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Review Article

Increased cancer risk for the disrupted co-evolution of *Helicobacter pylori* with hosts

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Abstract: *Helicobacter pylori* colonize the human stomach and increase the risk for gastric cancer. It is intriguing, however, that the incidence of gastric cancer is low in some geographical regions or ethnic groups with a high prevalence of *H. pylori*. During past hundreds of years, waves of human migration have resulted in mingled populations of multiple ethnicities in many geographical regions. The incidence of gastric cancer may vary among different ethnic groups in these regions. Studies have found newly acquired *H. pylori* infection or infection with strains replaced from other populations. For ethnic groups with a new acquisition of the bacterium, risk for gastric cancer is usually low owing to the low prevalence of *H. pylori*. In contrast, the risk is increased when an infection with strains from another population occurs. Currently, it appears that the differences in bacterial genomic contents are not responsible for the varied incidence of gastric cancer. Rather, the disruption of the original co-evolution of the bacterium and hosts could contribute to the gastric carcinogenesis. Further understanding the interaction between the bacterium and hosts would benefit for elucidating the carcinogenic mechanisms of *H. pylori*.

Keywords: *Helicobacter pylori*, gastric cancer, genome, evolution, mutation

The human pathogen *Helicobacter pylori* colonizes the stomach of nearly half of the world's population [1, 2]. Persist colonization of the bacterium in infected individuals leads to a variety of diseases. A disastrous outcome, although occurred in a minority of the infected individuals, is gastric cancer [3]. Different incidence of gastric cancer has been found between ethnic groups or geographical regions. It has been found that the incidence of gastric cancer in some geographical regions or ethnic groups is not always parallel to the prevalence of the *H. pylori* infection [4-6]. The occurrence of gastric cancer may be at a pretty low frequency despite of the high rate of *H. pylori* infection [7, 8].

Individuals from different ethnic groups are infected with different populations of *H. pylori* [9]. Over past hundreds of years, extensive human migration leads to mingled populations of multiple ethnicities in some geographical regions. These recent events of human migration have resulted in the alterations of the association of the bacterium with the host, leading to a variation in the incidence of gastric cancer among ethnic groups and geographical regions. It has been found that the interaction between the bacterium and the host is a determinant of cancer risk [10]. In this review, we will summarize the current understanding of the impact of the host-dependent lifestyle on the evolution of *H. pylori* genome, and discuss its roles and mechanisms in the development of gastric cancer.

Intimate association with human hosts

H. pylori have established its colonization in the human stomach since the origin of the anatomically modern human [11]. Along with the human migration, the bacterium segregated into two superlineages [12]. One *H. pylori* superlineage stayed mainly within South Africa. It is composed of only one modern *H. pylori* population, namely hpAfrica2. The other superlineage s-reads around the world with the migration of modern human out of Africa. It currently consists of six modern populations of *H. pylori*: hpAfrica1, hpNEAfrica, hpEurope, hpAsia2, hpEastAsia and hpSahul [13-15]. Each population may separate into a few of subpopulations.

Hosts of a particular ethnic group are generally infected with a specific population or subpopulation of *H. pylori*.

H. pylori spread through a mode of person to person transmission, mostly through a vertical transmission from parents to children [16, 17]. Transmission between siblings or spouses is uncommon. There has no environmental reservoir detected for *H. pylori* owing to that it is unable to proliferate in the environments other than human stomach. This mode of transmission leads to a host-dependent lifestyle of *H. pylori*. This intimate association has been established since the origin of the modern human, impacting profoundly on the bacterial genome [18, 19]. Variations in genomic sequences occur when infecting hosts of different genetic background. For individuals from a particular ethnic group, however, the infected bacteria may share some common genomic features because of the host genetic homogeneity. These adaptive genomic changes result in diversifications in the genome, constituting the basis for the evolution of *H. pylori* into various populations. The prolonged, intimate association of *H. pylori* with human, thus, comes to a situation that a particular ethnic group is generally colonized with a specialized group of *H. pylori*. It is unclear, however, what host responses are upon infection with a different population of *H. pylori*.

Recent acquired infection by nascent human populations

Over past hundreds of years, waves of human migration introduced the bacteria into ethic groups initially free of the bacterium. There are multi-racial groups in Malaysia. Indians, Chinese and native Malay constitutes the major part of Malay population. The infection rate of *H. pylori* in Chinese and Indians from Malaysia is as high as that from China or India [20, 21]. In contrast, native Malay has a very low prevalence of *H. pylori* [22]. Examination of the sequence of house-keeping genes of *H. pylori* reveals that Indians from Malaysia colonize with strains of hpAsia2 or hpEurope which are prevalent in India, while Chinese colonize with strains of hpEastAsia that is predominate in China [23]. In contrast, the most prevalent strains in Malaysia Malay cluster together with Indian strains of hpAsia2 or hpEurope [24]. Only a minority of Malay colonize with strains of hp East Asia. During the past two hundreds of years, Chinese and Indian migrated to Malaysia [25]. It is very likely that native Malay was initially free of *H. pylori* infection [23]. It appears that Malays acquired strains form these new immigrants. Introducing strains of hpEyrope, hpAsia2 and hpEastAsia has been found in other countries of Southeast Asia including Cambodia, Vietnam and Philippines [26]. Recent acquisition of *H. pylori* has also been found in South Africa, where hpAfrica2 is prevalent. Bantu, an isolated group within Cameron, has a low infection rate of approximately 20.8% [27]. Strains of *H. pylori* from Bantu belong unexpectedly to hpAfrica1, which is like those from neighboring regions with high prevalence. Therefore, a recent acquisition of *H. pylori* occurred in Bantu as well. The recent acquisition, both in Malay and Bantu, shows a feature of low prevalence of *H. pylori*. This is possibly attributed to facts that infection can only be acquired in susceptible individuals who get chances to access to infected patients. Besides, the time of the acquisition of the infection is not long enough for a wide spread of the pathogen. Therefore, the low prevalence of *H. pylori* may be caused by a recent acquisition. It would be expected that the low infection rate would cause a low incidence of gastric cancer for ethnic groups with a newly acquired infection.

Genomic differences by bacterial populations

In Malaysia, incidence of gastric cancer is much higher in Chinese than Indians or Malays [21]. This could not be attributed to differences in the infection rate between ethnic groups, since both Malaysian Chinese and Indians have high prevalence of *H. pylori*. Besides, the environmental exposure is much similar among these Malaysia ethnic groups. However, Malaysian Indians and Malays are mostly infected by strains of hpAsia2 or hpEurope [23]. In contrast, Chinese are generally infected with strains belonging to hpEastAsia [24]. Thus, it seems that the bacterial differences between *H. pylori* populations would contribute to the development of gastric cancer.

Genome sequences have been determined in *H. pylori* strains from Malaysian patients of different ethnicities [28-30]. Genomic comparisons identified genes specific to hpEastAsia including a gene encoding a lysozyme family

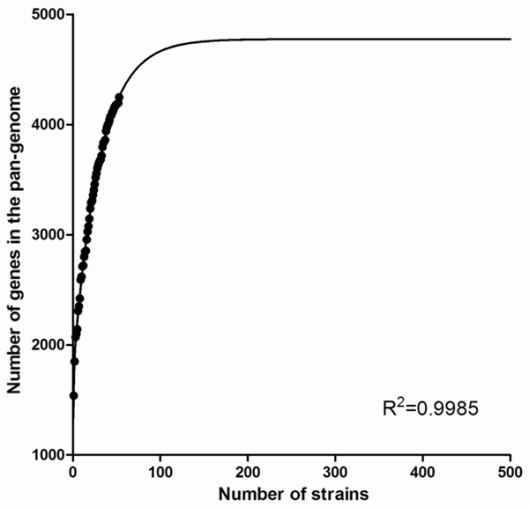

Figure 1. Extrapolated number of genes in the pan-genome of *H. pylori*. The genes in the pan-genome were identified with EDGAR tool based on 53 strains. The number of genes increased exponentially with the increasing number of strains analyzed. The curve showed a mathematical extrapolation of data to a larger number of strains. It showed the total number of genes in the pan-genome approached a maximum of 4774 when 460 strains were analyzed.

protein and three encoding hypothetical proteins [28]. In addition, seven genes were found to be associated with a potentially increased risk for gastric cancer [24]. To validate these findings, we have extracted genome sequences of 53 *H. pylori* strains from GenBank. They are all strains which have a complete genome sequence available at the time of analysis. Our results showed that these genes were not specific to hpEastAsia. Furthermore, none of genes were found to be specific to any particular *H. pylori* population. Therefore, it appears that differences in genomic contents would contribute little to the differential incidence of gastric cancer between ethnic groups.

Since only a small number of strains from each *H. pylori* population have been sequenced up to now, differences in genomic contents could be underestimated. To estimate how many strains would be sufficient for elucidating the virtual genomic differences between populations, we analyzed the pan-genome of *H. pylori*. The pan genome was identified with the EDGAR tool [31]. It was found that the pan-genome of *H. pylori* consisted of 4774 genes (95% confidence intervals, 4689 to 4859) (**Figure 1**), pretty larger than the average gene number (approximately 1600 genes) in a *H. pylori* genome. This is much higher than the previous estimate [32]. Our estimation demonstrated that sequencing 407 additional strains would be sufficient for identification of all genes in the pan-genome (**Figure 1**). This means that the true genomic differences between populations could only be clarified when more strains of *H. pylori* were sequenced, although currently it appears that there was no apparent difference in genomic contents.

Infection with strains of a different *H. pylori* population

South America is populated with Amerindians native to the local region. Over last centuries a mixed population appeared with the migration of European and African into this region [32]. *H. pylori* strains prevalent in South America belong to hpEastAsia (hspAmerind), hpAfrica1 or hpEurope populations [33]. Amerindians are thought to originate from East Asia. They carry *H. pylori* strains of hspAmerind, a subpopulation of hpEastAsia. This has been found in isolated regions of South America [34]. In contrast, hpAfrica1 and hpEurope population strains are prevalent in urban regions, where Amerindian descendents (Mestizos) are dominant [33]. Nearly no strain of hspAmerind was found in Mestizos. Thus, it has been suggested that hpEurope or hpAfrica1 replaced hspAmerind in South America. This replacement has been attributed to the reduced genetic diversity of hspAmerind and lowered competitive capacities [35].

The incidence of gastric cancer is high in South America [36]. There is, however, a variation in the incidence within this region, like that in East Asia [37, 38]. Gastric cancer is more prevalent in mountain areas than coastal regions. In Columbia, the incidence of gastric cancer in Tuquerres and Andean Mountains is 25 times higher than the neighboring coastal town of Tumaco [39, 40]. Since the prevalence of *H. pylori* in these two regions, is similarly high, genetic differences in bacteria and/or host are possibly responsible for the contrasting incidence of gastric cancer. Phylogenetic analyses initially found strains of hpEurope are predominant in mountain areas, while strains of hpAfrica1 in the coastal city [33]. Thus, it looks like that the infection replaced with hpEurope contributes to the increased cancer risk in mountain areas [33]. However, this explanation un-

dermines the impact of host genetic background. When host genotypes and bacterial population were determined simultaneously, findings are surprisingly different [10]. It was found that hosts with the least African ancestry have an augmented risk of gastric cancer if they infect with strains with highest proportion of AA ancestry (ancestral Africa population). In contrast, hosts with highest African ancestry have a lower risk if they infect with such strains. Geographical regions where strains were isolated have no influence on the risk for gastric cancer [10]. These findings demonstrate that the interaction between hosts and *H. pylori* determines the cancer risk. The difference in cancer incidence between mountain and coastal regions could be explained with host-bacteria interactions rather than the geographical locations. Therefore, the infection replaced with a different *H. pylori* population may increase the risk for gastric cancer.

Disrupted co-evolution of *H. pylori* with hosts

Individuals usually acquire *H. pylori* in their childhood from family members [41]. Once acquired, the bacterium persists in the human stomach for a life long time unless eradicated. When invading a new host, the bacterium needs to adapt to changed environments and different immune responses. Examination of the *H. pylori* genome revealed a remarkable increase in mutation rate shortly after the establishment of the infection [42]. This enables the rapid adaptation of the bacterium to the selection pressures from the new host. Thereafter, the bacterium persists in the stomach. However, the mutation rate decreases gradually [43]. Comparison of pairs of strains isolated from gastric antrum and corpus demonstrated that the genetic diversity reduced strikingly than expected [43]. Genetic drift has been suggested to be a major force to remove the genetic polymorphisms [44]. Thus *H. pylori* experience a cycle of genetic alterations during transmission. It presents as "mutation burst" followed by diminishment of diversity by genetic drift until invasion to another host. This becomes the foundation of the genomic evolution of *H. pylori*.

The intimate association of *H. pylori* with human promotes co-evolution of the bacterium. Examining the diversities and evolution of *H. pylori* can precisely illustrate the history of human migration [45]. This indicates the evolution of the bacterium is heavily influenced by the host genetic background. Comparison of strains of hpEastAsia with those from other populations identified dozens of positively selected genes [46]. This demonstrates genetic backgrounds of the Eastern could impose selection pressures over the bacterium, impacting the evolution of *H. pylori*. The major virulence associated genes factors *cagA* and *vacA* are among those positively selected genes [47, 48]. Others include outer membrane proteins which interact with host [49]. These findings indicate the host genetic background can influence the bacterial virulence via impacting on its evolution. Functional and genetic diversities of *babA* illustrate well the influence of host genetic background on the evolution of *H. pylori*. The *babA* gene encodes an outer membrane protein mediating the adhesion of *H. pylori* to epithelial cells [50]. It binds to glycoprotein on the epithelial cells [51]. Adherence to epithelial cells is essential for *H. pylori* to establish colonization, survival and proliferation in the stomach [52]. In South America, Amerindians is entirely of blood O group (expressing fucosylated H1 and Lewis b antigens) and rare of blood A group (expressing A-Lewis b antigen) [53]. 40% strains isolated from Amerindians and Mestizo of this region bind to Lewis b antigen only [52]. In contrast, this is rarely seen in strains from other regions. Thus, it appears that host background promotes the genetic diversification of *H. pylori*, driving the evolution of the bacterium towards a better adaptation to hosts of different ethnic groups.

Co-evolution of *H. pylori* with human appears to modifying the risk for gastric cancer in the infected individuals. This is supported by findings that individuals have an increased cancer risk when colonized with strains from a different *H. pylori* population. For a particular ethnic group, strains they carried have been co-evolved with them for a strikingly long time. Host responses and mucosal injuries are potentially reduced [54]. When they are infected with strains of different populations, the co-evolution is thus disrupted. Hosts may mount an excessive immune-inflammatory response, leading to an increase in the severity of mucosal damages [55]. Gastric microbiota has been suggested to be involved in the carcinogenesis [56]. It has been shown that *H. pylori* infection could influence the composition and structure

of gastric microbiota [57, 58]. Infection with a strain of different *H. pylori* population, thus, could alter the gastric microbiota. This may in turn result in an increased risk for gastric cancer.

In summary, a long history of intimate association of *H. pylori* with human leads to diversification of the bacterial genome towards adaptation to genetically different hosts. However, the impact of the intimate association on the outcomes of the infection remains unclear. Recent studies demonstrate infection with *H. pylori* of a different population disrupts the co-evolution of the bacterium and host, and augment the risk for gastric cancer. However, it is still unclear whether the co-evolution leads eventually to less severe outcomes of the infection. Understanding this issue would help in elucidating the carcinogenic mechanisms of *H. pylori* and guide the clinical practice of eradication.

Disclosure of conflict of interest

None.

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