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Short Communication

Non-detection of Epstein-Barr virus and Human Papillomavirus in a region of high gastric cancer risk indicates a lack of a role for these viruses in gastric carcinomas

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Abstract

Gastric mucosa tissue was collected from patients with gastroduodenal diseases in a region of norrteastern China showing a high risk of gastric cancer incidence. The presence of EBV and HPV were assayed to investigate the relationship between gastric carcinomas and virus infection. Neither EBV nor HPV DNA was detected in tissue from the patients. The role of EBV and HPV in gastric cancer is not well understood and still needs to be clarified.

Keywords: Human papillomavirus, Epstein-Barr virus, polymerase chain reaction, gastric carcinoma.

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Gastric carcinoma is the second leading cause of cancer-related deaths worldwide, with a widely varying geographic incidence. It is the most common malignant gastrointestinal tumor and is the primary factor contributing to the the overall mortality from malignant tumors in China. Recent research reported in Brazil reported the prevalence of Epstein-Barr virus (EBV) in patients with gastric carcinomas (Aquino *et al.*, 2012) . The authors obtained biopsies from six control subjects and 10 patients with gastric carcinomas living in Manaus to screen for EBV DNA by PCR. EBV DNA was detected in 8 out of the 10 tumors, but in none of the six control subjects. They suggested that EBV should be considered as a risk factor for this disease, but that an increased sample size would be necessary to obtain statistically more robust results.

A high risk of gastric carcinomas was also reported in a coastal region of northeastern China (Gao *et al.*, 2009), but there is currently no data concerning underlying mechanisms. The most important and common cause of gastric carcinoma is a long-term exposure to nitrosamines in food and drinking water, as well as microbial infections such as *Helicobacter pylori,* and certain viruses, including EBV and Human Papillomavirus (HPV) (Xu *et al.*, 2003; Backert, *et al.*, 2004; Fukayama and Shiku, 2011).

Herein, gastric mucosa tissue was collected from patients with gastroduodenal diseases in a region presenting high risk for occurrence of gastric cancer. The presence of EBV and HPV were assayed to investigate the relationship between gastric carcinomas and viral infection.

A total of 98 patients were involved in the study, including 44 patients with chronic gastritis (24 men and 20 women; mean age 48.5 years), 30 patients with peptic ulcer (17 men and 13 women; mean age 49.4 years), and 24 subjects with gastric carcinomas (10 men and 14 women; mean age,57.2 years).

Three biopsy samples were taken from each patient with chronic gastritis or peptic ulcer who underwent upper gastrointestinal endoscopy. Tumor samples, tissues with adjacent dysplastic epithelium, surrounding lymphocytes, and paired normal gastric mucosa were obtained from gastric carcinoma patients who underwent radical surgical resection at Weihai Municipal Hospital affiliated to Dalian Medical University between January 2011 and September 2012.

The histological subtypes of gastric carcinomas were classified according to the criteria of Lauren (1965) as intestinal type (n = 11) and diffuse type (n = 13). The clinical stage of gastric carcinomas was determined according to the tumor, node and metastasis (TNM) classification of the American Joint Committee on Cancer (Sobin and Fleming, 1997). Our cohort contained 10 patients at stage I or II and 14 patients at stage III or IV. Written and informed consent was obtained from all patients, and the study was conducted upon approval by the Ethical Committee of Weihai Municipal Hospital affiliated to Dalian Medical University. After tissue homogenization using a sterile tissue homogenizer, DNA was extracted using Chelex 100 chelating resin

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(BioRad) according to the manufacturer's protocol (Walsh *et al.*, 1991).

PCR analyses were carried out to determine the presence of EBV DNA as described previously (Aquino *et al.*, 2012). Additionally, a quantitative fluorescence polymerase chain reaction (QF-PCR) method was also utilized to analyze viral presence by means of the EBV Diagnostic Kit (DAAN Gene Co Ltd, Guangzhou). HPV genotypes were detected using the HPV GenoArray test kit (Hybribio Ltd, Hong Kong). The kit employs both DNA PCR-amplification and HybriBio's proprietary flow-through hybridization technique to simultaneously identify 21 HPV genotypes: 13 HR HPV genotypes, 5 LR HPV genotypes, and 3 HPV genotypes common in China, as previously described (Yuan *et al.*, 2011).

Neither EBV nor HPV DNA was detected in any of the patients' tissue, including gastric carcinoma cells, adjacent dysplastic epithelium, surrounding lymphocytes, and paired normal gastric mucosa. Our results place in evidence that the role of EBV and HPV in gastric carcinomas is still poorly understood and needs to be clarified in the future studies, especially in high risk regions such as northeastern China.

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