We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

5,300
Open access books available

130,000
International authors and editors

155M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Effect of Helicobacter pylori on Tight Junctions in Gastric Epithelia

Erika Patricia Rendón-Huerta,
Carlos Abraham García-García
and Luis Felipe Montaño Estrada

Abstract

Molecular complexes grouped under the names of tight, adherent or gap junction regulate the flow of water, ions and macromolecules through epithelium paracellular spaces. The main constituents of tight junctions are claudins, a family of 26 different proteins whose expression and distribution are tissue specific but varies in tumors. A change in claudin 1, 3, 4, 5, 6, 7, 9 and 18 expression, that contributes to lose epithelial cohesion, has been associated to enhanced cell proliferation, migration, and invasiveness in gastric neoplastic tissue. Chronic inflammation process induced by *H. pylori* infection, a major risk factor for gastric cancer development, disrupts tight junctions via CagA gene, Cag pathogenicity island, and VacA, but the effect upon the epithelial barrier of *H. pylori* lipopolysaccharides or *H. pylori*-induced up-regulation of mTOR and ERK signaling pathways by microRNA-100 establishes new concepts of proof.

Keywords: gastric epithelia, *H. pylori*, tight junctions, claudins

1. Introduction

Disruption of the epithelium apical-junctional complex is an initial step of the process which allows many bacteria and/or its toxins to permeate across an otherwise tight mucosa. Normally, the most likely target are claudins, a family of 27 different molecules [1], essential for the maintenance of intercellular tight junctions, that viruses and bacteria such as Hepatitis C virus or *Clostridum perfringens* enterotoxin, bind to mediate their entry in hepatocytes or in human ileum epithelial cells [2, 3]. The aim of this review is to recognize the mechanisms that *Helicobacter pylori* uses to disrupt the tight junctions and invade the gastric epithelial mucosa.

2. *Helicobacter pylori*

Helicobacter pylori (H. pylori) is a 3 micrometer long gram-negative spiral bacteria that colonizes the human gastric epithelium's luminal surface of approximately 50% of humans worldwide. Once acquired, it establishes a chronic persistent infection that
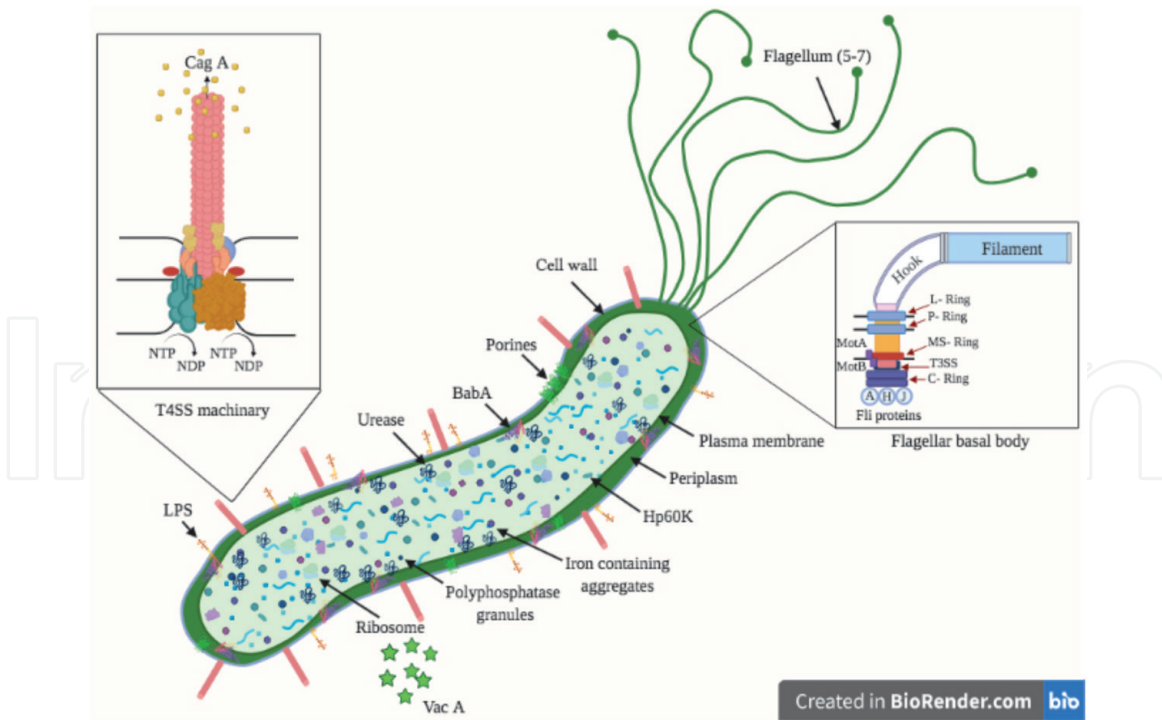
Figure 1.
Helicobacter pylori *components.*

leads to ulcer, cancer or MALT lymphoma [4]. *H. pylori* is conformed by BabA, SabA, OipA and HopQ bacterial colonization factors, and the effector proteins CagA, VacA, urease, catalase, flagellin, mucinase, lipase, neutrophil activating protein, lipopoly-saccharides, Cholesterol-Glucosyltransferase and HtrA considered as virulence/pathogenicity factors, and the outer membrane vesicles [5–9]. **Figure 1** shows the complete structure and components of *H. pylori*. Although it has been clearly established that *H. pylori* disrupts gastric epithelial barrier function [10, 11] the precise mechanism(s) remain elusive. A major structure of *H. pylori* is the syringe-like Type IV secretion system which is found in many species of bacteria [12, 13]; this system plays an essential role in the translocation of CagA into host cells [14].

3. Epithelial barrier

The epithelial barrier is a fence composed by intercellular structures termed tight junctions, located at the apical border between gastric epithelial cells, formed by four different transmembrane proteins [occludin, claudins, junction-adhesion-molecules, and CAR –Coxsackievirus and Adenovirus Receptor- proteins] anchored to actin filaments and myosin light chains (MLC) by the actin cytoskeleton and linker proteins zonula occludens ZO-1, ZO-2 and ZO-3 which are members of the membrane-associated guanylate kinase cytoplasmic adaptors. Other highly important members of the barrier are the Adherens Junctions, the Desmosome, the Gap junctions and the Hemidesmosomes. Occludin and claudins interact with adjacent cells through their extracellular loops, whereas JAMs and CAR contain extracellular IgG-like domains [15, 16]. Different proteins form the regulatory complex (Rac, Cdc42, Par3, Par6, PKC). **Figure 2** shows the structural conformation of tight junctions[1]. Claudins, a family of 27 different proteins, are essential to establish and maintain the barrier function as they regulate paracellular permeability [18] whereas occludin is important for epithelial differentiation but not for establishing

---

[1] A profound review of the gastric epithelal barrier can be found at Tegtmeyer and Backert [17].
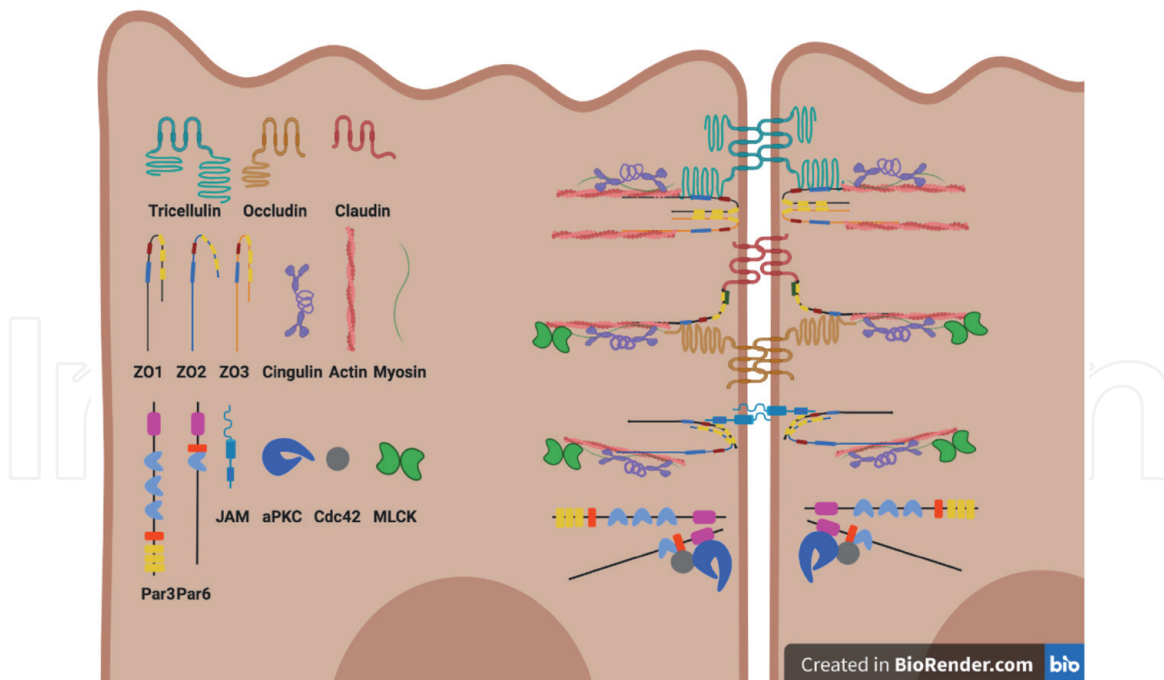
Figure 2.
Gastric epithelia tight junction structure.

the barrier [19]. Paracellular transport across the tight junctions is achieved through the leak pathway which is size-dependent and/or the pore pathway which is size and charge-dependent; size-dependance enables transportation of proteins and lipopolysaccharides and it is controlled by MLC kinase and occludin [20] whereas the pore pathway, controlled by claudins, enables the permeability of cations and anions across different epithelia and exclude molecules larger than 4A [21].

Claudins are responsible for watertight stability and transit of cations and anions. Claudins expression and regulation is tissue specific and their physiological and regulatory function varies according to the organ where they are being expressed [22, 23]. As an example, claudin-4 in ovarian cancer has a pro-angiogenic function whereas in pancreatic cancer it suppresses invasion [24, 25]. The expression of claudins is dysregulated in various cancers, and in gastric tissue the expression of claudin-1, −4, −6 and − 17 is modified when cancer develops but many other claudins such as −3, −5, −7 and − 18 have also been implicated; the loss or gain of claudins is linked to inflammation and inflammatory cytokines such as IFNy, IL-1, IL-6, IL-10, IL-17, IL-22, EGF, TGFb and TNF [26], as well as to several malignancies, drugs, antibiotics, toxins, pesticides, chemicals, microbiota imbalance and stress [27]. The integrity or modifications in tight junctions that affect claudin distribution is via the MAPK/ERK1/2 pathway [28–30]. It has been postulated that in *G. lamblia* infection the loss of epithelial barrier function could be caspase-3 dependent [31] but it does not seem the case in *H. pylori* infection.

The effect of the secretory molecules released by of *H. pylori* known to affect gastric mucosa tight junctions is discussed.

4. VacA

Amongst the major toxins that *H. pylori* possesses, the vacuolating cytotoxin A (VacA) contributes to host-pathogen interactions. After the 140 kDa VacA protein is translated, an active toxin of 88 kDa emerges after cleavage [32]. The toxin is conformed by two domains and three distinct segments: the signal region with
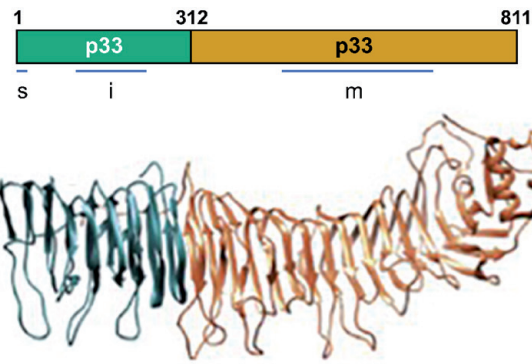
Figure 3.
Organization of VacA p88 protein. From Su et al. [33] and Foegeding et al. [34].

two allelic variations (s1, s2), the intermediate region, and the mid-region with two alleles (m1, m2) (**Figure 3**) [35, 36]; mosaicism has been reported for all the alleles (s1a, s1b, m1a, m1b) [34]. The relevance of these toxin components lays in the fact that s1 causes vacuolation of mammalian cells whereas s2 do not [37]; the discrepancy may be attributed to differences in channel-forming properties [38]. The combination of different VacA alleles is associated with more virulent strains and severe gastric disease: s1a/m2 strains are found in 87.5% of patients with peptic ulcer and in 93% of patients with gastric carcinoma [39]; other highly pathogenic associations include s1a/m1b, s1b/m1b, and s2/m2 [33]. VacA is involved in bacterial colonization of epithelial cells of the gastric mucosa via formation of low conductance membrane pores that are selective for anions over cations [40], and the induction of vacuole formation [41]. These vacuoles, once inside the epithelial cells, alter the transepithelial resistance but do not alter the localization or abundance of ZO-1 and occludin [42]. VacA exert other effects, mainly: endosomal, mitochondrial and epithelial barrier alterations, autophagy, atypical cell signaling and induction of apoptosis in epithelial cells [34]. AGS cells treated with *H. pylori* culture supernatants show rearrangement and disruption of the actin cytoskeleton due to a lack of actin stress fibers; these changes were not VacA dependent [43].

5. CagA

Of major relevance for this review is the effector protein CagA, one of the most important virulence factors [44, 45]. The cytotoxin-associated gene pathogenicity island (cagPAI) comprises 30 genes [46]. The cytotoxin-associated gene A is a 125-140 kDa protein encoded by the cag pathogenicity island [47], a chromosomal region that simultaneously encodes a type IV secretion system specialized in transferring peptidoglycan and CagA to the cytosol of the target cell in an ATP-dependent manner [45, 48]; once translocated, it interacts with numerous proteins in a phosphorylation dependent and independent manner within the epithelial cells, stimulating inflammatory responses, perturbing intracellular actin trafficking, and disrupting cellular tight junctions probably via the ERK1/2 signaling pathway [49–51]. Phosphorylated CagA interacts with Shp2, a host protein that binds to CagA, this complex dephosphorylates the focal adhesion kinase and in turn activates a signal pathway that involves ERK proteins [52, 53]. The transferred peptidoglycan promotes the activation of the pattern-recognition molecule Nod1 within the cytosol of the host cell [54] and subsequently induces the expression of IL-6 and IL-8 as well as MAPK phosphorylation [55–57]. The phosphorylation independent activity of CagA disrupts E-cadherin and ZO-1 and consequently cell-to-cell junctions in polarized epithelial cells [10, 49, 58, 59]. CagA modifies the

polarity of the infected cells by interacting with Par1b/MARK-2 [60, 61]. CagA also stimulates the expression of NfkB, which subsequently activates the IL-8 promoter and stimulates the release of the chemokine IL-8 into the gastric lumen [62], which disrupts epithelial tight junctions organization [63].

CagA is known to affect intercellular junctions and disrupt junction-mediated functions [64] as it causes an ectopic assembly of tight-junction components by recruiting ZO-1 and JAM to sites of bacterial attachment (Amieva 2003), and disrupts the epithelial barrier function [10]. CagA colocalizes with ZO-1 and JAM proteins, binds Par1b and, by inhibiting atypical PKC-mediated phosphorylation of Par1b, disrupts cell polarity and consequently tight junctions. CagA also targets Cdx2 and therefore claudin-2 expression thus suggesting a novel mechanism for gastric epithelial cells dedifferentiation [65]. Another pathophysiological mechanism by which *H. pylori* affect the epithelial barrier is by Rho kinase dependent manner that induces IL-1R type 1 phosphorylation and claudin-4 expression [66].

6. HtrA

One recently recognized mechanism by which CagA disrupts the barrier is mediated by a HtrA (high-temperature requirement A) serine protease [67]. This enzyme is part of a four proteases specific family identified in *E. coli*, *C. jejuni*, *C. coli* and *H. pylori*, all of which enhance adhesion, cellular invasion, and bacterial transmigration via the paracellular route [68]. The HtrA family of proteases contain a chymotrypsin-like protease domain and at least one C-terminal PDZ domain [69].

HtrA are bacterial proteins that provide tolerance to oxidative and heat stress; they undergo oligomerization when denatured proteins are encountered (**Figure 4**) [70]. HtrA can be expressed at the bacterial cell surface, or transported into the extracellular space, or shed in outer membrane vesicles. It favors bacterial paracellular transmigration by cleaving cell-to-cell junction factors such as components of tight junctions that leads to disruption of the epithelial barrier [71]. It has been shown that HtrA1 expression in gastric cancers correlates with better response to cisplatin-based chemotherapy [72].

Although *H. pylori*-infection and –related gastric diseases are clearly associated with downregulation of E-cadherin [73, 74], the mechanism remained elusive. The
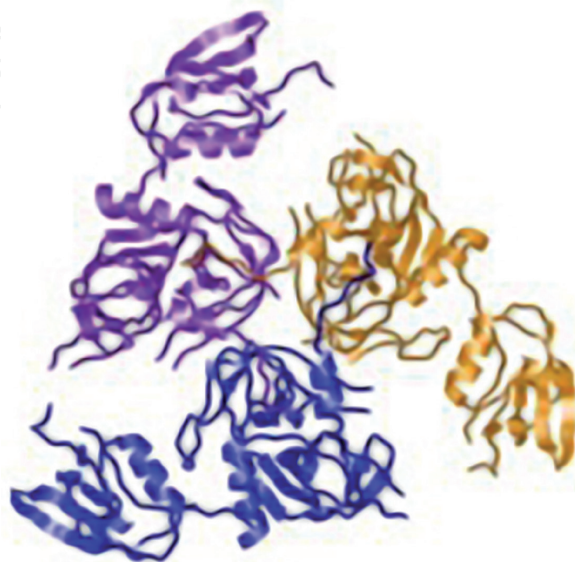

Figure 4.
Tridimensional modeling of H. pylori *trimeric HtrA. From Albrecht et al. [70].*

bacteria disrupts E-cadherin by upregulating the expression of several metallo-protease-1, −3, −7, −9, −10 and ADAM-10 and -15 all of which cleave E-cadherin on the cell surface [6, 68, 75–78]. It has recently been established that HtrA allows access of *H pylori* to the basolateral side of the gastric epithelium through cleavage of the N-terminal fragment domain of E-cadherin [79] apparently affecting occludin expression on the epithelial cell membrane leading to destruction of adherence junctions and downregulation of the barrier function thus facilitating CagA delivery [80–82]. Phosphorylation of MLC by the specific MLC kinase regulates paracellular permeability [83]. It has been shown that certain strains of *H. pylori* induce the rearrangement of claudin-4 and claudin-5 in a MLC Kinase dependent but in a CagA- and VacA-independent manner [84]; the exact mechanism was not determined although ammonium produced by *H. pylori* urease has been implicated [85, 86].

7. Lipopolysaccharide

Gut bacterial lipopolysaccharides (LPS) are known to affect intracellular signaling as well as tight junctions of the blood brain barrier [87] and the intestinal barrier [88]. LPS, an important structural component of bacterial walls´ outer membrane, is recognized by the membrane toll-like receptor 4, and alterations in permeability induced by LPS are via a TLR-4 dependent process associated to the adaptor protein focal adhesion kinase, which has been shown to co-localize with claudin-1 [89], and the activation of the MyD88-dependent pathway [90]. *H pylori* LPS has an agonist function upon TLR-2 and not TLR-4 [91, 92]. We have shown that *H pylori* LPS induces the expression of TLR-2 and that the greater expression of the receptor was accompanied by an initial increase in claudin-4 followed by claudin-6, −7 and − 9; this initial process was STAT3-dependent whereas the expression of claudin-6, −7 and − 9 was ERK1/2-dependent (**Figure 5**) [93]. The same pathway has been reported in claudin-1 downregulation in keratinocytes [94].
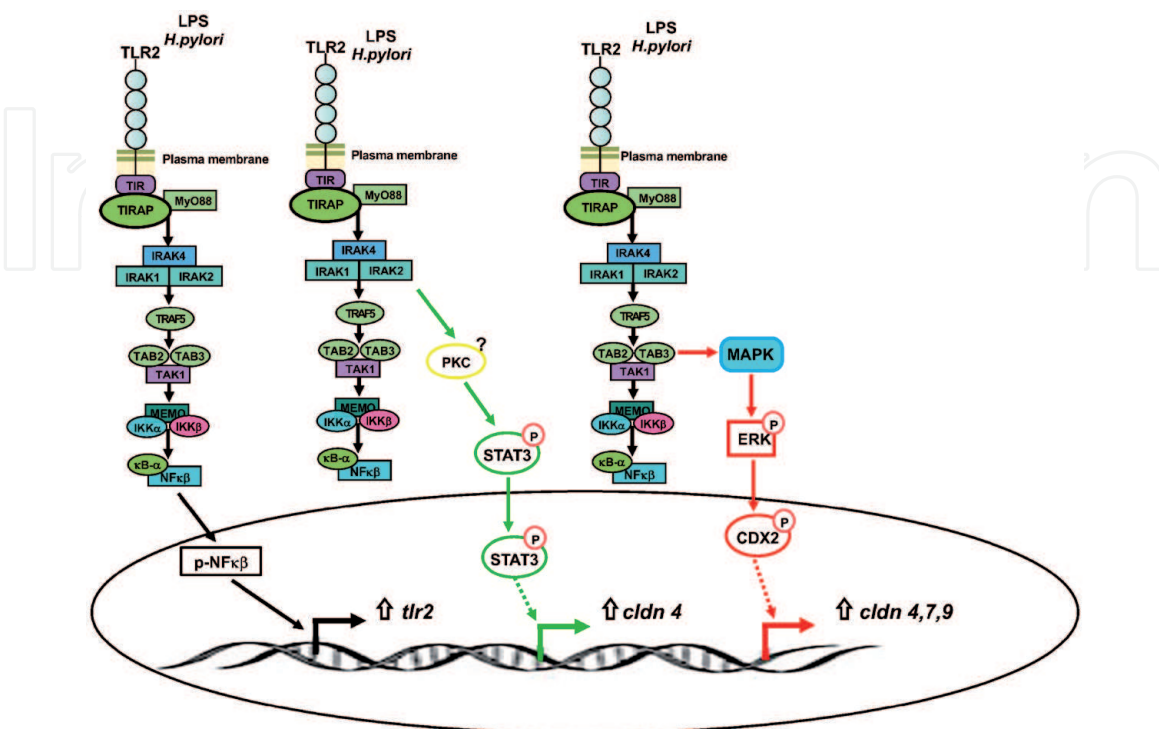

Figure 5.
Effect of H. pylori *LPS on TLR2 activation and claudin expression. From Chavarría-Velázquez et al. [93].*

8. Inflammation

Persistent *H. pylori* infection induces chronic inflammation, pro-inflammatory cytokines IL-1, Il-6, IL-8, TNF and micro RNAs, especially those of the let-7 family [95] that correlates significantly with one or various other pro-inflammatory cytokines [96]. Although it would be interesting to determine the role of pro-inflammatory cytokines in modulating tight junction dysfunction, it is clear that *H. pylori* infection does induce a local inflammatory process by activating nuclear transcription factors NFkB and the chemokine AP-1 [97] where IL-8 enhanced secretion plays an important role [98]. The phosphorylation of the IL-1 receptor after exposure to *H. pylori* reduces the expression of claudin-4 [66]. IL-8 exposure is known to disrupt the organization of epithelial tight junctions leading to "leaky" tight junctions due to a reduced expression of claudin-18 [63].

9. N-nitroso compounds

Exposure to N-nitroso compounds (NOCs) is clearly related to development and increased mortality of gastric cancer (**Figure 6**) [99, 100]. It has been established that nitrogenous constituents of gastric juice can be reduced and lead to the *in situ* formation of N-nitroso compounds [101] although the involvement of *H. pylori* in the development of NOCs and premalignant lesions was controversial until recently [102]. Gastric epithelial cells exposed to N-Nitroso compounds (NOCs) such as MNNG (N-methyl-N-nitro-N-nitrosoguanidine), N-nitrosodimetilamine, N-nitroso-N-ethylurea, or N-nitrosopiperidine through diet (bacon, smoked fish, sausages), high salt consumption, alcoholic beverages, and/or tobacco smoke[2], which also contains NOCs and favors the prevalence of *H. pylori* [103], induce
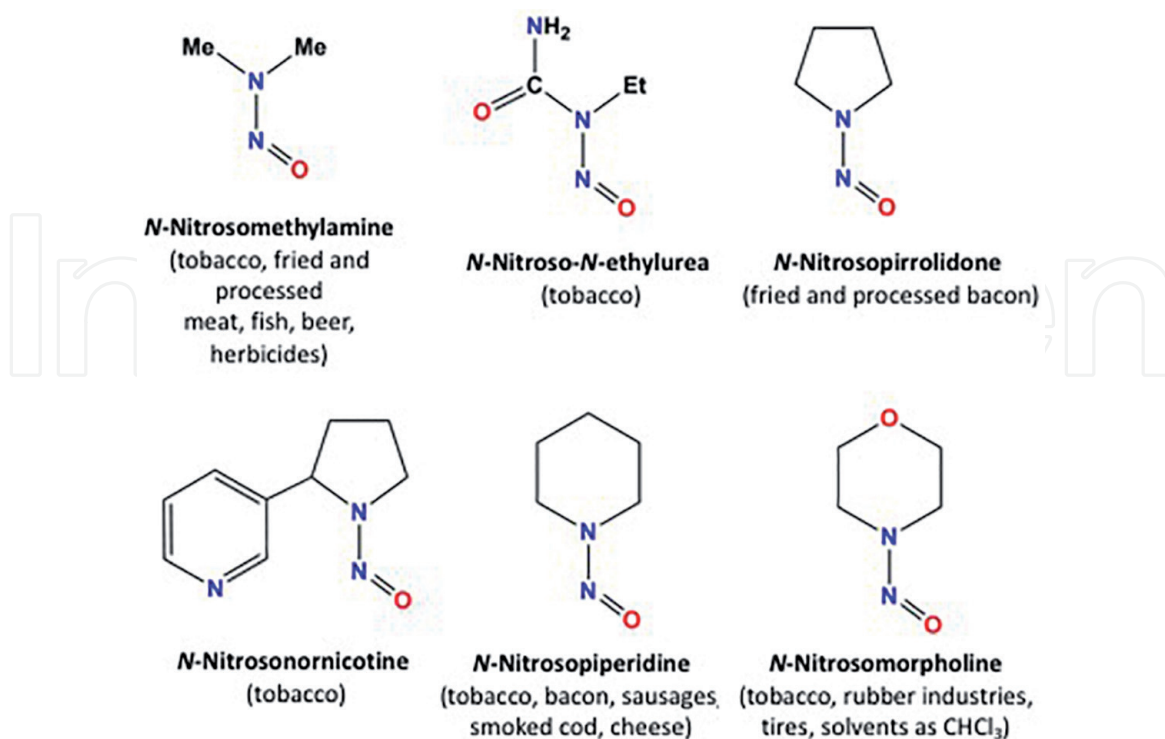
Figure 6.
Structure of relevant N-nitrosamine carcinogenic compounds. From NTP (National Toxicology Program), NIH, USA, 2014.

_____

[2] For a complete list of NOCs compounds go to http://ntp. niehs.nih.gov/pubhealth/ roc/roc13

the expression of epithelial-mesenchymal transition markers in the presence of CagA positive *H. pylori* strains [104] which is mediated by Akt or ERK activation [105], both of which are involved in tight junction assembly [28]. N-etil-N-nitro-N-nitrosoguanidine, a compound that behaves similar to MNNG [106] and induces gastric carcinoma in nonhuman primates [107], synergizes with *H. pylori*, especially CagA+ strains [108] and induces gastric carcinogenesis [109]. Therefore, protagonism of these compounds in individuals with *H, pylori* infection cannot be belittled.

10. Conclusions

Modulation of polarized gastric epithelial cells tight junctions by *H. pylori* involves not only the direct action of some of the most recognized virulence factors of the bacteria that target individual TJ components by different pathways, but also the effect of some *H. pylori*-induced secondary or indirect mechanisms. It is clear that *H. pylori* has developed several mechanisms to endure in an organism and that invasion of the gastric mucosa is just the beginning of the bacteria survival and replicative process where suppression of the immune response is a key component that needs to be continuously explored. Nevertheless, the adhesion and invasion of the gastric mucosa epithelial cells through mechanism that favor the opening of the cell-to-cell tight junction is a bacterial strategy that allows persistent colonization and enhances its ability to cause damage to the host.

Acknowledgements

Author details

Erika Patricia Rendón-Huerta[1*], Carlos Abraham García-García[1,2] and Luis Felipe Montaño Estrada[1]

1 Laboratorio Inmunobiología, Departamento Biología Celular y Tisular, Facultad de Medicina, UNAM, México, Mexico

2 Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edif. D, 1 piso, Circuito de Posgrados, Ciudad Universitaria, México, Mexico

*Address all correspondence to: erendon@unam.mx

IntechOpen

References

[1] Lal-Nag, M. & P. J. Morin (2009) The claudins. Genome Biol, 10, 235.

[2] Veshnyakova, A., J. Protze, J. Rossa, I. E. Blasig, G. Krause & J. Piontek (2010) On the interaction of Clostridium perfringens enterotoxin with claudins. Toxins (Basel), 2, 1336-1356.

[3] Zheng, A., F. Yuan, Y. Li, F. Zhu, P. Hou, J. Li, X. Song, M. Ding & H. Deng (2007) Claudin-6 and claudin-9 function as additional coreceptors for hepatitis C virus. J Virol, 81, 12465-12471.

[4] Ernst, P. B. & B. D. Gold (2000) The disease spectrum of Helicobacter pylori: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol, 54, 615-640.

[5] Ansari, S. & Y. Yamaoka (2019) Virulence Factors Exploiting Gastric Colonization and its Pathogenicity. Toxins (Basel), 11.

[6] Costa, A. M., R. M. Ferreira, I. Pinto-Ribeiro, I. S. Sougleri, M. J. Oliveira, L. Carreto, M. A. Santos, D. N. Sgouras, F. Carneiro, M. Leite & C. Figueiredo (2016) Helicobacter pylori Activates Matrix Metalloproteinase 10 in Gastric Epithelial Cells via EGFR and ERK-mediated Pathways. J Infect Dis, 213, 1767-1776.

[7] Mobley, H. L. T., G. L. Mendz & S. L. Hazell. 2001. : Physiology and Genetics.

[8] Odenbreit, S., J. Püls, B. Sedlmaier, E. Gerland, W. Fischer & R. Haas (2000) Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science, 287, 1497-1500.

[9] Sgouras, D., N. Tegtmeyer & S. Wessler (2019) Activity and Functional Importance of Helicobacter pylori Virulence Factors. Adv Exp Med Biol, 1149, 35-56.

[10] Amieva, M. R., R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson & S. Falkow (2003) Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. Science, 300, 1430-1434.

[11] Terrés, A. M., J. M. Pajares, A. M. Hopkins, A. Murphy, A. Moran, A. W. Baird & D. Kelleher (1998a) Helicobacter pylori disrupts epithelial barrier function in a process inhibited by protein kinase C activators. Infect Immun, 66, 2943-2950.

[12] Chung, J. M., M. J. Sheedlo, A. M. Campbell, N. Sawhney, A. E. Frick-Cheng, D. B. Lacy, T. L. Cover & M. D. Ohi (2019) Structure of the. Elife, 8.

[13] Grohmann, E., P. J. Christie, G. Waksman & S. Backert (2018) Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol, 107, 455-471.

[14] Backert, S., R. Haas, M. Gerhard & M. Naumann (2017) The Helicobacter pylori Type IV Secretion System Encoded by the cag Pathogenicity Island: Architecture, Function, and Signaling. Curr Top Microbiol Immunol, 413, 187-220.

[15] Guttman, J. A. & B. B. Finlay (2009) Tight junctions as targets of infectious agents. Biochim Biophys Acta, 1788, 832-841.

[16] Krause, G., L. Winkler, S. L. Mueller, R. F. Haseloff, J. Piontek & I. E. Blasig (2008) Structure and function of claudins. Biochim Biophys Acta, 1778, 631-645.

[17] N. Tegtmeyer and S. Backert (eds), *Molecular Pathogenesis and Signal Transduction by Helicobacter pylor*i, Current Topics in Microbiology and Immunology 400, Springer International Publishing AG, 2017. pp.: 195-226

[18] Krause, G., J. Protze & J. Piontek (2015) Assembly and function of claudins: Structure-function relationships based on homology models and crystal structures. Semin Cell Dev Biol, 42, 3-12.

[19] Schulzke, J. D., A. H. Gitter, J. Mankertz, S. Spiegel, U. Seidler, S. Amasheh, M. Saitou, S. Tsukita & M. Fromm (2005) Epithelial transport and barrier function in occludin-deficient mice. Biochim Biophys Acta, 1669, 34-42.

[20] Shen, L., C. R. Weber, D. R. Raleigh, D. Yu & J. R. Turner (2011) Tight junction pore and leak pathways: a dynamic duo. Annu Rev Physiol, 73, 283-309.

[21] Amasheh, S., N. Meiri, A. H. Gitter, T. Schöneberg, J. Mankertz, J. D. Schulzke & M. Fromm (2002) Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. J Cell Sci, 115, 4969-4976.

[22] Capaldo, C. T. & A. Nusrat (2015) Claudin switching: Physiological plasticity of the Tight Junction. Semin Cell Dev Biol, 42, 22-29.

[23] Van Itallie, C. M. & J. M. Anderson (2014) Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol, 36, 157-165.

[24] Li, J., S. Chigurupati, R. Agarwal, M. R. Mughal, M. P. Mattson, K. G. Becker, W. H. Wood, Y. Zhang & P. J. Morin (2009) Possible angiogenic roles for claudin-4 in ovarian cancer. Cancer Biol Ther, 8, 1806-1814.

[25] Michl, P., C. Barth, M. Buchholz, M. M. Lerch, M. Rolke, K. H. Holzmann, A. Menke, H. Fensterer, K. Giehl, M. Löhr, G. Leder, T. Iwamura, G. Adler & T. M. Gress (2003) Claudin-4 expression decreases invasiveness and metastatic potential of pancreatic cancer. Cancer Res, 63, 6265-6271.

[26] Han, X., E. Zhang, Y. Shi, B. Song, H. Du & Z. Cao (2019) Biomaterial-tight junction interaction and potential impacts. J Mater Chem B, 7, 6310-6320.

[27] Bhat, A. A., S. Uppada, I. W. Achkar, S. Hashem, S. K. Yadav, M. Shanmugakonar, H. A. Al-Naemi, M. Haris & S. Uddin (2018) Tight Junction Proteins and Signaling Pathways in Cancer and Inflammation: A Functional Crosstalk. Front Physiol, 9, 1942.

[28] Aggarwal, S., T. Suzuki, W. L. Taylor, A. Bhargava & R. K. Rao (2011) Contrasting effects of ERK on tight junction integrity in differentiated and under-differentiated Caco-2 cell monolayers. Biochem J, 433, 51-63.

[29] Dörfel, M. J. & O. Huber (2012) Modulation of tight junction structure and function by kinases and phosphatases targeting occludin. J Biomed Biotechnol, 2012, 807356.

[30] Kim, B. & S. Breton (2016) The MAPK/ERK-Signaling Pathway Regulates the Expression and Distribution of Tight Junction Proteins in the Mouse Proximal Epididymis. Biol Reprod, 94, 22.

[31] Chin, A. C., D. A. Teoh, K. G. Scott, J. B. Meddings, W. K. Macnaughton & A. G. Buret (2002) Strain-dependent induction of enterocyte apoptosis by Giardia lamblia disrupts epithelial barrier function in a caspase-3-dependent manner. Infect Immun, 70, 3673-3680.

[32] Telford, J. L., P. Ghiara, M. Dell'Orco, M. Comanducci, D. Burroni, M. Bugnoli, M. F. Tecce, S. Censini, A. Covacci & Z. Xiang (1994) Gene structure of the Helicobacter pylori cytotoxin and evidence of its key role in gastric disease. J Exp Med, 179, 1653-1658.

[33] Su, M., A. L. Erwin, A. M. Campbell, T. M. Pyburn, L. E. Salay, J. L. Hanks, D. B. Lacy, D. L. Akey, T. L. Cover & M. D. Ohi (2019) Cryo-EM Analysis Reveals Structural Basis of Helicobacter pylori VacA Toxin Oligomerization. J Mol Biol, 431, 1956-1965.

[34] Foegeding, N. J., R. R. Caston, M. S. McClain, M. D. Ohi & T. L. Cover (2016) An Overview of Helicobacter pylori VacA Toxin Biology. Toxins (Basel), 8.

[35] Atherton, J. C., P. Cao, R. M. Peek, M. K. Tummuru, M. J. Blaser & T. L. Cover (1995) Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem*, 270, 17771-17777.

[36] Go, M. F., L. Cissell & D. Y. Graham (1998) Failure to confirm association of vac A gene mosaicism with duodenal ulcer disease. Scand J Gastroenterol, 33, 132-136.

[37] McClain, M. S., P. Cao, H. Iwamoto, A. D. Vinion-Dubiel, G. Szabo, Z. Shao & T. L. Cover (2001) A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and alters membrane channel formation. J Bacteriol, 183, 6499-6508.

[38] Tombola, F., C. Pagliaccia, S. Campello, J. L. Telford, C. Montecucco, E. Papini & M. Zoratti (2001) How the loop and middle regions influence the properties of Helicobacter pylori VacA channels. Biophys J, 81, 3204-3215.

[39] Chen, X. J., J. Yan & Y. F. Shen (2005) Dominant cagA/vacA genotypes and coinfection frequency of H. pylori in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes,

coinfection and severity of the diseases. Chin Med J (Engl), 118, 460-467.

[40] Iwamoto, H., D. M. Czajkowsky, T. L. Cover, G. Szabo & Z. Shao (1999) VacA from Helicobacter pylori: a hexameric chloride channel. FEBS Lett, 450, 101-104.

[41] Junaid, M., A. K. Linn, M. B. Javadi, S. Al-Gubare, N. Ali & G. Katzenmeier (2016) Vacuolating cytotoxin A (VacA) - A multi-talented pore-forming toxin from Helicobacter pylori. Toxicon, 118, 27-35.

[42] Papini, E., B. Satin, N. Norais, M. de Bernard, J. L. Telford, R. Rappuoli & C. Montecucco (1998) Selective increase of the permeability of polarized epithelial cell monolayers by Helicobacter pylori vacuolating toxin. J Clin Invest, 102, 813-820.

[43] Bebb, J. R., D. P. Letley, J. L. Rhead & J. C. Atherton (2003) Helicobacter pylori supernatants cause epithelial cytoskeletal disruption that is bacterial strain and epithelial cell line dependent but not toxin VacA dependent. Infect Immun, 71, 3623-3627.

[44] Backert, S., N. Tegtmeyer & M. Selbach (2010) The versatility of Helicobacter pylori CagA effector protein functions: The master key hypothesis. Helicobacter, 15, 163-176.

[45] Noto, J. M. & R. M. Peek (2012) The Helicobacter pylori cag Pathogenicity Island. Methods Mol Biol, 921, 41-50.

[46] Essawi, T., W. Hammoudeh, I. Sabri, W. Sweidan & M. A. Farraj (2013) Determination of Helicobacter pylori Virulence Genes in Gastric Biopsies by PCR. ISRN Gastroenterol, 2013, 606258.

[47] Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang & N. Figura (1993)

Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. Proc Natl Acad Sci U S A, 90, 5791-5795.

[48] Backert, S. & N. Tegtmeyer (2012) Helicobacter pylori CagA tertiary structure reveals functional insights. Cell Host Microbe, 12, 3-5.

[49] Tohidpour, A. (2016) CagA-mediated pathogenesis of Helicobacter pylori. Microb Pathog, 93, 44-55.

[50] Wang, H. P., Y. L. Zhu & W. Shao (2013) Role of Helicobacter pylori virulence factor cytotoxin-associated gene A in gastric mucosa-associated lymphoid tissue lymphoma. World J Gastroenterol, 19, 8219-8226.

[51] Zhu, Y., C. Wang, J. Huang, Z. Ge, Q. Dong, X. Zhong, Y. Su & S. Zheng (2007) The Helicobacter pylori virulence factor CagA promotes Erk1/2-mediated Bad phosphorylation in lymphocytes: a mechanism of CagA-inhibited lymphocyte apoptosis. Cell Microbiol, 9, 952-961.

[52] Higashi, H., A. Nakaya, R. Tsutsumi, K. Yokoyama, Y. Fujii, S. Ishikawa, M. Higuchi, A. Takahashi, Y. Kurashima, Y. Teishikata, S. Tanaka, T. Azuma & M. Hatakeyama (2004) Helicobacter pylori CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. J Biol Chem, 279, 17205-17216.

[53] Higashi, H., R. Tsutsumi, S. Muto, T. Sugiyama, T. Azuma, M. Asaka & M. Hatakeyama (2002) SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. Science, 295, 683-6.

[54] Viala, J., C. Chaput, I. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. Mémet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott & R. L. Ferrero (2004) Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. Nat Immunol, 5, 1166-1174.

[55] Allison, C. C., T. A. Kufer, E. Kremmer, M. Kaparakis & R. L. Ferrero (2009) Helicobacter pylori induces MAPK phosphorylation and AP-1 activation via a NOD1-dependent mechanism. J Immunol, 183, 8099-8109.

[56] Guo, M., F. Wu, Z. Zhang, G. Hao, R. Li, N. Li, Y. Shang, L. Wei & T. Chai (2017) Characterization of Rabbit Nucleotide-Binding Oligomerization Domain 1 (NOD1) and the Role of NOD1 Signaling Pathway during Bacterial Infection. Front Immunol, 8, 1278.

[57] Gunawardhana, N., S. Jang, Y. H. Choi, Y. A. Hong, Y. E. Jeon, A. Kim, H. Su, J. H. Kim, Y. J. Yoo, D. S. Merrell, J. Kim & J. H. Cha (2017) -Induced HB-EGF Upregulates Gastrin Expression via the EGF Receptor, C-Raf, Mek1, and Erk2 in the MAPK Pathway. Front Cell Infect Microbiol, 7, 541.

[58] Bagnoli, F., L. Buti, L. Tompkins, A. Covacci & M. R. Amieva (2005) Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proc Natl Acad Sci U S A, 102, 16339-16344.

[59] Murata-Kamiya, N., Y. Kurashima, Y. Teishikata, Y. Yamahashi, Y. Saito, H. Higashi, H. Aburatani, T. Akiyama, R. M. Peek, T. Azuma & M. Hatakeyama (2007) Helicobacter pylori CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene, 26, 4617-4626.

[60] Lu, H. S., Y. Saito, M. Umeda, N. Murata-Kamiya, H. M. Zhang, H. Higashi & M. Hatakeyama (2008) Structural and functional diversity in

the PAR1b/MARK2-binding region of Helicobacter pylori CagA. Cancer Sci, 99, 2004-2011.

[61] Nesić, D., M. C. Miller, Z. T. Quinkert, M. Stein, B. T. Chait & C. E. Stebbins (2010) Helicobacter pylori CagA inhibits PAR1-MARK family kinases by mimicking host substrates. Nat Struct Mol Biol, 17, 130-132.

[62] Brandt, S., T. Kwok, R. Hartig, W. König & S. Backert (2005) NF-kappaB activation and potentiation of proinflammatory responses by the Helicobacter pylori CagA protein. Proc Natl Acad Sci U S A, 102, 9300-9305.

[63] Reynolds, C. J., K. Quigley, X. Cheng, A. Suresh, S. Tahir, F. Ahmed-Jushuf, K. Nawab, K. Choy, S. A. Walker, S. A. Mathie, M. Sim, J. Stowell, J. Manji, T. Pollard, D. M. Altmann & R. J. Boyton (2018) Lung Defense through IL-8 Carries a Cost of Chronic Lung Remodeling and Impaired Function. Am J Respir Cell Mol Biol, 59, 557-571.

[64] Hazell, S. L., A. Lee, L. Brady & W. Hennessy (1986) Campylobacter pyloridis and gastritis: association with intercellular spaces and adaptation to an environment of mucus as important factors in colonization of the gastric epithelium. J Infect Dis, 153, 658-663.

[65] Song, X., H. X. Chen, X. Y. Wang, X. Y. Deng, Y. X. Xi, Q. He, T. L. Peng, J. Chen, W. Chen, B. C. Wong & M. H. Chen (2013) H. pylori-encoded CagA disrupts tight junctions and induces invasiveness of AGS gastric carcinoma cells via Cdx2-dependent targeting of Claudin-2. Cell Immunol, 286, 22-30.

[66] Lapointe, T. K., P. M. O'Connor, N. L. Jones, D. Menard & A. G. Buret (2010) Interleukin-1 receptor phosphorylation activates Rho kinase to disrupt human gastric tight junctional claudin-4 during Helicobacter pylori infection. Cell Microbiol, 12, 692-703.

[67] Boehm, M., D. Simson, U. Escher, A. M. Schmidt, S. Bereswill, N. Tegtmeyer, S. Backert & M. M. Heimesaat (2018) Function of Serine Protease HtrA in the Lifecycle of the Foodborne Pathogen. Eur J Microbiol Immunol (Bp), 8, 70-77.

[68] Hoy, B., T. Geppert, M. Boehm, F. Reisen, P. Plattner, G. Gadermaier, N. Sewald, F. Ferreira, P. Briza, G. Schneider, S. Backert & S. Wessler (2012) Distinct roles of secreted HtrA proteases from gram-negative pathogens in cleaving the junctional protein and tumor suppressor E-cadherin. J Biol Chem, 287, 10115-10120.

[69] Pallen, M. J. & B. W. Wren (1997) The HtrA family of serine proteases. Mol Microbiol, 26, 209-221.

[70] Albrecht, N., N. Tegtmeyer, H. Sticht, J. Skórko-Glonek & S. Backert (2018) Amino-Terminal Processing of. Front Microbiol, 9, 642.

[71] Hoy, B., M. Löwer, C. Weydig, G. Carra, N. Tegtmeyer, T. Geppert, P. Schröder, N. Sewald, S. Backert, G. Schneider & S. Wessler (2010) Helicobacter pylori HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion. EMBO Rep, 11, 798-804.

[72] Chien, J., G. Aletti, A. Baldi, V. Catalano, P. Muretto, G. L. Keeney, K. R. Kalli, J. Staub, M. Ehrmann, W. A. Cliby, Y. K. Lee, K. C. Bible, L. C. Hartmann, S. H. Kaufmann & V. Shridhar (2006) Serine protease HtrA1 modulates chemotherapy-induced cytotoxicity. J Clin Invest, 116, 1994-2004.

[73] Terrés, A. M., J. M. Pajares, D. O'Toole, S. Ahern & D. Kelleher (1998b) H pylori infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control. J Clin Pathol, 51, 410-412.

[74] Zhang, Y., D. Li, Y. Dai, R. Li, Y. Gao & L. Hu (2019) The Role of E-cadherin

in Helicobacter pylori-Related Gastric Diseases. Curr Drug Metab, 20, 23-28.

[75] Kundu, P., A. K. Mukhopadhyay, R. Patra, A. Banerjee, D. E. Berg & S. Swarnakar (2006) Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by Helicobacter pylori infection. J Biol Chem, 281, 34651-34662.

[76] Pillinger, M. H., N. Marjanovic, S. Y. Kim, Y. C. Lee, J. U. Scher, J. Roper, A. M. Abeles, P. I. Izmirly, M. Axelrod, M. Y. Pillinger, S. Tolani, V. Dinsell, S. B. Abramson & M. J. Blaser (2007) Helicobacter pylori stimulates gastric epithelial cell MMP-1 secretion via CagA-dependent and -independent ERK activation. J Biol Chem, 282, 18722-18731.

[77] Sougleri, I. S., K. S. Papadakos, M. P. Zadik, M. Mavri-Vavagianni, A. F. Mentis & D. N. Sgouras (2016) Helicobacter pylori CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA- phosphorylation-dependent manner. FEBS J, 283, 206-220.

[78] Yin, Y., A. M. Grabowska, P. A. Clarke, E. Whelband, K. Robinson, R. H. Argent, A. Tobias, R. Kumari, J. C. Atherton & S. A. Watson (2010) Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7. Gut, 59, 1037-1045.

[79] Schmidt, T. P., C. Goetz, M. Huemer, G. Schneider & S. Wessler (2016) Calcium binding protects E-cadherin from cleavage by Helicobacter pylori HtrA. Gut Pathog, 8, 29.

[80] Boehm, M., B. Hoy, M. Rohde, N. Tegtmeyer, K. T. Bæk, O. A. Oyarzabal, L. Brøndsted, S. Wessler & S. Backert (2012) Rapid paracellular transmigration of Campylobacter jejuni across polarized epithelial cells without affecting TER: role of proteolytic-active HtrA cleaving E-cadherin but not fibronectin. Gut Pathog, 4, 3.

[81] Tegtmeyer, N., Y. Moodley, Y. Yamaoka, S. R. Pernitzsch, V. Schmidt, F. R. Traverso, T. P. Schmidt, R. Rad, K. G. Yeoh, H. Bow, J. Torres, M. Gerhard, G. Schneider, S. Wessler & S. Backert (2016) Characterisation of worldwide Helicobacter pylori strains reveals genetic conservation and essentiality of serine protease HtrA. Mol Microbiol, 99, 925-944.

[82] Waskito, L. A., N. R. Salama & Y. Yamaoka (2018) Pathogenesis of Helicobacter pylori infection. *Helicobacter,* 23 Suppl 1, e12516.

[83] Turner, J. R., B. K. Rill, S. L. Carlson, D. Carnes, R. Kerner, R. J. Mrsny & J. L. Madara (1997) Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation. Am J Physiol, 273, C1378-C1385.

[84] Fedwick, J. P., T. K. Lapointe, J. B. Meddings, P. M. Sherman & A. G. Buret (2005) Helicobacter pylori activates myosin light-chain kinase to disrupt claudin-4 and claudin-5 and increase epithelial permeability. Infect Immun, 73, 7844-7852.

[85] Lytton, S. D., W. Fischer, W. Nagel, R. Haas & F. X. Beck (2005) Production of ammonium by Helicobacter pylori mediates occludin processing and disruption of tight junctions in Caco-2 cells. Microbiology (Reading), 151, 3267-3276.

[86] Wroblewski, L. E., L. Shen, S. Ogden, J. Romero-Gallo, L. A. Lapierre, D. A. Israel, J. R. Turner & R. M. Peek (2009) Helicobacter pylori dysregulation of gastric epithelial tight junctions by urease-mediated myosin

II activation. Gastroenterology, 136, 236-246.

[87] Feng, S., L. Zou, H. Wang, R. He, K. Liu & H. Zhu (2018) RhoA/ROCK-2 Pathway Inhibition and Tight Junction Protein Upregulation by Catalpol Suppresses Lipopolysaccaride-Induced Disruption of Blood-Brain Barrier Permeability. Molecules, 23.

[88] Ghosh, S. S., H. He, J. Wang, T. W. Gehr & S. Ghosh (2018) Curcumin-mediated regulation of intestinal barrier function: The mechanism underlying its beneficial effects. Tissue Barriers, 6, e1425085.

[89] Ma, Y., S. Semba, R. I. Khan, H. Bochimoto, T. Watanabe, M. Fujiya, Y. Kohgo, Y. Liu & T. Taniguchi (2013) Focal adhesion kinase regulates intestinal epithelial barrier function via redistribution of tight junction. Biochim Biophys Acta, 1832, 151-159.

[90] Guo, S., M. Nighot, R. Al-Sadi, T. Alhmoud, P. Nighot & T. Y. Ma (2015) Lipopolysaccharide Regulation of Intestinal Tight Junction Permeability Is Mediated by TLR4 Signal Transduction Pathway Activation of FAK and MyD88. J Immunol, 195, 4999-5010.

[91] Nagashima, H., S. Iwatani, M. Cruz, J. A. Jiménez Abreu, T. Uchida, V. Mahachai, R. K. Vilaichone, D. Y. Graham & Y. Yamaoka (2015) Toll-like Receptor 10 in Helicobacter pylori Infection. J Infect Dis, 212, 1666-1676.

[92] Smith, M. F., A. Mitchell, G. Li, S. Ding, A. M. Fitzmaurice, K. Ryan, S. Crowe & J. B. Goldberg (2003) Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. J Biol Chem, 278, 32552-32560.

[93] Chavarría-Velázquez, C. O., A. C. Torres-Martínez, L. F. Montaño & E. P. Rendón-Huerta (2018) TLR2 activation induced by H. pylori LPS promotes the differential expression of claudin-4, −6, −7 and −9 via either STAT3 and ERK1/2 in AGS cells. Immunobiology, 223, 38-48.

[94] Ryu, W. I., H. Lee, H. C. Bae, J. Jeon, H. J. Ryu, J. Kim, J. H. Kim, J. W. Son, Y. Imai, K. Yamanishi, S. H. Jeong & S. W. Son (2018) IL-33 down-regulates CLDN1 expression through the ERK/STAT3 pathway in keratinocytes. J Dermatol Sci, 90, 313-322.

[95] Matsushima, K., H. Isomoto, N. Inoue, T. Nakayama, T. Hayashi, M. Nakayama, K. Nakao, T. Hirayama & S. Kohno (2011) MicroRNA signatures in Helicobacter pylori-infected gastric mucosa. Int J Cancer, 128, 361-370.

[96] Isomoto, H., K. Matsushima, N. Inoue, T. Hayashi, T. Nakayama, M. Kunizaki, S. Hidaka, M. Nakayama, J. Hisatsune, M. Nakashima, T. Nagayasu, K. Nakao & T. Hirayama (2012) Interweaving microRNAs and proinflammatory cytokines in gastric mucosa with reference to H. pylori infection. J Clin Immunol, 32, 290-299.

[97] Backert, S. & M. Naumann (2010) What a disorder: proinflammatory signaling pathways induced by Helicobacter pylori. Trends Microbiol, 18, 479-486.

[98] Fiorentino, M., H. Ding, T. G. Blanchard, S. J. Czinn, M. B. Sztein & A. Fasano (2013) Helicobacter pylori-induced disruption of monolayer permeability and proinflammatory cytokine secretion in polarized human gastric epithelial cells. Infect Immun, 81, 876-883.

[99] Hidajat, M., D. M. McElvenny, P. Ritchie, A. Darnton, W. Mueller, M. van Tongeren, R. M. Agius, J. W. Cherrie & F. de Vocht (2019) Lifetime exposure to rubber dusts, fumes and N-nitrosamines and cancer mortality in a cohort of British rubber workers with 49 years

.