Helicobacter pylori Associated Gastric Cancer

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Abstract: Gastric cancer is strongly associated with *Helicobacter pylori* infection but not all infected individuals develop carcinoma indicating that variety of factors are involved in the pathogenesis of disease which include diversified strains of *H. pylori* as well as host and environmental factors. Extraordinary diversity in *H. pylori* strains makes it hard to link its particular virulence factor with the progression of gastric carcinoma.

Key Words: Helicobacter pylori, cagA, vacA, babA, gastric cancer.

INTRODUCTION

Second leading cause of cancer related death in the world is gastric carcinoma which is strongly believed to be associated with *Helicobacter pylori* infection, based on the epidemiological and interventional studies on animals as well as on humans. Variety of *H. pylori* virulence factors in addition with host factors are involved in initiating neoplastic transformation which is however only observed in small proportion (3-10%) of the *H. pylori* infected patients population with gastroduodenal ulcerations.

H. pylori Virulence Genes

The cagA gene

One of the most important virulence factors of *H. pylori* is the cytotoxin associated gene A (*cagA*) which now indicates the pathogenicity island (PAI) of *H. pylori* genome. Cag PAI comprises of approximately 37000 bases, around 31 genes, divided into two regions each having 14 and 16 open reading frames and termed as cagI and cagII respectively. CagA is 120-145 kDa protein which is cytotoxic in nature and expressed by almost all *cagA +ve H. pylori*, causes an increased production of antibodies against cagA in infected subjects [1]. In East Asian countries approximately 90% of *H. pylori* strains are positive for *cagA* gene [2], while in western countries *cagA +ve H. pylori* prevalence was reported to be around 70% [3]. Meta-analysis studies have shown the strong association of *cagA +ve H. pylori* with the increased risk of gastric cancer [4-7].

CagA proteins are injected into the host epithelial cells *via* Type IV Secretion System (TFSS), which is encoded by number of genes including cagα, cagβ, cagE, cagT, cagW, cagX, cagY genes [8, 9]. These genes are required to be intact because it has been reported that any mutation or inactivation of these genes may not result in cagA translocation in to the host cells. After being translocated, CagA is then phosphorylated on tyrosine residues and brings morphological changes within the cells [10]. Phosphorylated cagA binds with Src homology-2 domain-containing protein-tyrosine phosphatase (SHP-2) of host cells and disturbs the normally ongoing processes of motility, momrphogenesis and proliferation [11]. *CagA +ve H. pylori* are also involved in inducing enhanced production of proinflammatory cytokines mainly IL-8 results in the cellular damage by initiating inflammatory response [12].

Genetic Analysis of *cagA* revealed that there are two subtypes of this protein. Variation in its tyrosine phosphorylation site has been observed and on this basis *cagA* was classified into Western *cagA* and East Asian *cagA* [13]. East Asian strains are considered more pathogenic and strongly associated with severe gastric inflammation, atrophy and gastric cancer than western type [14]. It was also reported to have more binding affinity towards SHP-2 than western type *cagA* [15].

The vacA gene

Vacuolating cytotxin A is one of the main virulence factors of *H. pylori*. Almost all *H. pylori* carry *vacA* gene, but approximately half of them produce vacuolating cytotoxin [16]. VacA precursor is 140kDa containing an active toxin of 87 to 94 kDa, which includes N-terminal region which is mainly responsible for vacA activity, and C terminal region which facilitates the binding of protein to the host cells [17]. There are two regions in *vacA* gene on the basis of diversity i.e. signal peptide coding region (s) and middle region (m). S region is further categorized into four allelic subtypes; s1a, s1b, s1c and s2 while middle region consists of three allelic subtypes; m1, m2a and m2b [18]. Previous studies revealed that genotype s1-m1 has higher vacuolating activity as compared to s1-m2, while s2-m2 genotype was reported for exhibiting very low or no vacuolating activity [19].

Initially, vacA form chloride ion channels in plasma membrane of epithelial cells, which allows more chloride to enter into the cell resulting in the serial increase of chloride concentration, ATPase activity and proton pump activity.

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Ammonia being a membrane-permeable weak base also enters into late endocytic compartments, gets protonated and trapped there. Increase in the concentration of ions causes osmotic swelling which finally leads to vacuole formation [20, 21].

Based on the findings of several studies it has also been suggested that s1 genotype is more associated to gastric cancer than s2 [3, 22]. Moreover, *H. pylori cagA* positivity is correlated with expression of *vacA* s1-m1 or s1-m2 genotypes [23].

The babA Gene

*BabA*1 and *babA*2 are the two *BabA* adhesin encoding genes. B*abA*1 is suppressive while *babA*2 is responsible for the expression of *BabA*. Disease outcome in relation with *babA* positive *H. pylori* is to some extent conflicting with variation in findings of number of researchers. Couple of studies suggested the that there is strong relation of *babA*2 with duodenal ulcer [24] and gastric cancer [25]. Yamaoka *et al*. and Mizushima did not find strong correlation between *babA* positive *H. pylori* and gastric cancer [26] and other *H. pylori* related diseases [27]. Another study conducted on Brazilian patients didn't support the existence of any relation of *babA*2 with *cagA*, *vacA* genotype of *H. pylori* and worsening of gastric inflammation, but *babA*2 positive *H. pylori* were frequently isolated from patients of peptic ulcer disease [28].

H. pylori and Gastric Cancer

Gastric cancer is a malignant epithelial tumor which basically originated from glandular epithelium of gastric mucosa. 90% of gastric cancers are adenocarcinoma [29]. According to Lauren classification, gastric cancer can be mainly classified into two histological types: Intestinal type and Diffuse type [30].

Intestinal type can be identified as irregular tubular structures, with stratification, reduced stroma which surrounds multiple lumens. Initially, tumor overruns into the gastric wall, infiltrate muscularis mucosa, sub mucosa and then muscularis propria. Intestinal type is usually associated with intestinal metaplasia and can be graded as well, moderate and poorly differentiated on the basis of glandular architecture, cellular pleomorphism and mucosecretion [31], while diffuse type can be identified histologically by discohesive and mucus secreting tumor cells, mucus is delivered in interstitium which produces optically empty spaces full of mucus. Inside the cell, nucleus is forced against the cell membrane by the mucus, forming 'signet-ring cell' structures [30, 31]. Decision made by World Health Organization (WHO) and International Agency for Research on Cancer to designate *H. pylori* as group I carcinogen in the year of 1994 was based on number of studies, which proved seroepidemiologicaly that previously positively reported subjects for *H. pylori* infection were later diagnosed for gastric cancer. Intestinal type of gastric cancer is observed more associated with *H. pylori* infection than diffuse type. Steps involved in the transformation of normal mucosa to adenocarcinoma include initiation with chronic superficial gastritis followed by atrophic gastritis and intestinal metaplasia and then adenocarcinoma. No mutational events consistently appeared in any intermediate step of the transformation into intestinal type gastric adenocarcinoma therefore the ability of *H. pylori* to induce superficial gastritis is considered most important and highly associated with the progression into intestinal type adenocarcinoma [32].

The largest study to find out the association between *H. pylori* infection and gastric cancer was conducted by Eurogast Study Group over 17 populations in 13 countries which showed six fold increase in gastric cancer risk in *H. pylori* infected population as compared to H. pylori uninfected population [33]. In numbers of studies many evidences were collected about the role of *H. pylori* in carcinogenesis and identified 2 to 40% incidence rate of gastric cancer development in *H. pylori* infected Mongolian gerbils [34]. New approaches were also adopted in the direction of eradicating *H. pylori*. Treatment of *H. pylori* resulted in the regression of gastric atrophy and intestinal metaplasia, which further supported the association between *H. pylori* and gastric cancer [35]. It is an important fact that regression will only occur if treatment for eradicating *H. pylori* is started in early stages because similar to regression observed in MALT lymphoma case, in already diagnosed gastric cancer cases, regression doesn't occur on the eradication of *H. pylori* [36].

Pathogenesis of *H. pylori* in Gastric Cancer

H. pylori, despite of infecting more than half of the world's population and showing strong association with peptic ulcer disease and gastric cancer, only little proportion of the population develop peptic ulcer disease or gastric cancer. It is reported that only 3% and 10-20% of *H. pylori* infected people are at higher risk of developing gastric cancer and peptic ulcer disease respectively [37], if *H. pylori* eradication is not successful.

There are also so many factors involved in progression of gastritis into precancerous and cancerous outcomes; therefore, majority of *H. pylori* infected population doesn't show severe diseased conditions. Variety of host factors and environmental factors including host genetic polymorphisms are mainly given importance to find out any possible correlation of these factors with the presence of *H. pylori*, which in combination enhance the risk of developing gastric cancer [38-43]. Host genetic factors play a very important role in the progression of gastric cancer from H.pylori associated gastric inflammation and pre-cancerous lesions In our own study of more than 330 gastric biopsies from dyspeptic patients, normal individuals and 100 gastric cancer patients, more than 55% of cancer patient's biopsies were positive for *H.pylori Urease* A gene . In more than half of the cases included, we found that genotype vacA s1a/m1 of *H.pylori* with virulence factors cagA and babA were highly associated with severity of gastric inflammation and gastric cancer particularly intestinal type. Cag+ *H.pylori* was highly

associated with increased production of reactive oxygen species (ROS) affecting DNA damage repair systems resulting in the accumulation of DNA damages and mutations which lead to carcinogenesis . We also found that *H.pylori* infected patients carrying IL1B-511+T and IL1RN+2 genotypes have high risk of gastric cancer. Further research is required to get insight knowledge to determine more precise molecular mechanism(s) of pathogenesis of *H. pylori* which will not only help to design effective treatment against *H. pylori* infection but will also lead to effective control in progression towards gastric adenocarcinoma particularly intestinal type.

REFERENCES

[1] Hayashi, T., *et al*., Tertiary Structure-Function Analysis Reveals the Pathogenic Signaling Potentiation Mechanism of Helicobacter pylori Oncogenic Effector CagA. Cell host & microbe. **12**(1): p. 20-33.

[2] Graham, D.Y., H. Lu, and Y. Yamaoka, *African, Asian or Indian enigma, the East Asian Helicobacter pylori: facts or medical myths.* Journal of Digestive Diseases, 2009. **10**(2): p. 77-84.

[3] C. Figueiredo, L.J.V.D.C.N.J.M.S.C.P.P.F.W.G.V.Q.F.C., Helicobacter pylori Genotypes Are Associated with Clinical Outcome in Portuguese Patients and Show a High Prevalence of Infections with Multiple Strains. Scandinavian Journal of Gastroenterology, 2001. **36**(2): p. 128-135.

[4] Loh, J.T., *et al*., Analysis of cagA in Helicobacter pylori Strains from Colombian Populations with Contrasting Gastric Cancer Risk Reveals a Biomarker for Disease Severity. Cancer Epidemiology Biomarkers & Prevention, 2011. **20**(10): p. 2237-2249.

[5] Naoko, M.-K., Pathophysiological functions of the CagA oncoprotein during infection by Helicobacter pylori. Microbes and Infection, 2011. **13**(10): p. 799-807.

[6] Batista, S., *et al*., Higher number of Helicobacter pylori CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. BMC Microbiology, 2011. **11**(1): p. 61.

[7] Figura, N., *et al*., cagA structural types of Helicobacter pylori strains isolated from patients with gastric carcinoma and chronic gastritis only. Internal and Emergency Medicine, 2012: p. 1-3.

[8] Backert, S., *et al*., Phosphorylation of tyrosine 972 of the Helicobacter pylori CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Molecular Microbiology, 2001. **42**(3): p. 631-644.

[9] Covacci, A. and R. Rappuoli, *Tyrosine-Phosphorylated Bacterial Proteins.* The Journal of Experimental Medicine, 2000. **191**(4): p. 587-592.

[10] Stein, M., 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, 2000. **97**(3): p. 1263-1268.

[11] Neel, B.G., H. Gu, and L. Pao, *The â€˜Shp'ing news: SH2 domain-containing tyrosine phosphatases in cell signaling.* Trends in Biochemical Sciences, 2003. **28**(6): p. 284-293.

[12] Crabtree, J.E., *et al*., Modulation of Helicobacter pylori induced interleukin-8 synthesis in gastric epithelial cells mediated by cag PAI encoded VirD4 homologue. Journal of Clinical Pathology, 1999. **52**(9): p. 653-657.

[13] Higashi, H., *et al*., Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proceedings of the National Academy of Sciences, 2002. **99**(22): p. 14428-14433.

[14] Tokudome, S., *et al., Re: Helicobacter pylori infection and gastric cancer: Facing the enigmas.* International Journal of Cancer, 2004. **112**(1): p. 166-167.

[15] Azuma, T., *et al*., Association between Diversity in the Src Homology 2 Domain-Containing Tyrosine Phosphatase Binding Site of Helicobacter pylori CagA Protein and Gastric Atrophy and Cancer. journal of infectious diseases, 2004. **189**(5): p. 820-827.

[16] GonzÃ¡lez-Rivera, C., *et al*., The Intermediate Region of Helicobacter pylori VacA Is a Determinant of Toxin Potency in a Jurkat T Cell Assay. Infection and Immunity. **80**(8): p. 2578-2588.

[17] de Bernard, M., *et al*., Identification of the Helicobacter pylori VacA Toxin Domain Active in the Cell Cytosol. Infection and Immunity, 1998. **66**(12): p. 6014-6016.

[18] Van Doorn, L.J., *et al*., *Geographic distribution of vacA allelic types of Helicobacter pylori.* Gastroenterology, 1999. **116**(4): p. 823-30.

[19] Atherton, J.C., *et al*., *Mosaicism in Vacuolating Cytotoxin Alleles of Helicobacter pylori.* Journal of Biological Chemistry, 1995. **270**(30): p. 17771-17777.

[20] Cover, T.L. and S.R. Blanke, *Helicobacter pylori VacA, a paradigm for toxin multifunctionality.* Nat Rev Micro, 2005. **3**(4): p. 320-332.

[21] de Bernard, M., *et al*., *The multiple cellular activities of the VacA cytotoxin of Helicobacter pylori.* International Journal of Medical Microbiology, 2004. **293**(7â€“8): p. 589-597.

[22] Miehlke, S., *et al.*, Helicobacter pylori vacA, iceA, and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. Am J Gastroenterol, 2001. **96**(4): p. 1008-1013.

[23] Kidd, M., *et al*., Conservation of the cag pathogenicity island is associated with vacA alleles and gastroduodenal disease in South AfricanHelicobacter pylori isolates. Gut, 2001. **49**(1): p. 11-17.

[24] Oliveira, A.G.a., *et al.*, babA2- and cagA-Positive Helicobacter pylori Strains Are Associated with Duodenal Ulcer and Gastric Carcinoma in Brazil. 2003, American Society for Microbiology.

[25] Talebi Bezmin Abadi, A., *et al*., High correlation of <i>babA</i>_{<i>2</i>}-positive strains of <i>Helicobacter pylori</i> with the presence of gastric cancer. Internal and Emergency Medicine: p. 1-5.

[26] Yamaoka, Y., *et al*., Helicobacter pylori outer membrane proteins and gastroduodenal disease. Gut, 2006. **55**(6): p. 775-781.

[27] Mizushima, T., *et al*., Clinical Relevance of the babA2 Genotype of Helicobacter pylori in Japanese Clinical Isolates. Journal of clinical microbiology, 2001. **39**(7): p. 2463-2465.

[28] Mattar, R., *et al.*, No Correlation of babA2 with vacA and cagA Genotypes of Helicobacter pylori and Grading of Gastritis from Peptic Ulcer Disease Patients in Brazil. Helicobacter, 2005. **10**(6): p. 601-608.

[29] Kumar, *Pathologic Basis of Disease*. 8th ed. 2010: Saunders Elsevier. 784.

[30] Lauren, P., The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. An Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand, 1965. **64**: p. 31-49.

[31] Danciu, M. and M.-S. Mihailovici. *Atlas of Pathology* 01/30/2009 [cited; 2nd:[Available from: http://www.pathologyatlas.ro/.

[32] Peek, R.M. and M.J. Blaser, *Helicobacter pylori and gastrointestinal tract adenocarcinomas.* Nature reviews. Cancer, 2002. **2**(1): p. 28-37.

[33] Group, T.E.S., An international association between Helicobacter pylori infection and gastric cancer. Lancet, 1993. **341**: p. 1359-1362.

[34] Hirayama, F., *et al.*, Development of poorly differentiated adenocarcinoma and carcinoid due to long-term <i> Helicobacter pylori</i> colonization in Mongolian gerbils. Journal of Gastroenterology, 1999. **34**(4): p. 450-454.

[35] Leung, W.K., *et al*., Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. Gut, 2004. **53**(9): p. 1244-1249.

[36] Annibale, *et al*., Cure of Helicobacter pylori infection in atrophic

body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. Alimentary Pharmacology & Therapeutics, 2000. **14**(5): p. 625-634.

[37] Uemura, N., *et al*., *Helicobacter pylori Infection and the Development of Gastric Cancer*. New England Journal of Medicine, 2001. **345**(11): p. 784-789.

[38] Mohri, Y., *et al*., Prognostic Significance of Host- and Tumor-Related Factors in Patients with Gastric Cancer. World Journal of Surgery. **34**(2): p. 285-290.

[39] El Omar, E.M., *et al*., Interleukin-1 polymorphisms associated with increased risk of gastric cancer. 2000, Nature Publications Group.

[40] Stoicov, C., *et al*., Molecular biology of gastric cancer: Helicobacter infection and gastric adenocarcinoma: bacterial and host factors responsible for altered growth signaling. Gene, 2004. **341**(0): p. 1-17.

[41] Ha, D.M., *et al*., Ethnic Differences in Dietary Intake and the Association between Dietary Intake and Gastric Cancer Risk in Chinese Subjects Resident in Malaysia The Third International Conference on the Development of Biomedical Engineering in Vietnam, R. Magjarevic, Editor, Springer Berlin Heidelberg. p. 255-258.

[42] Peleteiro, B.r., *et al*., *Association between environmental factors and CDX2 expression in gastric cancer patients.* European Journal of Cancer Prevention. **Publish Ahead of Print**: p. 10.1097/CEJ.0b013e32834ef1aa.

[43] Shin, C.M., *et al*., Intrafamilial aggregation of gastric cancer: a comprehensive approach including environmental factors, Helicobacter pylori virulence, and genetic susceptibility. European journal of gastroenterology & hepatology. **23**(5): p. 411-417 10.1097/MEG.0b013e328343b7f5.