Research Article

**Helicobacter pylori** CagA and IL-1β Promote the Epithelial-to-Mesenchymal Transition in a Nontransformed Epithelial Cell Model

Haruki Arévalo-Romero,1,2 Isaura Meza,1 Gabriela Vallejo-Flores,2 and Ezequiel M. Fuentes-Pananá2

1Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Avenida Instituto Politécnico Nacional 2508, San Pedro Zacatenco, 07360 Ciudad de México, DF, México
2Unidad de Investigación en Virología y Cancer, Hospital Infantil de México Federico Gómez, Dr. Márquez 162, Colonia Doctores, 06720 Ciudad de México, DF, México

Correspondence should be addressed to Ezequiel M. Fuentes-Pananá; empanana@yahoo.com

Received 11 February 2016; Revised 13 May 2016; Accepted 26 June 2016

Academic Editor: Oz Helieh

Copyright © 2016 Haruki Arévalo-Romero et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Gastric cancer is the third cause of cancer death worldwide and infection by *Helicobacter pylori* (*H. pylori*) is considered the most important risk factor, mainly by the activity of its virulence factor CagA. *H. pylori*/CagA-induced chronic inflammation triggers a series of gastric lesions of increased severity, starting with gastritis and ending with cancer. IL-1β has been associated with tumor development and invasiveness in different types of cancer, including gastric cancer. Currently, it is not clear if there is an association between CagA and IL-1β at a cellular level. In this study, we analyzed the effects of IL-1β and CagA on MCF-10A nontransformed cells. We found evidence that both CagA and IL-1β trigger the initiation of the epithelial-to-mesenchymal transition characterized by β-catenin nuclear translocation, increased expression of Snail1 and ZEB1, downregulation of CDH1, and morphological changes during MCF-10A acini formation. However, only CagA induced MMP9 activity and cell invasion. Our data support that IL-1β and CagA target the β-catenin pathway, with CagA leading to acquisition of a stage related to aggressive tumors.

1. Introduction

Gastric cancer (GC) is the fifth most frequently diagnosed malignancy in the world and the third cause of cancer death worldwide [1]. GC is strongly associated with infection by *Helicobacter pylori* (*H. pylori*), a microaerophilic Gram-negative bacterium that persistently colonizes the gastric mucosa of at least 50% of the world’s population [2]. Due to its association with GC, *H. pylori* was classified as a class 1 carcinogen by the International Agency for Research on Cancer (IARC) [3–7].

*H. pylori* expresses several virulence and colonization factors [8–11]. The pathological effects of *H. pylori* in the gastric mucosa are associated with the presence of CagA (cytotoxin associated gene A), which is encoded in the cag pathogenicity island (cagPAI), a chromosomal DNA segment of about 40 kb encoding genes for a type IV secretion system (T4SS) [6, 12–14]. Different bacteria use T4SS to release effector molecules into host cells [15, 16]. Studies in GC cell lines showed that, after the adhesion of *H. pylori* to the epithelial cell, this secretion system is used to translocate the CagA protein [17]. Once CagA is inside the cell, it is phosphorylated in EPIYA motifs by members of the Src family of kinases and by the Abelson murine leukemia viral oncogene homolog 1 (c-Abl) kinase [18–23]. Phosphorylated and unphosphorylated CagA then activate a complex network of signaling molecules directly affecting cellular process related to cellular transformation, such as proliferation, cell survival, cell polarity, and the epithelial-to-mesenchymal transition (EMT) [24–31].

Chronic inflammation is an important driver of different types of cancer [32, 33]. Particularly, GC evolves through progressive inflammatory lesions, starting with superficial gastritis, followed by atrophic gastritis, intestinal metaplasia,
and dysplasia, to finally become a cancerous lesion [34, 35]. Precancerous gastric lesions are characterized by prominent infiltration of mononuclear and polymorphonuclear immune cells and the presence of inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-8 [36]. Epidemiological data also support an association between GC and polymorphisms in the genes encoding IL-8 [36]. Epidemiological data also support an association between GC and polymorphisms in the genes encoding IL-8 [36].

5%CO₂ Biosciences, San Jose, CA, USA, number 211037) for 48h at 3000 cells/cm² and cultured for 48 hrs to reach 70% confluence and then switched to DMEM-F12 without fetal bovine serum (FBS). Afterwards, cells were infected with an H. pylori multiplicity of infection (MOI) of 100 and/or stimulated with 20 ng/mL of human recombinant IL-1β (Peprotech, Rocky Hill, NJ, USA) for 48 hrs. As a control for all experiments, cells were similarly handled but were not infected nor treated with IL-1β (mock infected/treated control cells). For 3D culture infection-stimulation assays, single cells suspensions were treated as mentioned above and then seeded in Matrigel (BD Biosciences, San Jose, CA, USA, number 354230). Cells were grown for 14 days changing medium every other day. In infected and/or IL-1β treated cells, media of days 2, 4, and 6 also contained bacteria and IL-1β to maintain the stimulus while the acini were growing and shaping.

2. Material and Methods

2.1. H. pylori Strains and Culture. Two CagA positive H. pylori strains were used in this study: strain 365A3 with a Western type CagA (EPIYA ABCCC) that was obtained from the American Type Culture Collection (ATCC CRL-10317, Manassas, VA, USA). MCF-10A three-dimensional (3D) or monolayer (2D) cultures were performed as previously reported by us and Debnath et al. [47, 51]. For infection assays, MCF-10A cells were seeded at 3000 cells/cm² and cultured for 48 hrs to reach 70% confluence and then switched to DMEM-F12 without fetal bovine serum (FBS). Afterwards, cells were infected with an H. pylori multiplicity of infection (MOI) of 100 and/or stimulated with 20 ng/mL of human recombinant IL-1β (Peprotech, Rocky Hill, NJ, USA) for 48 hrs. As a control for all experiments, cells were similarly handled but were not infected nor treated with IL-1β (mock infected/treated control cells). For 3D culture infection-stimulation assays, single cells suspensions were treated as mentioned above and then seeded in Matrigel (BD Biosciences, San Jose, CA, USA, number 354230). Cells were grown for 14 days changing medium every other day. In infected and/or IL-1β treated cells, media of days 2, 4, and 6 also contained bacteria and IL-1β to maintain the stimulus while the acini were growing and shaping.

2.2. MCF-10A Culture, Infection, and IL-1β Stimulation. MCF-10A cells are human mammary epithelial cells that were obtained from the American Type Culture Collection (ATCC CRL-10317, Manassas, VA, USA). MCF-10A three-dimensional (3D) or monolayer (2D) cultures were performed as previously reported by us and Debnath et al. [47, 51]. For infection assays, MCF-10A cells were seeded at 3000 cells/cm² and cultured for 48 hrs to reach 70% confluence and then switched to DMEM-F12 without fetal bovine serum (FBS). Afterwards, cells were infected with an H. pylori multiplicity of infection (MOI) of 100 and/or stimulated with 20 ng/mL of human recombinant IL-1β (Peprotech, Rocky Hill, NJ, USA) for 48 hrs. As a control for all experiments, cells were similarly handled but were not infected nor treated with IL-1β (mock infected/treated control cells). For 3D culture infection-stimulation assays, single cells suspensions were treated as mentioned above and then seeded in Matrigel (BD Biosciences, San Jose, CA, USA, number 354230). Cells were grown for 14 days changing medium every other day. In infected and/or IL-1β treated cells, media of days 2, 4, and 6 also contained bacteria and IL-1β to maintain the stimulus while the acini were growing and shaping.

2.3. Immunofluorescence. Cells grown under 3D conditions were fixed with paraformaldehyde (PFA Electron Microscopy Sciences Cat. 15713) at 3.7% for 20 minutes at room temperature, washed, and permeabilized with PBS-0.2% Triton X-100 for 20 minutes and then washed again and treated with PBS-0.02% Triton X-100 plus 10% goat-serum and 1% BSA (blocking buffer). Cells were then incubated overnight with anti-GM130 antibody (Genetex number GTX61445; 1:50 dilution in blocking buffer), washed, and incubated with anti-rabbit IgG labeled with Alexa-488 (Invitrogen, Carlsbad, CA, USA, number A11008, 1:100 dilution) for one hour. Cells were then stained with DAPI (Invitrogen, Carlsbad, CA, USA, number D1306). Finally, the preparations were washed and mounted in the 3D culture chambers adding 12 μL of VECTASHIELD (Vector Laboratories, Burlingame, CA, number H-1000) and observed in a confocal microscope Leica SP2 (Leica Microsystems, Wetzlar, Germany). Confocal images were analyzed with the Leica LAS AF-Lite 2.6.0 software.

For 2D assays, cells were grown on sterile glass coverslips in DMEM-F12 for 48 hours. Cells were then serum starved and IL-1β-stimulated or infected for 48 hours. Then, cells were subjected to the same staining process as described for the 3D cultures by using antibodies against β-catenin (Invitrogen, Carlsbad, CA, USA, number 138400), E-cadherin (BD Biosciences, San Jose, CA, number 610082), and ZO-1 (Genetex, Irvine, CA, number GTX108631). Finally, cell preparations were mounted on slides and observed with the inverted epifluorescence microscope Olympus IX50. Images were recorded with the DP72 digital camera and analyzed with Image-Pro Plus software (V7.0) averaged cybernetics (Silver Spring, MD, USA).

2.4. RNA Extraction and RT-PCR. Total RNA was obtained from IL-1β stimulated and H. pylori infected cells lysed with 1mL of TRIzol (Invitrogen, Carlsbad, CA, USA, number...
Complementary DNA synthesis was performed using 2.5 μg of total RNA in a reaction mixture with Super-Script Kit V1LO Master Mix (Invitrogen, Carlsbad, CA, USA, number 11755-050). ZEB1 gene was amplified by using the oligonucleotide pairs, sense: GGG AAT GCT AAG AACT TGC TG and antisense: GGT GTA ACT GCA CAG GGA GC. For Snail1 gene the oligonucleotides were as follows: sense: TGG GAA GCC TAA CTA CAG CGA and antisense: AGA TGA GCA TTG GCA GCC AG; for CDH1 sense: CCC ACC AGC TAG AAG GGT C and antisense: CTG GGG TAT TGG GGG GCA TC; for RPLP0 sense: ATG GGG AAG CTG AAG GTC GG and antisense: GTG GCA GTG ATG GCA TGG ACT; for GAPDH sense: ATG GGG AAG GTG AAG GTC GG and antisense: GTG GCA GTG ATG GCA TGG ACT. The 20 μL PCR mixture contained 200 μM of dNTPs mix, 2.0 mM of MgCl₂, 200 nM of each primer, Taq Polymerase buffer, and 1.0 U of recombinant Taq Polymerase (Invitrogen, Carlsbad, CA, USA, number 11615-010). The reaction was performed with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final extension of 72°C for 5 min.

2.5. Zymography. MCF-10A cells were infected with H. pylori or treated with IL-1β for 48 h and culture supernatants were recovered and concentrated using 30 K cutoff Amicon Centricon filters (Millipore, Billerica, MA, USA, number UFC503024). Protease activity was revealed in 8% SDS-PAGE gels copolymerized with 1 mg/ml gelatin and activation buffer (50 mM Tris-HCl, pH 7.4, 4.5 mM CaCl₂). Gels were stained with Coomassie Blue and densitometric analyses were performed with the ImageJ software.

2.6. Invasion Assays. 1 × 10³ IL-1β-stimulated or H. pylori infected MCF-10A cells were seeded in the inner chamber of Transwell units (Corning, NY, USA, number 3422) with Matrigel-coated polycarbonate filters as a substrate for degradation and filled up with DMEM-F12 free of serum. The outer chamber of the Transwell unit was loaded with DMEM-F12 supplemented with 10% of FBS as the chemoattractant. Cells were allowed to migrate for 36 h at 37°C. The porous membranes containing migrating cells were cut out from the inner chambers and fixed with 3.4% PFA for 30 min. Fixed cells were permeabilized with PBS-0.2% Triton X-100 and stained with DAPI.

2.7. SDS-PAGE and Western Blot. 30 μg of protein extracts was separated in 10% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and then transferred onto nitrocellulose membranes. Membranes were blotted with antibodies against E-cadherin, β-catenin, and β-actin as loading control (kindly provided by Dr. J. M. Hernandez; CINVESTAV-IPN, Mexico). HRP-conjugated secondary antibody was from Invitrogen (Carlsbad, CA, USA, number G21040). Positive bands were revealed by enhanced chemiluminescence.

2.8. Statistical Analysis. Statistical analyses were performed by one-way ANOVA test. Data are presented as mean ± standard deviation. p values ≤ 0.05 were considered significant.

3. Results

We addressed whether CagA alone and/or cooperating with the inflammatory cytokine IL-1β promotes β-catenin translocation, one of the first steps of EMT. In mock control cells β-catenin was localized to cellular membrane. In contrast, after IL-1β treatment or infection with cagA positive strains we observed that β-catenin was translocated to the nucleus (Figure 1(a)). We did not find evidence that the combination of the two stimuli was able to induce the translocation of β-catenin into the nuclei of cells with an additive or synergistic effect (Figure 1(b)). When cells were infected with H. pylori strains lacking cagA or with a defective cagPAI, the membrane localization of β-catenin was not altered (Figure 1(b)).

We analyzed the effect of CagA on other membrane proteins located at adherent junctions. We found that E-cadherin was distributed on the cell membrane homogeneously in uninfected cells, while in cells infected with the cagA positive strains there were some areas in which the fluorescent signal decreased (arrows) (Figure 2(a)). CagA was also able to redistribute ZO-1 (arrows) (Figure 2(b)). We then evaluated the effect of CagA and IL-1β on MCF-10A 3D acini morphogenesis. In mock cells, GM130 localized towards the center of the acini in the apical face. However, when cells were stimulated with IL-1β or infected with H. pylori cagA positive strains a redistribution of GM130 protein was observed surrounding acini central cells (Figure 2(c) top panels and bottom enhanced images). These results show that CagA and IL-1β interfere with GM130 localization during acini morphogenesis.

When β-catenin and E-cadherin were analyzed by Western blot, we observed that both proteins remain stable after both CagA and IL-1β stimuli at early experimental time-points (Figure 3(a)). On the other hand, the expression levels of transcription factors ZEB1 and Snail1, two of the master regulators of EMT initiation, and of CDH1, the gene that encodes for E-cadherin, were significantly changed after both stimuli. We found an increased expression of Snail1 and ZEB1 after stimulation with IL-1β or infection (Figures 3(b) and 3(c)), but Snail1 expression was significantly larger for the H. pylori strains (Figure 4(b)). An invasion assay showed that CagA has the ability to confer migratory properties on H. pylori infected MCF-10A cells (Figures 4(c) and 4(d)). Interestingly, similar to the metalloproteinase assay, IL-1β was not as efficient to induce cell invasion. Finally, we found that the stimulation of invasion is dependent on AKT and Src kinase activities (Figures 4(c) and 4(d)). Src is the main kinase that phosphorylates CagA and we have
previously shown that CagA-induced activation of AKT relies on Src activity [47]. Overall these results agree with a CagA and IL-1β-induced onset of EMT, with CagA promoting more aggressive cancer features.

4. Discussion

The nontransformed cell line MCF-10A recapitulates several traits of the architecture of glandular epithelium providing a system in which to ask questions about the mechanisms of cell growth, proliferation, growth factors independence, and cell polarity. This model has been used to study mechanisms of transformation triggered by viral and cellular oncogenes, including some that are not related to the development of breast carcinomas [51–54].

ZO-1, E-cadherin, and β-catenin are part of the epithelial apical junctional complexes that regulate cell polarity, proliferation, and differentiation [55–57]. *H. pylori* infection induces loss of polarity and relocation of ZO-1 protein in MDCK cells, which causes impairment of the epithelial barrier integrity [26, 30, 58]. E-cadherin is frequently lost in EMT-induced metastatic cancer cells [59]. We observed that although protein levels of E-cadherin remained unchanged, there was downregulation of the CDH1 transcript after both IL-1β and CagA stimulus. E-cadherin degradation may be activated at later times than the ones analyzed in this study.

---

**Figure 1:** *H. pylori* CagA and IL-1β induce β-catenin nuclear translocation. (a) MCF-10A cells were infected with CagA positive strains (ABCCC or ABD) or stimulated with IL-1β. (b) MCF-10A cells were infected with CagA positive strains and stimulated with IL-1β or single infected with CagA negative variants CagA(−) and cagPAI(−). Immunofluorescence images show β-catenin (green) and nuclei (DAPI, blue). Arrows indicate nuclear staining (a) and membrane staining (b) of β-catenin. Figures are representative of three independent experiments performed in duplicate or triplicate.
Figure 2: Localization of ZO-1 and GM130 is altered by *H. pylori* CagA. (a-b) Cells were infected with the CagA positive strain EPIYA ABCCC and were stained with anti-ZO-1 or anti-E-cadherin antibodies (green) and DAPI (blue). Arrows in panel (a) indicate the loss of signal of E-cadherin and in panel (b) show delocalization of ZO-1. (c) MCF-10A cells were infected with *H. pylori* positive strains ABCCC and ABD, stimulated with IL-1β, or both infected and stimulated during acini morphogenesis. The acini structures were stained with anti-GM130 antibody (green) and DAPI (blue) and confocal microscopy sections were made at 50% depth of the acini. The bottom panels show an enhanced fragment of the acini to see in more detail the GM130 distribution. Figures are representative of three independent experiments performed in duplicate or triplicate.
or EMT may happen without affecting levels of E-cadherin as it has been shown in other studies [60, 61].

A link between CagA and EMT has been shown in previous studies. In AGS and MKN74 cells H. pylori induced expression of mesenchymal markers ZEB1, vimentin, Snail1, Snail3, and MMP9 with concomitant decreasing of the epithelial marker keratin-7 [62]. EMT also generated cells with characteristics of cancer stem cells (CSC) and expression of CD44 [63]. However, AGS and MKN74 cells are transformed; hence the signaling pathways and biological processes associated with cancer are already altered before the expression of CagA [62, 63]. In the past two years, several primary human gastric organoid models have emerged to assess H. pylori infection or CagA activity. In one study, H. pylori was found to induce secretion of TNFα and IL-1β inflammatory cytokines, as well as several chemokines, through activation of NFκB [64]. In other studies, H. pylori altered the cell polarity through relocalization of claudin-7 by activation of β-catenin and Snail [65].

It is thought that resistance to anoikis is responsible for the survival of invading tumor cells upon undergoing EMT and detaching from the basal membrane [66]. We previously showed that CagA induced anoikis resistance via AKT phosphorylation and inactivation of the proapoptotic proteins BIM and BAD [47]. AKT is also an important inducer of β-catenin nuclear translocation through inactivation of GSK3β; nuclear β-catenin then initiates the EMT transcriptional program [67]. Other studies have shown CagA activity leading to β-catenin nuclear accumulation [31]. Our results show that translocation of β-catenin correlates with an increased expression of the Snail1 and ZEB1 EMT genes, which are involved in deregulation of adherens junctions by transcriptional repression of the CDH1 promoter [68]. Furthermore, we also observed increased cell invasion and MMP9 protease activity promoted by CagA. Metalloproteinases degrade ECM components facilitating cell invasiveness. Interestingly, we only observed a CagA-mediated increased MMP9 activity and cell invasiveness, in spite of IL-1β efficiently promoting β-catenin translocation, transcriptional upregulation of ZEB1 and Snail1, and downregulation of CDH1. This may be due to a more chronic requirement on IL-1β to achieve a similar activity. A recent report showed that stable EMT-related changes were only induced after >3 weeks of IL-1β treatment [69], while in our study treatment went only for 2 to 6 days. Also relevant is the lack of cooperation between CagA and IL-1β. This could be because infection by H. pylori may directly induce an inflammatory response in which IL-1β is present, thus already saturating the need for IL-1β activity.

5. Conclusions

Our findings support that CagA oncoprotein from H. pylori and the inflammatory stimulus of IL-1β guide the onset of EMT in nontransformed cells using a model of acini morphogenesis. CagA but not IL-1β was found to induce cell

Figure 3: Nuclear translocation of β-catenin correlates with increased expression of ZEB1 and Snail. (a) Immunoblotting of whole cell lysates of infected or IL-1β-stimulated cells with anti-β-catenin and anti-E-cadherin antibodies. The relative expression of (b) Snail1 and CDH1 and (c) ZEB1 genes was determined by semiquantitative RT-PCR. Expression of housekeeping genes RPLP0 and GAPDH was used as internal control. Figures are representative of three independent experiments performed in duplicate or triplicate.
invasion and formation of an aggressive phenotype related to cancers.

**Competing Interests**

The authors state that they do not have any competing interests.

**Acknowledgments**

This paper constitutes the fulfillment of the Graduate Program in Molecular Biomedicine, CINVESTAV (IPN), to Arévalo-Romero. The authors thank R. Bonilla-Moreno, I. Galvan-Mendoza, M. C. Domínguez-Robles, and A. Trejo for technical assistance. This study was supported by scholarships from CONACyT and IMSS (Arévalo-Romero) and Grants 176880 (Fuentes-Panana) and 166462 (Meza) from CONACyT and Grant HIM-2013-051 (Fuentes-Panana) from Fondo de Apoyo a la Investigación HIMFG.

**References**

[1] J. Ferlay, I. Soerjomataram, M. Ervik et al., "GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11," 2013.

[2] D. N. Taylor and M. J. Blaser, "The epidemiology of *Helicobacter pylori* infection," *Epidemiologic Reviews*, vol. 13, no. 1, pp. 42–59, 1991.
[3] D. Forman, D. G. Newell, F. Fullerton et al., “Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation,” British Medical Journal, vol. 302, no. 6788, pp. 1302–1305, 1991.

[4] D. M. Parkin, “The global health burden of infection-associated cancers in the year 2002,” International Journal of Cancer, vol. 118, no. 12, pp. 3030–3044, 2006.

[5] The EUROGAST Study Group, “An international association between Helicobacter pylori infection and gastric cancer,” The Lancet, vol. 341, no. 8857, pp. 1359–1363, 1993.

[6] A. Nomura, G. N. Stemerman, P.-H. Chyou, I. Kato, G. I. Perez-Perez, and M. J. Blaser, “Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii,” The New England Journal of Medicine, vol. 325, no. 16, pp. 1132–1136, 1991.

[7] J. Parsonnet, G. D. Friedman, D. P. Vandersteen et al., “Helicobacter pylori infection and gastric carcinoma,” The New England Journal of Medicine, vol. 325, no. 16, pp. 1127–1131, 1991.

[8] K. A. Eaton, C. L. Brooks, D. R. Morgan, and S. Krakowka, “Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets,” Infection and Immunity, vol. 59, no. 7, pp. 2470–2475, 1991.

[9] J. G. Kusters, A. H. M. van Vliet, and E. J. Kuipers, “Pathogenesis of Helicobacter pylori infection,” Clinical Microbiology Reviews, vol. 19, no. 3, pp. 449–490, 2006.

[10] K. A. Eaton, J. V. Gilbert, E. A. Joyce et al., “In vivo complementation of ureB restores the ability of Helicobacter priori to colonize,” Infection and Immunity, vol. 70, no. 2, pp. 771–778, 2002.

[11] K. A. Eaton and S. Krakowka, “Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by Helicobacter pylori,” Infection and Immunity, vol. 62, no. 9, pp. 3604–3607, 1994.

[12] M. J. Blaser, G. I. Perez-Perez, H. Kлененшт et al., “Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach,” Cancer Research, vol. 55, no. 10, pp. 2111–2115, 1995.

[13] J. Parsonnet, G. D. Friedman, N. Orentreich, and H. Vogelman, “Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection,” Gut, vol. 40, no. 3, pp. 297–301, 1997.

[14] S. Censini, C. Lange, Z. Xiang et al., “Cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors,” Proceedings of the National Academy of Sciences of the United States of America, vol. 93, no. 25, pp. 14648–14653, 1996.

[15] E. Cascales and P. J. Christie, “The versatile bacterial type IV secretion systems,” Nature Reviews Microbiology, vol. 1, no. 2, pp. 137–149, 2003.

[16] K. Walldén, A. Rivera-Calzada, and G. Waksman, “Type IV secretion systems: versatility and diversity in function,” Cellular Microbiology, vol. 12, no. 9, pp. 1203–1212, 2010.

[17] S. Odenbreit, J. Püls, B. Sedlmaier, E. Gerland, W. Fischer, and R. Haas, “Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion,” Science, vol. 287, no. 5457, pp. 1497–1500, 2000.

[18] M. Asahi, T. Azuma, S. Ito et al., “Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells,” The Journal of Experimental Medicine, vol. 191, no. 4, pp. 593–602, 2000.

[19] E. D. Segal, J. Cha, J. Lo, S. Falkow, and L. S. Tompkins, “Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori,” Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 25, pp. 14559–14564, 1999.

[20] M. Stein, F. Bagnoli, R. Halenbeck, R. Rappuoli, W. J. Fantl, and A. Covacci, “c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs,” Molecular Microbiology, vol. 43, no. 4, pp. 971–980, 2002.

[21] M. Stein, R. Rappuoli, and A. Covacci, “Tyrosine phosphorylation of the Helicobacter pylori CagA antigen after cag-driven host cell translocation,” Proceedings of the National Academy of Sciences of the United States of America, vol. 97, no. 3, pp. 1263–1268, 2000.

[22] M. Selbach, S. Moese, C. R. Hauck, T. F. Meyer, and S. Backert, “Src is the kinase of the Helicobacter pylori CagA protein in vitro and in vivo,” The Journal of Biological Chemistry, vol. 277, no. 9, pp. 6775–6778, 2002.

[23] D. Mueller, N. Tegtmeyer, S. Brandt et al., “c-Src and c-Ab1 kinases control hierarchical phosphorylation and function of the CagA effector protein in Western and East Asian Helicobacter pylori strains,” Journal of Clinical Investigation, vol. 122, no. 4, pp. 1533–1566, 2012.

[24] H. Higashi, R. Tsutsumi, S. Muto et al., “SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein,” Science, vol. 295, no. 5555, pp. 683–686, 2002.

[25] S. Yamazaki, A. Yamakawa, Y. Ito et al., “The CagA protein of Helicobacter pylori is translocated into epithelial cells and binds to SHP-2 in human gastric mucosa,” Journal of Infectious Diseases, vol. 187, no. 2, pp. 334–337, 2003.

[26] M. R. Amieva, R. Vogetmann, A. Covacci, L. S. Tompkins, W. J. Nelson, and S. Falkow, “Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA,” Science, vol. 300, no. 5624, pp. 1430–1434, 2003.

[27] I. Saadat, H. Higashi, C. Obuse et al., “Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity,” Nature, vol. 447, no. 7142, pp. 330–333, 2007.

[28] H.-S. Lu, Y. Saito, M. Umeda et al., “Structural and functional diversity in the PAR1b/MARK2-binding region of Helicobacter pylori CagA,” Cancer Science, vol. 99, no. 10, pp. 2004–2011, 2008.

[29] H. Lu, N. Murata-Kamiya, Y. Saito, and M. Hatakeyama, “Role of partitioning-defective 1/microtubule affinity-regulating kinases in the morphogenetic activity of Helicobacter pylori CagA,” The Journal of Biological Chemistry, vol. 284, no. 34, pp. 23024–23036, 2009.

[30] S. Tan, L. S. Tompkins, and M. R. Amieva, “Helicobacter pylori usurps cell polarity to turn the cell surface into a replicative niche,” PLoS Pathogens, vol. 5, no. 5, Article ID e1000407, 2009.

[31] A. T. Franco, D. A. Israel, M. K. Washington et al., “Activation of beta-catenin by carcinogenic Helicobacter pylori CagA,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 30, pp. 10646–10651, 2005.

[32] A. Mantovani, P. Allavena, A. Sica, and F. Balkwill, “Cancer-related inflammation,” Nature, vol. 454, no. 7203, pp. 436–444, 2008.

[33] A. Mantovani, C. Garlanda, and P. Allavena, “Molecular pathways and targets in cancer-related inflammation,” Annals of Medicine, vol. 42, no. 3, pp. 161–170, 2010.

[34] P. Correa and Y.-H. Shiao, “Phenotypic and genotypic events in gastric carcinogenesis,” Cancer Research, vol. 54, no. 7, supplement, pp. 1941s–1943s, 1994.
[35] P. Correa, W. Haenszel, C. Cuello et al., “Gastric precancerous process in a high risk population: cross-sectional studies,” Cancer Research, vol. 50, no. 15, pp. 4731–4736, 1990.

[36] F. Colotta, P. Allavena, A. Sica, C. Garlanda, and A. Mantovani, “Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability,” Carcinogenesis, vol. 30, no. 7, pp. 1073–1081, 2009.

[37] L. E. Wroblewski, R. M. Peek Jr., and K. T. Wilson, “Helicobacter pylori and gastric cancer: factors that modulate disease risk,” Clinical Microbiology Reviews, vol. 23, no. 4, pp. 713–739, 2010.

[38] B. J. Dicken, D. L. Bigam, C. Cass, J. R. Mackey, A. A. Joy, and S. M. Hamilton, “Gastric adenocarcinoma: review and considerations for future directions,” Annals of Surgery, vol. 241, no. 1, pp. 27–39, 2005.

[39] E. M. El-Omar, M. Carrington, W.-H. Chow et al., “Interleukin-1 polymorphisms associated with increased risk of gastric cancer,” Nature, vol. 404, no. 6776, pp. 398–402, 2000.

[40] R. N. Apte, S. Dotan, M. Elkebats et al., “The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumor-host interactions,” Cancer and Metastasis Reviews, vol. 25, no. 3, pp. 387–408, 2006.

[41] V. Serelli-Lee, K. L. Ling, C. Ho et al., “Persistant helicobacter pylori specific th17 responses in patients with past H. pylori infection are associated with elevated gastric mucosal IL-1β,” PLoS ONE, vol. 7, no. 6, Article ID e39199, 2012.

[42] H. C. Jung, J. M. Kim, I. S. Song, and C. Y. Kim, “Helicobacter pylori induces an array of pro-inflammatory cytokines in human gastric epithelial cells: quantification of mRNA for interleukin-8, -la/β, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1 and tumour necrosis factor-α,” Journal of Gastroenterology and Hepatology, vol. 12, no. 7, pp. 473–480, 1997.

[43] D. Basso, M. Scrigner, A. Toma et al., “Helicobacter pylori infection enhances mucosal interleukin-1β, interleukin-6, and the soluble receptor of interleukin-2,” International Journal of Clinical and Laboratory Research, vol. 26, no. 3, pp. 207–210, 1996.

[44] S. Tu, G. Bhagat, G. Cui et al., “Overexpression of interleukin-1β induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice,” Cancer Cell, vol. 14, no. 5, pp. 408–419, 2008.

[45] R. Kalluri and R. A. Weinberg, “The basics of epithelial-mesenchymal transition,” The Journal of Clinical Investigation, vol. 119, no. 6, pp. 1420–1428, 2009.

[46] N. Kalluri, J. K. Fornari, and R. A. Weinberg, “The role of apoptosis in creating and maintaining luminal space within normal and oncogene-expressing mammary acini,” Cell, vol. 111, no. 1, pp. 29–40, 2002.

[47] S. K. Muthuswamy, D. Li, S. Lelievre, E. B. Porter, and J. S. Brugge, “ErBβ2, but not ErBβ1, reinitiates proliferation and induces luminal repopulation in epithelial acini,” Nature Cell Biology, vol. 3, no. 9, pp. 785–792, 2001.

[48] E. Knust and O. Bossinger, “Composition and formation of intercellular junctions in epithelial cells,” Science, vol. 298, no. 5600, pp. 1955–1959, 2002.

[49] C. Jamora and E. Fuchs, “Intercellular adhesion, signalling and the cytoskeleton,” Nature Cell Biology, vol. 4, no. 4, pp. E101–E108, 2002.

[50] F. Bagnoli, L. Butti, L. Tompkins, A. Covacci, and M. R. Amieva, “Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 45, pp. 16339–16344, 2005.

[51] G. Christofori and H. Semb, “The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene,” Trends in Biochemical Sciences, vol. 24, no. 2, pp. 73–76, 1999.

[52] F.-M. Wang, H.-Q. Liu, S.-R. Liu, S.-P. Tang, L. Yang, and G.-S. Feng, “SHP-2 promotes migration and metastasis of MCF-7 with loss of E-cadherin, dephosphorylation of FAK and secretion of MMP-9 induced by IL-1β in vivo and in vitro,” Breast Cancer Research and Treatment, vol. 89, no. 1, pp. 5–14, 2005.

[53] R. Martinez-Orozco, N. Navarro-Tito, A. Soto-Guzman, L. Castro-Sanchez, and E. Perez Salazar, “Arachidonic acid promotes epithelial-to-mesenchymal-like transition in mammary epithelial cells MCF-10A,” European Journal of Cell Biology, vol. 89, no. 6, pp. 476–488, 2010.

[54] M. T. Nieman, R. S. Prudoff, K. R. Johnson, and M. J. Wheelock, “E-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression,” The Journal of Cell Biology, vol. 147, no. 3, pp. 631–644, 1999.

[55] J. Baud, C. Varon, S. Chabas, L. Chambonnier, F. Darfeuille, and C. Staedel, “Helicobacter pylori CagA initiates a mesenchymal transition through ZEB1 in gastric epithelial cells,” PLoS ONE, vol. 8, no. 4, Article ID e60315, 2013.

[56] E. Bessède, C. Staedel, L. A. Acuña Amador et al., “Helicobacter pylori generates cells with cancer stem cell properties via epithelial-mesenchymal transition-like changes,” Oncogene, vol. 33, no. 32, pp. 4123–4131, 2014.

[57] P. Schlaermann, B. Toelle, H. Berger et al., “A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro,” Gut, vol. 65, no. 2, pp. 202–213, 2016.
[65] L. E. Wroblewski, M. B. Piazuelo, R. Chaturvedi et al., “Helicobacter pylori targets cancer-associated apical-junctional constituents in gastriods and gastric epithelial cells,” *Gut*, vol. 64, no. 5, pp. 720–730, 2015.

[66] P. Paoli, E. Giannoni, and P. Chiarugi, “Anoikis molecular pathways and its role in cancer progression,” *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1833, no. 12, pp. 3481–3498, 2013.

[67] R. L. Daugherty and C. J. Gottardi, “Phospho-regulation of β-catenin adhesion and signaling functions,” *Physiology*, vol. 22, no. 5, pp. 303–309, 2007.

[68] A. G. de Herreros, S. Peiró, M. Nassour, and P. Savagner, “Snail family regulation and epithelial mesenchymal transitions in breast cancer progression,” *Journal of Mammary Gland Biology and Neoplasia*, vol. 15, no. 2, pp. 135–147, 2010.

[69] T. Leibovich-Rivkin, Y. Liubomirski, B. Bernstein, T. Meshel, and A. Ben-Baruch, “Inflammatory factors of the tumor microenvironment nduce plasticity in nontransformed breast epithelial cells: EMT, invasion, and collapse of normally organized breast textures,” *Neoplasia*, vol. 15, no. 12, pp. 1330–1346, 2013.