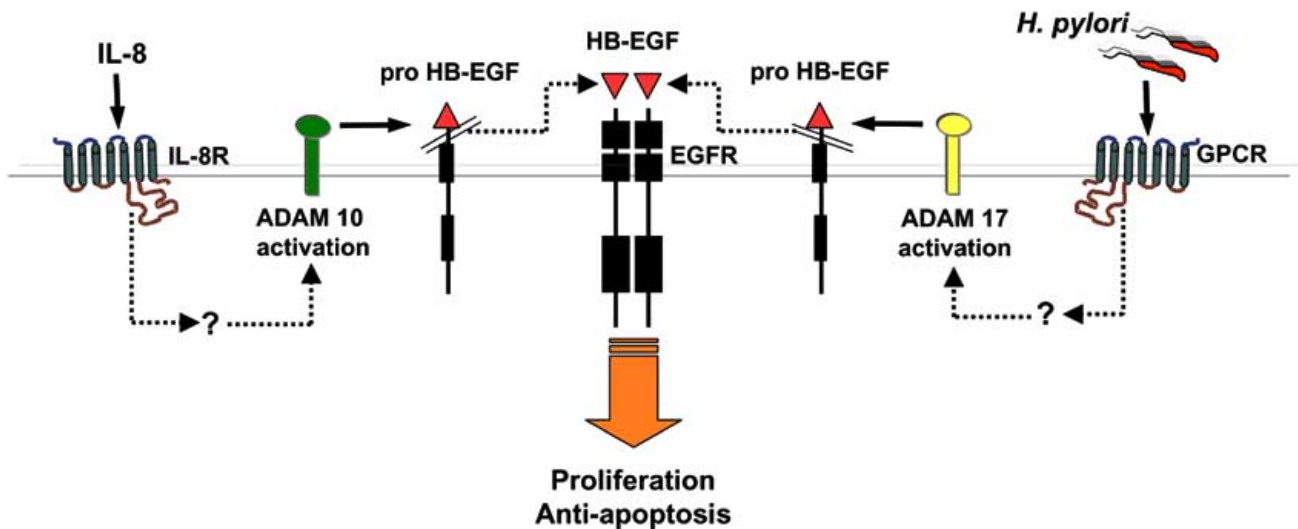


Fig. (2). Stimulus specific transactivation of the EGFR in gastric epithelial cells by *H. pylori* and IL-8.

The EGFR activation by *H. pylori* involves GPCR activity and the “triple membrane passing signal” (TMPS) for EGFR transactivation which is dependent on extracellular transmembrane metalloprotease cleavage of pro-heparin binding epidermal growth factor (proHB-EGF) and signaling by mature HB-EGF as described by Wallasch *et al.* (2002) [99]. IL-8 mediated EGFR transactivation via a TMPS signaling pathway is dependent on ADAM 10 as described by Tanida *et al.* (2004) [131]. ADAM 17 is required for *H. pylori* dependent EGFR transactivation [124].

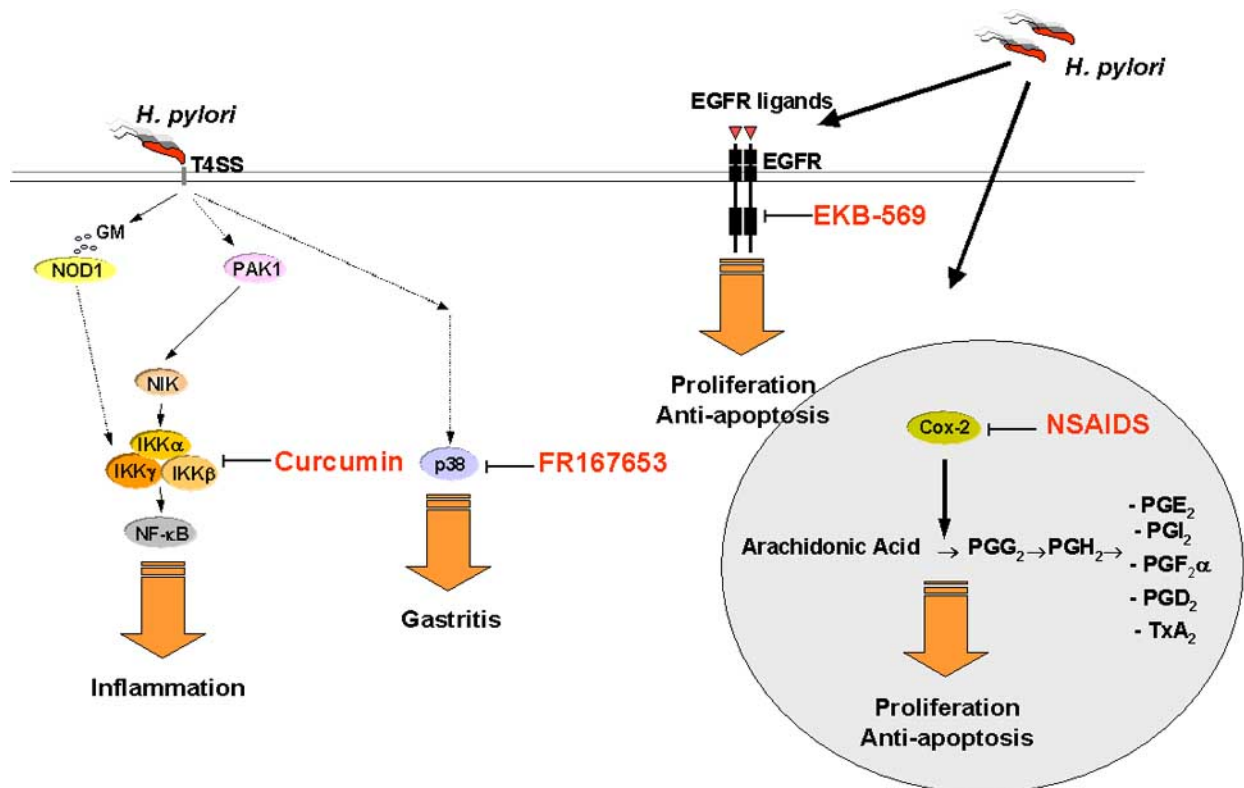


Fig. (3). Potential therapeutic interventions in *H. pylori* epithelial signaling pathways.

In vivo and *in vitro* experiments indicate the potential of several pharmacological factors and dietary components to inhibit *H. pylori*-induced inflammatory and oncogenic signaling pathways. NSAIDs, including selective COX-2 inhibitors (Nimesulide and Etodolac), block *H. pylori* and N-methyl-N-nitrosurea induced cancer in rodent models [152,153]. Selective inhibitors of EGFR (e.g. EKB-569) have chemopreventative potential [158], and *H. pylori* induced acute gastritis is attenuated by p38 mitogen-activated protein kinases inhibitors (e.g. FR167653) [149]. Dietary factors such as curcumin, a component of tumeric, also have block *H. pylori*-induced inflammatory signaling pathways [160].

with *H. pylori* strains with a functional *cag* PAI, and *cag* PAI isogenic mutants with defective signaling responses [34,49, 148], results in clear pathological differences in the gerbil model. This model thus is suitable to examine the therapeutic potential of inhibiting *cag* PAI signaling pathways.

To date there have been few studies evaluating the therapeutic potential of blocking *H. pylori* stimulated signaling pathways. Short term two week administration of FR167653, a p38 kinase-selective kinase inhibitor, to gerbils two weeks post-infection with *H. pylori* inhibited neutrophil infiltration, mucosal injury and the local production of neutrophil chemokines CINC/KC, but not *H. pylori* infection density or acid secretion, suggesting p38 kinase has a role in the induction of gastritis [149] Fig. (3). *H. pylori* infection is associated with increased Cox-2 expression *in vitro* and *in vivo* [150,151]. Magari *et al.* [152] evaluated the effects of a selective cyclooxygenase-2 inhibitor, etodolac, on *H. pylori* and N-methyl-N-nitrosurea induced carcinogenesis in the gerbil. Etodolac dose dependently inhibited epithelial cell proliferation, the development of intestinal metaplasia and cancer, but had no effect on the extent of inflammation. Similar chemopreventative effects on *H. pylori* and N-methyl-N-nitrosurea induced gastric carcinogenesis and increased gastric epithelial apoptosis were observed in mice treated with nimesulide, a selective inhibitor of COX-2 [153]. Whether COX-2 inhibitors prevent *H. pylori* gastric carcinogenesis in the absence of the carcinogen N-methyl-N-nitrosurea remains to be determined. These findings in rodent models are consistent with epidemiological studies indicating that non-steroidal anti-inflammatory drugs (NSAIDs) have a chemopreventative role clinically, reducing the risk of gastric cancer [154]. The chemopreventative mechanisms of NSAIDs in gastric cancer are currently unclear, but limited *in vivo* studies suggest alterations in epithelial proliferation and apoptosis both clinically [31], and in animal models [152,153]. However other recent studies have highlighted the critical role of COX-2/PGE₂ in promotion of FOXP3 expression and T regulatory cell function [155,156]. COX-2 inhibition may thus increase anti-tumour immune responses [156].

EGFR transactivation by *H. pylori* [98,99], and by molecules upregulated by *H. pylori* infection such as gastrin [133], IL-8 [131] and COX-2 derived PGE₂ [132], may have an important role in the initiation of gastric cancer. EGFR tyrosine kinase inhibitors may thus have therapeutic potential as chemopreventive agents in *H. pylori* infection. Oral administration of EKB-569, a potent irreversible specific inhibitor of the EGFR's tyrosine kinase activity [157], reduces intestinal polyp formation in APC^{Min/+} mice [158] and combination therapies, involving EKB-569 and inhibitors of the cyclooxygenase signaling, result in synergistic activity in the prevention of tumour development [158] Fig. (3). As EKB-569 blocks *H. pylori* stimulated ERK1/2 activation in epithelial cells *in vitro* [159], the chemo-preventative potential of EGFR inhibitors on *H. pylori* related disease is being actively explored. Such studies should shed light on the significance of EGFR trans-activation in infection related tumourigenesis.

Given the diversity of epithelial signaling responses induced by *H. pylori* several natural dietary agents may also

have chemopreventive potential. Dietary differences in human populations with a high incidence of *H. pylori* infection may, in addition to bacterial and host genotype, influence clinical outcome. Curcumin, the major component of tumeric powder, with anti-inflammatory properties, which is used extensively in the Indian sub continent, blocks *H. pylori* induced NF- κ B activation and IL-8 synthesis, but not activation of ERK1/2 and p38 [160]. Other dietary factors, which may be relevant are tea polyphenols, such as epigallocatechin-3-gallate (EGCG), which in some systems has been shown to inhibit EGR signaling pathways [161,162]. The impact of dietary factors on *H. pylori* induced epithelial signaling requires further investigation. Dietary factors have the potential to modify *H. pylori* induced epithelial responses and impact on clinical outcome.

CONCLUSIONS/PERSPECTIVES

The log-in of *H. pylori* into the intracellular communication network of the host epithelial cell allows this human microbial pathogen to modulate defined host target molecules (e.g. receptors, kinases etc.). Eradication of *H. pylori* infection with a combination of two antibiotics and a proton pump inhibitor in subjects without premalignant lesions decreases the risk of subsequently developing severe gastric diseases like gastric cancer [163]. *H. pylori* eradication therapy however is complicated by increasing global antibiotic resistance in the pathogen [164] and global eradication of *H. pylori* using standard therapy is not a realistic option. In addition, *H. pylori* infection is often lost in those with severe gastric atrophy and intestinal metaplasia [165], a group with the greatest risk of developing gastric cancer [20]. Understanding the key epithelial signaling pathways initiated by *H. pylori*, and how autocrine signaling loops may be augmented in premalignant conditions such as gastric atrophy, will delineate potential chemopreventative agents to target oncogenic pathways in at risk populations. Based on these studies the discovery of novel therapeutic drugs targeted at signaling pathways has the advantage that the microorganism can hardly develop resistance.

ABBREVIATIONS

ADAM	=	A disintegrin and metalloprotease
AP-1	=	Activator protein 1
BabA2	=	Blood group antigen-binding adhesin A2
CagA	=	Cytotoxin associated antigen
<i>cag</i> PAI	=	<i>cag</i> pathogenicity island
CSK	=	Carboxy-terminal Src kinase
CDK	=	Cyclin-dependent kinase
ECL	=	Enterochromaffin-like cells
EGFR	=	Epidermal growth factor receptor
EMT	=	Epithelial-mesenchymal transition
ERK	=	Extracellular signal regulated kinase
Gab1	=	Grb2-associated binder 1

Git1	= G protein-coupled receptor kinase-interactor 1
GM	= Meso-diaminopimelate-containing N-glucosamine-N-acetylmuramic acid tripeptide
GPCR	= G-protein-coupled receptor
Grb2	= Growth factor receptor bound 2
HB-EGF	= Heparin-binding EGF
HGF	= Hepatocyte growth factor
IKK β	= I κ B kinase β
IL-8	= Interleukin-8
JAM	= Junctional adhesion protein
JNK	= C-terminal Jun-kinase
LPS	= Lipopolysaccharide
LRRs	= Leucine-rich repeats
MALT lymphoma	= Mucosa-associated lymphoid tissue (MALT) lymphoma
MAPK	= Mitogen activated protein kinase
MDCK	= Marbin-Darby canine kidney cells
MEK	= Mitogen-activated protein kinase/extracellular signal regulated kinase
MKK4	= MAP kinase kinase 4
NACHT-domain	= Present in <u>NAIP</u> , <u>CIITA</u> , <u>HET-E</u> , <u>TP-1</u>
NF- κ B	= Nuclear factor kappaB
NIK	= NF- κ B inducing kinase
NLRs	= NACHT-LRR proteins
NOD1	= Oligomerisation domain protein 1
NSAIDS	= Non-steroidal anti-inflammatory drugs
PAK1	= p21-activated kinase 1
PGN	= Peptidoglycan
PI3-K	= Phosphatidylinositol 3-OH kinase
PLC γ	= Phospholipase C γ
proHB-EGF	= Pro-heparin binding epidermal growth factor
PGE ₂	= Prostaglandin E ₂
PTP- β	= Tyrosine phosphatase receptor β
SHP-2	= Src homology 2 (SH2)-domain containing protein tyrosine phosphatase
T4SS	= Type IV secretory system
TLR2	= Toll-like receptor 2
VacA	= Vacuolating cytotoxin A

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