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The Gastric Cardia is not a Target for Human Papillomavirus-induced Carcinogenesis

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Abstract

Background—Thousands of people in central Asia die every year from gastric cardia adenocarcinoma (GCA). GCA arises in the transformation zone between the esophagus and the stomach, similar to cervical and oropharyngeal carcinoma, which arise in areas with transformation zone characteristics. The analogous biology of the gastric cardia to the cervix and oropharynx, where human papillomavirus (HPV) is known to cause cancer, raises the possibility that GCA could be an HPV-associated cancer. Given the availability of an effective HPV vaccine and its potential to prevent HPV-associated cancer, we decided to evaluate the prevalence of HPV DNA in GCA.

Methods—We collected tumor tissue from 144 histopathologically-confirmed GCA patients at Yaocun Commune Hospital, Linxian, China, with rigorous attention to prevent DNA contamination. We tested for the presence of HPV DNA in fresh-frozen tumor specimens using PCR with sensitive *L1*, *E6*, and *E7*-based primers.

Results—DNA was adequate, as indicated by β-globin positivity, in 108 cases. Of these, all (100%, 95% confidence interval: 97%–100%) were negative for HPV DNA

Conclusions—These results suggest that HPV does not contribute to gastric cardia carcinogenesis in north central China.

Impact—Since GCA does not appear to be an HPV-associated cancer, prophylactic HPV vaccination is unlikely to affect rates of GCA in China.

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Disclosure of Potential Conflicts of Interest

Dr. Gravitt has been a paid consultant and has received research funding from Roche Molecular Systems, who manufacture the HPV Linear Array test used in this study. None of the other authors has a personal financial conflict of interest to report.

Introduction

Human papillomavirus (HPV) has been suggested as a risk factor for gastric cardia adenocarcinoma (GCA); one previous study detected HPV16 DNA in 67.6% of 74 GCA tumor tissues (1). Involvement of HPV infection in the development of GCA seems plausible given the biologic similarity between the gastroesophageal junction and the cervix (i.e., a transformation zone); the existence of HPV-associated cervical adenocarcinoma (2); and the shared exposure of gastroesophageal and tonsillar tissue, where HPV is an established risk factor (3). However, there is concern over the potential for contamination by environmental HPV DNA (4,5), which is resistant to cleaners and desiccation (6–8). Given the availability of an effective HPV vaccine and the potential for cancer prevention through use of the vaccine if HPV is involved, we collected fresh frozen tumor specimens from 144 histopathologically-confirmed GCA cases in Linxian, China, with rigorous attention to prevent DNA contamination and tested them using multiple sensitive HPV DNA detection methods.

Materials and Methods

From October 2006 through March 2007, we recruited 144 consecutive incident adult GCA cases undergoing surgical resection at the Yaocun Commune Hospital in Linxian, China, at the same time that we recruited esophageal squamous cell carcinoma (ESCC) cases, as previously described (9). All patients gave written informed consent. Both the National Cancer Institute and Cancer Institute Chinese Academy of Medical Sciences (CICAMS) Institutional Review Boards approved the protocol. With over 100 cases, this study had over 99% power to detect at least one positive sample for an HPV prevalence as low as 5%.

Details of the specimen collection and processing have been published previously (9). In brief, we used a standardized protocol to minimize the potential for environmental HPV to contaminate the tissues. Tissues were snap frozen in liquid nitrogen within 30 minutes of removal from the vasculature in over 90% of the cases and stored in a −70°C freezer. CICAMS and NCI pathologists (DML, MJR, SMD) read H&E stained slides for all cases to confirm the histology and the presence of tumor.

As previously described (9), frozen tissues were homogenized and digested until the tissue was fully dissolved. Phenol:chloroform:isoamyl organic extraction was used to purify DNA in Qiagen high density MaXtract microcentrifuge tubes, and precipitated DNA was pelleted by centrifugation at 4°C, washed with 70% ethanol, and resuspended in 100μL of loTE.

We used the Roche HPV Linear Array Assay to test for HPV DNA in 50μL aliquots of purified DNA according to the manufacturer's instructions (10). This assay uses consensus amplification with PGMY primers for the highly sensitive detection of 37 carcinogenic and noncarcinogenic HPV types. The β-globin gene was co-amplified to assess DNA quality. Samples positive for β-globin were included in the analysis (11). Four positive controls, 10 and 100 HPV16-plasmids and 10 and 100 HPV18-plasmids diluted in a background of 50ng of human placental DNA, were placed directly into PCR. We also tested for the *E6* and *E7* oncogenes of HPV16 and HPV18, the two major carcinogenic types in cervical and oropharyngeal cancer (12,13), using type-specific TaqMan assays targeting the *E6/E7* open reading frame (14,15).

Results

We collected specimens from 144 GCA cases, who came from 9 Provinces throughout north central China. Forty percent came from the Taihang mountain region where the rate of GCA is high, and 60% came from outside this region. The median age was 61 years (range: 40–79),

and 81% (116) were male (Table 1). Sixty-six percent (N=95) of patients were ever smokers, and 49% (N=70) of patients were ever drinkers. All smokers or drinkers were male.

The β-globin signal was strong for 71% of cases (N = 102), weak for 4% (N = 6), and absent for 25% (N = 36). Thus, β-globin, and therefore DNA quality, was adequate in 75% of cases (108/144).

Among the 108 cases with adequate β-globin, all were negative for HPV DNA by Linear Array and *E6/E7*-based PCR (100%, 95% confidence interval: 97%-100%).

Discussion

We found no evidence of HPV DNA in GCA tumor tissue from 108 evaluable resection specimens. These results do not support a previous report of a high prevalence of HPV16 DNA in GCA tumor tissue from China (1). They do, however, support our previous serology-based findings of no association between HPV and GCA in Linxian (16). Patients in our study came from 9 Provinces with high and low rates of GCA, suggesting that our cases may be broadly representative of GCA throughout China. Despite the analogous biology of the gastroesophageal junction and the cervix and the established involvement of HPV in oropharyngeal cancer, HPV does not appear to be involved in gastric cardia carcinogenesis.

A limitation of our study is the high proportion of cases without adequate β-globin. In contrast, 98% (267/272) of ESCC cases that we collected at the same time had adequate β-globin (9), suggesting that the lack of adequate DNA was specifically related to the GCA tissue. It is possible that acid from the stomach began fixing the tissues before they were snap frozen since we required that the stomach section remain unopened until it was received in the tissue processing room to minimize contamination. Alternatively, organs with high connective tissue content might have higher DNA degradation, as has been shown for RNA degradation in gastrointestinal-like calf tissue (17). Despite this limitation, our rigorous effort to prevent DNA contamination in collecting, processing, and testing the specimens is a major strength of this study. In addition, we used a highly sensitive assay for HPV DNA detection, Linear Array, which compared to other systems, better distinguishes between types and has improved detection of multiple HPV types (18). Finally, this study is, to our knowledge, the largest study of HPV in GCA tumor tissue to date.

Taken together, our results suggest that HPV does not contribute to gastric cardia carcinogenesis in this part of China.

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Table 1

Distribution of case characteristics among 144 gastric cardia adenocarinoma cases presenting for surgery Yaocun Commune Hospital in Linxian, China, from October 2006 through March 2007.

Characteristic	N	%
Male gender	116	81
Married	141	98
Current smokers	9	6
Ever smokers	95	66
Current drinkers	6	4
Ever drinkers	70	49
Family history of cancer	38	26