Chapter 7
Biomarkers of Gastric Premalignant Lesions

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Epidemiology

Despite decreasing incidence in the last 50 years, gastric cancer remains the fifth most common cancer in the world, representing 6.8% of the total global cancer cases [1], and ranks third as the most common cause of cancer-related death in men. Almost one million new cases of stomach cancer were estimated to have occurred in 2012 [1, 2]. There is a wide geographic variation in gastric cancer incidence and mortality rates, with more than 70% of gastric cancer cases occurring in less developed countries [1]. In Eastern Asia and South and Central America, gastric cancer is a significant health burden [1, 2]. In addition, both gastric cancer incidence and mortality vary widely among different race/ethnic groups in the United States. Asian, Hispanic, non-Hispanic black, and Native American populations have up to 50% higher risk for gastric cancer than non-Hispanic white populations [3–5]. Similarly, gastric cancer survival is better in Asians than in Caucasian Americans, African Americans, and Hispanics [4, 6, 7]. Hispanics are younger and more often with stage IV disease when gastric cancer is diagnosed, and they present a shorter survival time than non-Hispanic whites [8]. Lower survival rates for non-Hispanic blacks compared to non-Hispanic whites have also been reported [9].
Etiology

H. pylori

*Helicobacter pylori* (*H. pylori*) is among several factors associated with non-cardia intestinal-type gastric cancer development. It is the primary cause of the initiation of the disease and has been classified as a class I carcinogen [10]. Infection with *H. pylori* occurs mainly during childhood [11], and in a proportion of those chronically infected, it results in the transformation of the normal gastric mucosa into non-atrophic gastritis (NAG), followed by multifocal atrophic gastritis without intestinal metaplasia (MAG), intestinal metaplasia (IM), dysplasia, and finally cancer [12, 13].

Current estimates of *H. pylori* prevalence in the world range from 24 to 79% [14]. The highest prevalence is in Africa (79%) and Latin America and the Caribbean region (63.4%), and the lowest in Oceania (24.4%) and Northern America (37.1%). In regions of South and Central America, which include those with high gastric cancer risk, *H. pylori* prevalence can reach up to 80–85%, some of the highest prevalence in the world [15]. In the United States, the estimated *H. pylori* prevalence is 30% [15]. However, while *H. pylori* prevalence ranges from 18.4 to 26.9% in non-Hispanic whites, it can be as high as 51.1%, 57.9%, and 75% in non-Hispanic blacks, Hispanics, and Alaskan Native Americans, respectively [14, 16, 17]. This high prevalence likely contributes to the high incidence and mortality from gastric cancer in non-Hispanic blacks and Hispanics. Despite this high prevalence of infection, it is estimated that approximately 1% of those chronically infected with *H. pylori* will develop gastric cancer [18, 19]. In fact, the majority of the population will remain asymptomatic.

In the last decades, *H. pylori* prevalence has decreased around the world, especially in the more developed regions, mostly due to improved hygienic conditions, improved diet and food preservation, and broader access to antibiotics [2]. Recently, Hooi et al. [14] compared *H. pylori* prevalence from two time periods (1970–1999 and 2000–2016) and found that from one time period to the next, *H. pylori* prevalence significantly decreased in Europe (from 48.8 to 39.8%), Northern America (from 42.7 to 26.6%), and Oceania (from 26.6 to 18.7%) [14]. In contrast, *H. pylori* prevalence remained similar during the same periods in Asia (53.6% vs. 54.3%) and Latin America (62.8% vs. 60.2%) [14]. This geographical variability in *H. pylori* prevalence explains in part the higher gastric cancer incidence and mortality observed in Latin American countries compared to more developed countries as the United States. Furthermore, Porras et al. in a recent study of the epidemiology of *H. pylori* infection in six countries of Latin America did not observe any significant changes in *H. pylori* prevalence between the oldest and youngest participants in their study, suggesting that gastric cancer incidence is not going to decrease in those countries in the near future [15].
Environmental Factors

Even though infection with *H. pylori* is considered necessary for the development of gastric cancer, it is not determinant; just 1–3% of those infected with *H. pylori* will develop gastric cancer in their lifetime [18, 19]. Additional environmental factors are associated with gastric cancer risk, including smoking, alcohol use, and a diet low in fresh produce and high in meats and salt [20]. In a recent meta-analysis, Bonequi et al. found that in Latin America, smoking and alcohol use were associated with a 47% and 61% increase of gastric cancer risk, respectively [21]. Regarding diet, the same study found that consumption of red and processed meats were associated with a 73% and 64% increase of gastric cancer risk, respectively. High salt intake was associated with 2.24-fold increase. In contrast, consumption of fruits and vegetables were associated with a 32% and 42% reduction of gastric cancer risk, respectively [21]. There is a high prevalence of smoking and alcohol use in Latin American populations [22, 23], and in regions with high gastric cancer rates as in the Andean mountains, the diet is poor in fruits and vegetables and excessively high in consumption of salt [24]. Data from the US National Health Interview Survey indicate that Hispanics have the lowest prevalence of smoking in all racial/ethnic populations and the highest consumption of fresh fruits and vegetables [25]. These habits are not in concordance with their gastric cancer incidence and mortality rates.

Genetic Bases of the Gastric Inflammatory Cascade (Correa’s Cascade)

**Single-Nucleotide Polymorphisms (SNPs)**

In 1975, Correa et al. analyzed 1500 stomachs obtained at autopsy to estimate the prevalence of intestinal metaplasia [26]. As a result of that analysis and later updates, Correa et al. proposed that gastric adenocarcinoma is the final stage of an inflammatory cascade that leads the normal gastric epithelia to non-atrophic gastritis (NAG), multifocal atrophic gastritis (MAG), complete intestinal metaplasia (IM), incomplete intestinal metaplasia, dysplasia, and cancer [13, 27–30]. It was shown that single-nucleotide polymorphisms (SNPs) in the cytokine gene encoding interleukin-1β (IL1B) are associated with the risk of gastric cancer [31]. Since then, others have shown the association of cytokine SNPs with gastric cancer risk in several populations [32–36]; however, very few works have centered on defining the association of cytokine SNPs and the presence of advanced gastric lesions as precursors of gastric cancer. Our work has led to the identification of SNPs and haplotypes in the *IL1B* gene associated with advanced gastric premalignant stages in African American and Caucasian individuals [37, 38]. Our studies have shown that African American individuals have a higher prevalence of MAG as well as a higher rate of *H. pylori* infection [37, 38]. Using DNA samples from healthy African
Stage-Specific and Evolution-Associated Gene Profiles

The pioneer studies by Correa et al. led to the identification of a premalignant cascade suggested to precede gastric carcinogenesis [27]. However, the molecular basis for the intricate relationship between the different stages and their evolution over time is not fully known. Using baseline and 6-year follow-up samples from a cohort study established by Correa et al. in Colombia [40], we extracted RNA and performed a microarray analysis to find genes associated with stage and progression of premalignant lesions. Analyzing the genomics of lesion evolution over time, we found that the genes CD44, NUMA, and LCN2 were associated with progression [41]. Interestingly, these three genes have been associated with several types of cancer and with advanced premalignant lesions [42–46]. Using mouse models of H. pylori infection in wild-type and Cd44−/− H. pylori mice, we found a significant activation of immune-related pathways in response to the infection, among them was the IFNγ pathway [41]. Interestingly, the gastric mucosa of Cd44−/− mice had significantly lower expression of Ifng and Ifng-related genes including Irf7, Ifit3, Ifit2, Nos2, and Stat1 [41]. Reduction in Stat1 expression was paralleled with reduction in phosphorylation of the Stat1 protein [41]. In order to correlate the differences found in global and immune gene expression with pathological changes in the gastric mucosa, we determined and compared the presence of gastric lesions between wild-type and Cd44−/− H. pylori-infected mice. We found that compared to the wild-type mice, the H. pylori infection did not induce tissue damage in the gastric mucosa of Cd44−/− H. pylori-infected mice. These data suggest that this gene, and the protein encoded by it, is essential to mount the Th1 responses associated with tissue damage induced by the infection [41, 47–49].

Using baseline samples from the same cohort of individuals described for our work with CD44 [40], we identified 37 samples with MAG, 25 with IM, and 12 with dysplasia. Using the less advanced gastric precancerous lesion as reference (MAG), we identified 16 genes with at least a 30% change in their expression levels when compared with dysplasia [50]. However, the only one showing significantly higher expression was the gene Deleted in Malignant Brain Tumor 1 (DMBT1), which was able to separate most dysplasia from MAG cases [50]. Interestingly, gastric tissue from African American and Caucasian individuals with advanced gastric lesions also had increased levels of expression of the gene [50], suggesting that this response is conserved across ethnicities. We also found that the expression of the DMBT1 gene was significantly higher in individuals with advanced gastric lesions who also had infection with H. pylori, which highlights the role of the DMBT1 protein as an agglutinin [51–53]. Mouse models of H. pylori infection show that this gene acts as...
a tumor suppressor by limiting tissue damage in response to the infection and through the activation of interleukin 33 (IL33) and pERK [50].

In summary, gastric cancer is a disease of disparities, with minority groups having increased prevalence and mortality of the disease. We have shown that precancerous lesions and their evolution over time are associated with specific patterns of genes that may be used as the basis to devise strategies for the prediction of disease aggressiveness and outcome.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
3. Dong E, Duan L, Wu BU. Racial and ethnic minorities at increased risk for gastric cancer in a regional US population study. Clin Gastroenterol Hepatol. 2017;15(4):511–7.
4. Lui FH, Tuan B, Swenson SL, Wong RJ. Ethnic disparities in gastric cancer incidence and survival in the USA: an updated analysis of 1992–2009 SEER data. Dig Dis Sci. 2014;59(12):3027–34.
5. Wu X, Chen VW, Andrews PA, Ruiz B, Correa P. Incidence of esophageal and gastric cancers among Hispanics, non-Hispanic whites and non-Hispanic blacks in the United States: substate and histology differences. Cancer Causes Control. 2007;18(6):585–93.
6. Kim J, Sun CL, Mailey B, Prendergast C, Artinyan A, Bhatia S, et al. Race and ethnicity correlate with survival in patients with gastric adenocarcinoma. Ann Oncol. 2010;21(1):152–60.
7. Al-Refaie WB, Tseng JF, Gay G, Patel-Parekh L, Mansfield PF, Pisters PWT, et al. The impact of ethnicity on the presentation and prognosis of patients with gastric adenocarcinoma. Results from the National Cancer Data Base. Cancer. 2008;113(3):461–9.
8. Duma N, Sanchez LJ, Castro YS, Jennis AA, McCain DA, Gutierrez ME, et al. Gastric adenocarcinoma: clinicopathologic differences among Hispanics and non-Hispanic whites. A single Institution’s experience over 14 years. Ann Gastroenterol. 2016;29(3):325–31.
9. Jinjuvadia R, Jinjuvadia K, Liangpunsakul S. Racial disparities in gastrointestinal cancers-related mortality in the U.S. population. Dig Dis Sci. 2013;58(1):236–43.
10. Schistosomes, liver flukes and Helicobacter pylori. IARC working group on the evaluation of carcinogenic risks to humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum. 1994;61:177–240.
11. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and Helicobacter pylori. J Infect Dis. 1993;168(1):219–21.
12. Correa P, Houghton J. Carcinogenesis of Helicobacter pylori. Gastroenterology. 2007;132(2):659–72.
13. Correa P, Piazuelo MB. The gastric precancerous cascade. J Dig Dis. 2012;13(1):2–9.
14. Hooi JKY, Lai WY, Ng WK, Sue MM, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology. 2017;153(2):420–9.
15. Porras C, Nodora I, Sexton R, Ferreccio C, Jimenez S, Dominguez RL, et al. Epidemiology of Helicobacter pylori infection in six Latin American countries (SWOG trial S0701). Cancer Causes Control. 2013;24(2):209–15.
16. Nguyen T, Ramsey D, Graham D, Shaib Y, Shiota S, Velez M, et al. The prevalence of Helicobacter pylori remains high in African American and Hispanic veterans. Helicobacter. 2015;20(4):305–15.
17. Everhart JE, Kruszon-Moran D, Perez-Perez GI, Tralka TS, McQuillan G. Seroprevalence and ethnic differences in *Helicobacter pylori* infection among adults in the United States. J Infect Dis. 2000;181(4):1359–63.

18. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med. 2002;347(15):1175–86.

19. Wrobleski LE, Peek RM Jr, Wilson KT. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23(4):713–39.

20. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Washington, DC: AICR; 2007.

21. Bonequi P, Meneses-Gonzalez F, Correa P, Rabkin CS, Camargo MC. Risk factors for gastric cancer in Latin America: a meta-analysis. Cancer Causes Control. 2013;24(2):217–31.

22. Muller F, Wohbe L. Smoking and smoking cessation in Latin America: a review of the current situation and available treatments. Int J Chron Obstruct Pulmon Dis. 2008;3(2):285–93.

23. Monteiro MG. Alcohol and public health in the Americas: a case for action. Washington, DC: PAHO; 2007. http://www.who.int/substance_abuse/publications/alcohol_public_health_americas.pdf

24. Camargo MC, Burk RF, Bravo LE, Piazuelo MB, Hill KE, Fontham ET, et al. Plasma selenium measurements in subjects from areas with contrasting gastric cancer risks in Colombia. Arch Med Res. 2008;39(4):443–51.

25. Thompson FE, Midthune D, Subar AF, McNeel T, Berrigan D, Kipnis V. Dietary intake estimates in the National Health Interview Survey, 2000: methodology, results, and interpretation. J Am Diet Assoc. 2005;105(3):352–63.

26. Correa P, Cuello C, Duque E. Carcinoma and intestinal metaplasia of the stomach in Colombian migrants. J Natl Cancer Inst. 1970;44(2):297–306.

27. Correa P, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. Lancet. 1975;2(7924):58–60.

28. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol. 1996;20(10):1161–81.

29. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988;48(13):3554–60.

30. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res. 1992;52(24):6735–40.

31. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream J, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404(6776):398–402.

32. Duraes C, Munoz X, Bonet C, Garcia N, Vencesla A, Carneiro F, et al. Genetic variants in the IL1A gene region contribute to intestinal-type gastric carcinoma susceptibility in European populations. Int J Cancer. 2014;135(6):1343–55.

33. Canedo P, Corso G, Pereira F, Lunet N, Suriano G, Figueiredo C, et al. The interferon gamma receptor 1 (IFNGR1) -56C/T gene polymorphism is associated with increased risk of early gastric carcinoma. Gut. 2008;57(11):1504–8.

34. Machado JC, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, et al. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology. 2003;125(2):364–71.

35. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, et al. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology. 2001;121(4):823–9.

36. Sicinschi LA, Lopez-Carrillo L, Camargo MC, Correa P, Sierra RA, Henry RR, et al. Gastric cancer risk in a Mexican population: role of *Helicobacter pylori* CagA positive infection and polymorphisms in interleukin-1 and -10 genes. Int J Cancer. 2006;118(3):649–57.

37. Zabaleta J, Camargo MC, Piazuelo MB, Fontham E, Schneider BG, Sicinschi LA, et al. Association of interleukin-1beta gene polymorphisms with precancerous gastric lesions in African Americans and Caucasians. Am J Gastroenterol. 2006;101(1):163–71.
38. Zabaleta J, Camargo MC, Ritchie MD, Piazuelo MB, Sierra RA, Turner SD, et al. Association of haplotypes of inflammation-related genes with gastric preneoplastic lesions in African Americans and Caucasians. Int J Cancer. 2011;128(3):668–75.

39. Zabaleta J, Schneider BG, Ryckman K, Hooper PF, Camargo MC, Piazuelo MB, et al. Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. Cancer Immunol Immunother. 2008;57(1):107–14.

40. Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, et al. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-\textit{Helicobacter pylori} therapy. J Natl Cancer Inst. 2000;92(23):1881–8.

41. Garay J, Piazuelo MB, Majumdar S, Li L, Trillo-Tinoco J, Del Valle L, et al. The homing receptor CD44 is involved in the progression of precancerous gastric lesions in patients infected with \textit{Helicobacter pylori} and in development of mucous metaplasia in mice. Cancer Lett. 2016;371(1):90–8.

42. Washington K, Gottfried MR, Telen MJ. Expression of the cell adhesion molecule CD44 in gastric adenocarcinomas. Hum Pathol. 1994;25(10):1043–9.

43. Dammrich J, Vollmers HP, Heider KH, Muller-Hermelink HK. Importance of different CD44v6 expression in human gastric intestinal and diffuse type cancers for metastatic lymphogenic spreading. J Mol Med (Berl). 1995;73(8):395–401.

44. Bruning-Richardson A, Bond J, Alsiaiy R, Richardson J, Cairns DA, McCormac L, et al. NuMA overexpression in epithelial ovarian cancer. PLoS One. 2012;7(6):e38945.

45. Sier CF, Kubbenn FJ, Ganesh S, Heerding MM, Griffioen G, Hanemaaijer R, et al. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer. 1996;74(3):413–7.

46. Fan XG, Fan XJ, Xia HX, Keeling PW, Kelleher D. Up-regulation of CD44 and ICAM-1 expression on gastric epithelial cells by \textit{H. pylori}. APMIS. 1995;103(10):744–8.

47. Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, et al. Lymphocytes in the human gastric mucosa during \textit{Helicobacter pylori} have a T helper cell 1 phenotype. Gastroenterology. 1998;114(3):482–92.

48. Mohammadi M, Nedrud J, Redline R, Lycke N, Czinn SJ. Murine CD4 T-cell response to helicobacter infection: TH1 cells enhance gastritis and TH2 cells reduce bacterial load. Gastroenterology. 1997;113(6):1848–57.

49. Nedrud JG, Mohammadi M, Blanchard T, Redline R, Czinn SJ. Th1/Th2 lymphocyte responses in helicobacter infections. In: Hunt RH, Tytgat GNJ, editors. \textit{Helicobacter pylori}: basic mechanisms to clinical cure 1998. Dordrecht: Springer; 1998. p. 101–9.

50. Garay J, Piazuelo MB, Lopez-Carrillo L, Leal YA, Majumdar S, Li L, et al. Increased expression of deleted in malignant brain tumors (DMBT1) gene in precancerous gastric lesions: findings from human and animal studies. Oncotarget. 2017;8(29):47076–89.

51. Kukita K, Kawada-Matsuo M, Oho T, Nagatomo M, Oogai Y, Hashimoto M, et al. Staphyloccocus aureus SasA is responsible for binding to the salivary agglutinin gp340, derived from human saliva. Infect Immun. 2013;81(6):1870–9.

52. Edwards AM, Manetti AG, Falugi F, Zingaretti C, Capo S, Buccato S, et al. Scavenger receptor gp340 aggregates group a streptococci by binding pili. Mol Microbiol. 2008;68(6):1378–94.

53. Chu Y, Li J, Wu X, Hua Z, Wu Z. Identification of human immunodeficiency virus type 1 (HIV-1) gp120-binding sites on scavenger receptor cysteine rich 1 (SRCR1) domain of gp340. J Biomed Sci. 2013;20:44.
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