Helicobacter pylori infection and lung cancer: a review of an emerging hypothesis

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Helicobacter pylori (Hp) is one of the most common bacteria infecting humans. Recently, certain extragastric manifestations, linked to Hp infection, have been widely investigated, suggesting that Hp infection might be a ‘systemic’ disease. Accumulating, yet limited, evidence points to a potential association between Hp infection and lung cancer risk. Epidemiologic studies have shown that odds ratios (estimated relative risks) of lung cancer with Hp infection range from 1.24 to 17.78 compared with the controls, suggesting an increased lung cancer risk in the population exposed to Hp infection although far from supporting a causal relationship between Hp and lung cancer. Many studies have demonstrated the existence of Hp in the mucosa of the upper respiratory tract with no direct evidence of Hp-localization in lung tissue in the published literatures, rendering the possible functional mechanism underlying the association an open question. We followed the classic hypothesis-generating path, where we have thoroughly reviewed the publications on lung cancer and Hp infection from serological association to possible mechanisms as: (i) p130cas activated by Src kinase following Hp–host communication and p130cas-related carcinogenesis as in various malignancies; and (ii) gastroesophageal reflux and inhalation of urease or gastrin, which are Hp-related carcinogenic factors and present in lung tissues. We propose rigorous investigations regarding the Hp–lung cancer association and, if confirmed, the mechanisms of Hp infection leading to lung cancer development and progression. Clarification on Hp–lung cancer association is important for the understanding of lung cancer beyond tobacco-smoking-related carcinogenesis.

Introduction

Despite primary lung cancer being known as a disease caused by environmental carcinogens—inhaled either voluntarily via tobacco smoking or involuntarily from the ambient air—other causes are to be revealed for the victims who had little or minimal such environmental exposures. Globally, 10–15% of lung cancer cases diagnosed in Western countries and approximately one of every four cases in Asia is a never smoker (1–5).

Infectious agents and their potential roles in carcinogenesis have been extensively investigated, e.g. tuberculosis, human papilloma virus, epstein-barr virus and Helicobacter pylori (Hp). More specifically, tuberculosis has been confirmed to be a risk factor for certain lung cancer histologic types decades after the initial epidemiologic observations (6); human papilloma virus has been demonstrated to be correlated with carcinoma of the head and neck (7), oropharyngeal cancer (8) and cervical cancer (9); whereas, epstein-barr virus has been found to be associated with nasopharyngeal carcinoma (10), gastric carcinoma (11), breast cancer (12) and Hodgkin’s lymphoma (13). All of these recognized connections between infectious agents and various cancers have gone through observational and serological epidemiologic investigations.

Along the same line of research, growing attention has been drawn to Hp infection and cancer risks (13,14), based on the estimated odds ratio (OR), which is an effective measure of relative risk to show the higher or lower incidence of a disease in the population exposed to a factor, although it is not used for supporting a causal relationship. Hp is one of the most common bacteria infecting humans. It has been estimated that 20–40% of the USA population is infected with Hp, varied by ethnic groups (15,16). Recently, the extragastric manifestations and carcinogenesis of Hp infection have been studied and investigated widely (14). A growing body of literature has shown an association of Hp with lung cancer.

Intriguingly and importantly, the reported association of lung cancer risk with Hp infection is 5–10 times stronger than with passive smoking exposure (17,18); therefore, Hp infection could potentially be a significant risk factor among non-smoking-related lung cancer. However, the evidence and potential mechanisms supporting a role of Hp in lung cancer are far from clear. Herein, we present an overview regarding the association between Hp infection and lung cancer and a series of hypotheses on the mechanisms possibly underlying this association.

Pathogenesis and carcinogenesis of Hp infection in the stomach

Hp is a spiral-shaped, Gram-negative, microaerophilic bacterium found in the stomach (19). Hp infects approximately 50% of the world’s population, varying widely by geographic area, age, race, ethnicity and social economic status (20), especially with a general trend of higher prevalence in developing countries and higher in non-White populations (16). Data from molecular epidemiological studies indicated that Hp strains from different geographical areas show diverse regional features (21–23). The most likely mode of Hp transmission for the general population is from person to person, by either the oral–oral or the fecal–oral route. Tobacco use or alcohol consumption has not been found to be risk factors for Hp infection. Over 80% of the infected people are asymptomatic, and the remaining 20% are linked to the development of various diseases including chronic gastritis, gastric duodenal ulcers, mucosa-associated lymphoid tissue lymphoma and gastric cancer (24,25). In 1994, the International Agency for Cancer Research (IACR) classified Hp as a class-I human carcinogen for gastric cancer (26). Hp-infected individuals have an up to 20% lifetime risk of developing peptic ulcers and a 1–2% risk of acquiring gastric cancer (27). More specifically, Hp-induced inflammation of the pyloric antrum is more likely to lead to duodenal ulcers; whereas, the inflammation of the gastric corpus is more likely to lead to gastric ulcers and carcinoma (25).

The pathogenesis of Hp mainly depends on the exposure of several bacterial factors to the host, including cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), type IV secretion system (T4SS), outer inflammatory protein A and adherance factors (28). Because of their critical roles in Hp-induced pathogenesis, these factors are currently being intensively studied for their carcinogenic mechanisms.

Cytotoxins and carcinogenesis-associated proteins of Hp

The cytotoxin-associated protein and the vaculating cytotoxin, encoded by CagA and VacA, respectively, are important virulence determinants.
of Hp and may involve complex cellular responses of epithelial cells in Hp pathogenesis and carcinogenesis (29). Based on the phenotypic analyses of clinical isolates of Hp, most of the strains can be classified into two broad groups—those expressing both VacA and CagA (type I) and those producing neither (type II). The remaining Hp strains have an intermediate phenotype (type III), expressing CagA independently of VacA (CagA+VacA−) or vice versa (CagA−VacA+) (30).

CagA has attracted much attention because its expression is closely associated with the development of severe diseases in vivo. The CagA gene is located within the Cag pathogenicity island region on the bacterial chromosome, which encodes proteins important for structure and function of T4SS (31). Approximately, 60–70% of Western Hp strains and almost 100% of East Asian strains express CagA (29). Many studies described that CagA+ strains are closely connected with the development of acute gastritis and pre-neoplastic and neoplastic lesions (32–34). Causative associations between CagA and neoplasia were demonstrated in animal models (35), providing strong evidence for the role of CagA as a bacterium-derived oncoprotein.

VacA, the second most extensively studied Hp factor, enhances Hp virulence though its pleiotropic functions in vivo. The gene encoding VacA is present in virtually all Hp strains (36). However, some strains without VacA activity are found to produce inactive cytotoxin and/or have defective secretion mechanisms, although they possess the gene (37). An association has been shown between VacA and gastroduodenal diseases (e.g. peptic ulcer, atrophic gastritis and gastric cancer) (38). The differences in the VacA structure at the signal region (s1 and s2) and the middle region (m1 and m2) lead to variations in the vaculating activity (39). In general, VacA s1m1 strains produce a larger amount of toxin and induce higher vaculating activity in gastric epithelial cells than other strains; s1m2 strains produce moderate amounts, and s2m2 strains produce very little or no toxin (39). Many studies in Western countries showed that individuals infected with VacA s1 or m1 strains have an increased risk of peptic ulcer or gastric cancer compared with those with VacA s2 or m2 strains (40,41). The m1 strains are common in Northeast Asia, e.g. Japan and South Korea; whereas, the m2 strains are predominant in Southeast Asia, e.g. Taiwan and Vietnam (42,43).

Hp infection and extragastric manifestations
Recently, certain extragastric manifestations, linked to Hp infection, have been investigated. The interesting association has been unveiled between Hp and many abnormal conditions, e.g. cardiovascular (44–46), hematologic (47–49), eye and skin (50–52) and hepatobiological diseases (53–55), diabetes mellitus (56–58) and neurological disorders (59–61). Furthermore, Hp infection has been uncovered to be involved in autoimmune pancreatitis and pancreatic cancer (62), and can increase the risk of transforming growth factor-β1-mediated tumorigenesis by disturbing the balance between apoptosis and proliferation of hepatocytes (53). There is also accumulating evidence for a role of Hp infection in colorectal and laryngo–hypopharyngeal carcinogenesis (63,64). All these above-mentioned data imply that Hp infection might be a ‘systemic’ disease. Equally intriguing, an inverse association between the infection of some Hp strains and esophageal adenocarcinoma in Western countries has been confirmed; whereas, the association with esophageal squamous cell carcinoma is still inconclusive (21–23); thus, the magnitude and mechanism of an Hp-infection-associated tumor may vary by both Hp strain and tumor histological subtype and location (65).

Hp infection and respiratory system localization
Hp is known to localize in the gastrointestinal tract due to its selective advantage of protection against highly acidic environments. It is still unknown whether Hp has the microbiological advantage in the upper or lower respiratory tract; however, many studies have demonstrated the existence of Hp in the mucosa of the upper respiratory tract and the potential role in the development of upper respiratory system diseases, e.g. sinusitis, adenotonsillar hypertrophy, pharyngitis and laryngitis (66–75) (Table I). To date, two attempts to detect Hp in bronchoalveolar lavage fluid and resected lung tissue, respectively, failed to identify Hp (76,77). Serological studies have demonstrated the close associations between Hp infection and lower respiratory system diseases, e.g. chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis and asthma (Table II), though, currently, there is no definite proof of a causal relationship between Hp infection and these diseases (78,79).

It is known that the gastric content can easily reach the airways through gastroesophageal reflux, which is a physiological phenomenon and the key discomfort symptom in a pathological condition, i.e. gastroesophageal reflux disease. If Hp is present in the gastric reflux, it may colonize in the respiratory system through the oral–pharyngeal–laryngeal routes (57). Hp has been isolated from tracheal secretion of intubated patients (88). Equally important is persistent Hp infection in the gastric epithelium, which can trigger chronic inflammation, inducing systemic effects and immune responses that can cause lesions distant from the primary infection site (89). The cytokines and mediators induced by Hp infection include, but not limited to, interleukins-1, -17 and -23, tumor necrosis factor-α, interferon-γ, leukotriene C4 and platelet-activating factor (89,90). In addition, both Hp infection and the respiratory diseases are characterized by their attraction of granulocytes as well as B- and T-cell-mediated responses (78).

All available data on Hp infection in the lungs are based on epidemiological cross-sectional or case-control studies; there are some discrepancies among the studies, likely due to the different patient characteristics and diversity of the methods used to detect Hp (e.g. enzyme-linked immunosorbent assay [ELISA] on serum and IHC, PCR or culture on tissue). It is imperative to test the presence of Hp in lung tissue, as well as the pathogenetic link and mechanism between Hp infection and respiratory diseases.

Hp infection and lung cancer
In the past decade, some investigators have endeavored to clarify the serological association of lung carcinoma risk with Hp infection, with inconsistent results (Table III). Between 2000 and 2010, five regional case-control studies (two from Iran and one from Poland, Turkey and Greece, respectively) have reported an association of lung cancer with Hp infection: three showed a significant OR of 2.51, 5.06 and 17.78, respectively (17,91,92). Two indicated a trend of positive association (OR: 1.24 and 1.35) without reaching a statistical significance threshold (93,94). All five studies were based on small sample sizes, ranging from 40 to 72 cases and 28 to 100 controls. In a meta-analysis that produced the above-mentioned studies, Zhuo et al. (18) found that lung cancer risk among Hp-infected individuals was 3.24-fold compared with the Hp-non-infected controls. There are obvious limitations of these currently published studies. First, the small sample sizes were likely underpowered in the evaluation of the relationship between Hp infection and lung cancer risk; second, not of the all controls were comparable with the cases; third, an important confounder, smoking, was not considered nor fully adjusted as a strong risk factor for lung cancer and fourth, none of the studies explored the lung tissue Hp+ rate and differential association with different lung tumor types. In a recent nested, case-control study, Koshiol et al. (95) reported null results, which were based on participants of a cancer prevention trial in Finland. Two notable strengths of the Finnish study were (i) careful case-control matching and (ii) stratification by tumor histology, which were not considered by all five earlier studies. However, several drawbacks were identified from this study: (i) only cancer-free male smokers were eligible to enroll; (ii) the mean age of the participants was approximately 50 years and (iii) the sero-IgG-Hp positivity was nearly 80% for both the cases and controls, which were substantially higher than other Caucasian populations and particularly in disease-free controls. We hypothesize that Hp infection–lung cancer association may be modified by other known factors, e.g. gender and previous gastric and pulmonary conditions, leading to the conflicting published results.

Hp eradication therapy has revolutionized the concept of treatment and outcome prediction for microbial infection-related malignancies, specifically gastric cancers. Whether Hp eradication therapy can bring beneficial outcomes in Hp–lung cancer patients warrants further study.
Possible functional mechanisms underlying Hp-induced lung cancer

The lungs develop from the same endodermal cells that form the lining of the gastrointestinal tract and also contain cells that release a variety of hormonal peptides (95). We postulate that Hp may induce lung carcinogenesis partly via the similar mechanisms as in gastric cancer.

CagA and lung cancer. In the gastric epithelium, CagA is transported via the T4SS into the host cytoplasm where it activates signal transduction pathways, leading to cancer-associated processes (96). The Glu-Pro-Ile-Tyr-Ala sequences in the CagA protein (CagAα) can induce the development of a dramatic cell elongation, also known as 'hummingbird phenotype', (97) in cultured Hp-infected gastric epithelial cells, which might influence immune response, wound healing, metastasis or invasive growth of cancer cells in vivo. CagA-positive Hp strains have a closer association with the progress of both peptic ulcers and gastric cancer than CagA-negative strains (98). So far, only two studies have investigated the association of CagA and lung cancer; but the results are conflicting (92,95). Specifically, one study points out that the seroprevalence of CagA was significantly higher in cases than healthy controls (92); whereas, the other study shows that the seroprevalence of CagA was similar in cases and in healthy controls (95). So far, there is no report on the association of VacA and lung cancer.

Src/p130cas signal cascades and lung cancer. Hp–host communication is believed to be targeted on focal adhesions, which are strictly regulated during cell migration. Focal adhesions are comprised of α and β integrin heterodimers that form a bridge between the intracellular actin cytoskeleton and the extracellular matrix. Bacterial adhesin CagL located on the tip of the T4SS, can bind with β1 integrin receptor of epithelial cells, which may activate focal adhesion kinase (FAK) and Src kinase (99,100). The activation of FAK and Src leads to the injection of CagA. CagA is initially phosphorylated by Src, which then interacts with Src homology 2 domain-containing tyrosine phosphatase and C-terminal Src kinase to inactivate FAK and Src. P130cas, the substrate of Src kinase, can be recruited and activated by the activation of Src; thereafter, p130cas can recruit a Crk (v-crk sarcoma virus CT10 oncogene homolog)/DOCK180 (dedicator of cytokinesis) complex that has guanine nucleotide exchange factor activity toward Rac1 (101). The critical role of the Crk/p130cas complex in the actin cytoskeleton rearrangement of Hp-infected gastric epithelial cells has been demonstrated, i.e. a promotion of Hp-induced migration and invasive growth of gastric epithelial cells (28). Furthermore, p130cas is implicated in the carcinogenesis and prognosis in a variety of malignancies including lung cancer (102,103), e.g. p130cas RNA interference can cause cell arrest, cell migration inhibition and cell cycle arrest of lung cancer cells (102). P130cas mediates a cell survival signal from cell–matrix interaction and alterations, and prevents lung cancer cells from anoikis (103). Overexpression of p130cas correlates with poor overall survival in lung cancer cases (102). Therefore, the involvement of p130cas in Hp-mediated carcinogenesis in lung cancer deserves further investigation (Figure 1).

Other possible carcinogenic factors and lung cancer

Urease, a surface enzyme of Hp, is involved in Hp infection and survival, and urease messenger RNA was found to be highly expressed...
| First author (ref) | Publication year | Country of study | Number of cases/controls | Type of controls | Case histology | Method | Hp seropositivity cases/controls | OR (95%CI) | Strength and weakness
|-------------------|------------------|------------------|--------------------------|------------------|---------------|--------|---------------------------------|-----------|-----------------------------
| Gocyk (92)        | 2000             | Poland           | 50/100                   | Not specified    | 40 SCC, 10 ADC | ELISA  | 90%/60%                          | 5.06 (1.84–13.90) | Strength: the possible mechanisms of Hp in lung cancer were explored. Weaknesses: small sample size, the comparability between cases and controls was doubted and did not adjust important confounders.
| Philippou (94)    | 2004             | Greece           | 72/68                    | Participants of courses designed for public health education | NA            | ELISA  | 61.1%/55.9%                      | 1.24 (0.63, 2.43) | Weaknesses: small sample size, did not adjust important confounders.
| Ece (91)          | 2005             | Turkey           | 43/28                    | Smokers from healthy patient family members | 22 SCC, 21 ADC | ELISA  | 93%/42%                          | 17.78 (4.42, 71.49) | Weaknesses: small sample size, did not adjust important confounders.
| Najafizadeh (93)  | 2007             | Iran             | 40/40                    | Patients' family members | NA            | ELISA  | 52.5%/45.0%                      | 1.35 (0.56–3.25) | Strength: patient's family members were matched to each case subject on the basis of age to control for socioeconomic status. Weaknesses: small sample size, did not adjust important confounders, such as smoking.
| Behroozian (17)   | 2010             | Iran             | 66/66                    | Patients without lung cancer | 25 ADC, 21 SCC, 16 SC, 4 others | ELISA  | 73%/51%                          | 2.51 (1.14–5.54) | Strength: matched controls with the patients for sex, age and smoking. Weakness: small sample size.
| Koshiol (95)      | 2012             | Finland          | 700/700                  | Cancer-free, matched controls | 350 ADC, 350 SCC | ELISA  | 79.7%/78.5%                      | ADC: 1.1 (0.75–1.6), SCC: 1.1 (0.77–1.7) | Strength: controls were matched one-to-one by age and date of baseline serum draw, adjusted potential confounders. Weaknesses: only cancer-free male smokers were eligible to enroll, mean age of participants was around 50 years and the sero-IgG-Hp positivity was nearly 80% for both cases and controls.

ADC, adenocarcinoma; NA, not applicable/available. SCC, squamous cell carcinoma; SC, small cell lung cancer.

*aWe analyzed the ‘strength (s) and weakness(es)’ of design of each case-control study, considering epidemiologic study design evaluation, sample sizes, confounders and matching strategies.*
in gastric cancer tissues (104). Cell proliferation rate of gastric cancer cells was higher after stimulation of Hp exudates with strong urease activity than with weak urease activity (104). These results suggest that urease may hold an important role in the development of gastric mucosal hyperproliferation. Recently, Hp urease proteins were found to access the lung by gastroesophageal reflux disease, providing an antigenic trigger for the initiation of pulmonary granuloma (105); thus, Hp-associated urease may also have an important role in proliferation and carcinogenesis of pulmonary mucosa.

Hp infection in the stomach is known to remarkably enhance release of gastrin in gastric tissue (106). One study (92) demonstrated that Hp infection is associated with higher levels of gastrin, its receptors and cyclooxygenase-2 than intact bronchial mucosa. Hence, we concur with the hypothesis that Hp infection may result in lung cancer by upregulating gastrin and cyclooxygenase-2, which could stimulate tumor growth and angiogenesis (92).

Fig. 1. Hypothesis of Src/p130cas signal cascades and lung cancer. Hp–host communication is targeted on focal adhesions, which are comprised of α and β integrin heterodimers. Bacterial adhesin CagL, located on the tip of the T4SS can bind with α1 integrin receptor of epithelial cells, which may activate FAK and Src kinase. The activation (phosphorylation) of FAK and Src leads to the injection of CagA. CagA is initially phosphorylated by Src, which then interacts with Src homology 2 domain-containing tyrosine phosphatase and C-terminal Src kinase to inactivate FAK and Src at later time points. p130cas, which is the substrate of Src kinase can be recruited and activated by the activation of Src, holds the carcinogenesis roles in cell invasiveness and migration of lung cancer. α and β, integrin heterodimers; Csk, C-terminal Src kinase; ECM, extracellular matrix; L, CagL; PY, phosphorylation; Src, Src kinase; Shp-2, Src homology 2 domain-containing tyrosine phosphatase.

**Hypothesis of Src/p130cas signal cascades and lung cancer.**

**Conclusion**

Growing attention has been drawn to the potential association between lung cancer risk and infection of Hp, which is one of the most common bacteria infecting humans. The purpose of this review is to summarize what we know to date for an emerging new hypothesis, rather than providing evidence for a causal relationship between Hp and lung cancer. The challenge we face is to further investigate whether, at what magnitude, and at which direction that Hp may be linked to lung cancer risk; and to which subtypes and in which populations. Based on published information, we hypothesize that (i) there is an association between Hp infection and lung cancer; (ii) the magnitude and mechanism of Hp infection-associated lung cancer may vary by both Hp strain and tumor histological subtype and location; (iii) the Hp infection–lung cancer association may be modified by other known factors, e.g., gender and previous gastritis and pulmonary conditions and (iv) Hp infection status or Hp eradication therapy may influence lung cancer treatment responses and clinical outcomes. Considering the public health importance of lung cancer, the promising yet debatable link to Hp infection and the plausible mechanisms of Hp leading to lung cancer, we believe it is time now to rigorously and robustly test the observed association between Hp and lung cancer. Well-designed, prospective and retrospective, multiple-population studies should be conducted including (i) comparing Hp infection rates between cases and controls, between populations and by cigarette smoking history; (ii) evaluating the magnitude of Hp–lung cancer association by tumor staging and histologic features; (iii) assessing the role of effect modifiers from other known risk factors and (iv) evaluating short- and long-term outcomes of Hp(+) and Hp(−) lung cancer patients, with and without Hp eradication therapy. Furthermore, experiments in vitro should be conducted to elucidate possible functional mechanisms of Hp-induced lung cancer.

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