

***In situ* detection of Epstein Barr virus in gastric carcinoma tissue in China highrisk area**

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Subject headings stomach neoplasms; Epstein-Barr virus; *In situ* hybridization; LMP-1 protein

INTRODUCTION

Epstein-Barr virus (EBV), a gammaherpesvirus, has been strongly associated with African Burkitt's lymphoma and nasopharyngeal carcinoma. Recently it has been identified in lymphoepithelioma-like carcinoma of thymus, tonsil, lung and in some gastric carcinoma^[1-4]. The development of very sensitive methods for detection of EBV infection in archival pathologic tumor sections has allowed us to study the association of EBV with gastric adenocarcinomas by using *In situ* hybridization with EBER-1 oligoprobes and immunohistochemistry with anti-LMP1 antibodies.

MATERIALS AND METHODS

Materials

Cases were selected from a series of primary gastric carcinomas collected at the Department of Pathology, Fujian Medical University, Fuzhou, Fujian. The specimens included 58 primary gastric carcinomas, 5 chronic peptic ulcer and 10 additional specimens of normal gastric mucosa obtained from postmortem patients without gastrointestinal disease. Formalin-fixed, paraffin-embedded tissues were prepared for light microscopic examination.

Method

***In situ* hybridization** The EBV sequence, EBER-1, was detected with a complementary digoxigeninlabeled 30 -base oligomer using a procedure previously described^[5]. A blue-brown or brown color within

the nucleus over background levels was considered positive. In each case, hybridization was applied to section that contained both neoplastic and adjacent non-neoplastic mucosa.

A known EBV-positive nasopharyngeal carcinoma served as positive control and *In situ* hybridization without EBER probe was taken as negative control.

Immunohistology

Paraffin-embedded sections were stained with monoclonal antibodies (CS1-4, DAKO) to evaluate the expression of LMP-1. Immunostaining was performed with the avidin-biotin complex (ABC) method as previously described. For these antibodies, the method of antigen retrieval was used by microwave oven pretreatment in place of proteolytic digestion before immunostaining. LMP-1 positive nasopharyngeal carcinomas were used as positive controls.

RESULTS

Clinical data and histological subtype

The age range of patients was 37-74 years, with a median age of 54.5 years. Fifty patients were males and 8 were females, the ratio of males to females being 6.3:1. Among the 58 gastric adenocarcinomas, 22 were poorly-differentiated, 18 tubular, 10 mucinous, 7 signet-ring cell carcinoma, 1 papillary adenocarcinoma.

EBV gene expression

EBER-1 expression *In situ* hybridization, signals were strong and limited to the nucleus of carcinoma cell (Figure 1). A blue-brown or brown color was considered a positive signal. Six cases (10.3%) showed EBER-1 expression, including 5-poorly-differentiated and 1 papillary adenocarcinoma. In positive cases, virtually all malignant cells were strongly labeled with EBER probes, while the infiltrating lymphocytes, blood vessels and smooth muscle were EBER-1 negative. No EBER-1 signals were observed in adjacent non-neoplastic epithelial cells, dysplastic epithelial cells and normal gastric mucosa.

LMP-1 expression No expression of LMP-1 was seen in both EBER-1 positive and negative cases.

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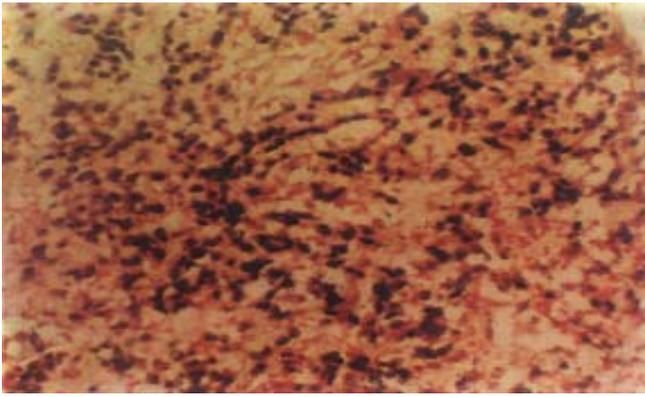


Figure 1 EBER-1 expression in nuclei of essentially all gastric malignant cells. $\times 200$

DISCUSSION

EBER-1 is EBV encoded small RNA, of which high levels expression can be up to 10^6 - 10^7 copies per cell exceeding DNA in EBV-infected cell. EBER oligoprobe may combine with EBER-1 enabling for detecting EBV on paraffin-embedded tissues using non-isotopic labeling and *In situ* hybridization technique which are now considered the most sensitive methods.

EBV is an oncogenic virus which has neoplastic transforming properties. The incidence of EBV infection is high in Chinese population especially in east and south China. In the current study, we had demonstrated the presence of viral RNA in 10.3% of our cases of gastric carcinomas by this method. The frequency of EBV gene expression in gastric malignant epithelium in Fuzhou was higher than that (1.6% to 6.1%) reported in Changsha and Shenyang^[6]. Our study suggested that the frequency of EBV infection was different in different regions of China.

The presence of EBV in neoplasms seemed to be related to the histological subtype of neoplasms. Raab-Traub *et al*^[7], found EBV in undifferentiated and poorly differentiated nasopharyngeal carcinomas, which generally contained a relatively large number of EBV genome equivalents. In Hodgkin's disease, presence of EBV was often in mixed cellularity (HD-MC, 91%) and nodular sclerosis (HD-NS, 43%) subtypes^[8]. In our study, most of the EBV-positive cases were poorly

differentiated gastric carcinomas. The relation between EBV infection and poorly differentiated carcinomas is unknown. Many believed that EBV virus might multiply easier in the poorly differentiated carcinoma, EBV genome amplification favored growth of malignant cells, promote infiltration and metastasis^[7]. We failed to detect LMP-1 expression in this tumor due to the methylation of coding and regulatory regions of this protein^[9].

Shibata *et al*^[10] and Gulley *et al*^[11], found that the EBER-1 was present in dysplastic epithelium of gastric mucosa before malignancy transformation. But our result showed no EBER-1 expression in the adjacent non-neoplastic epithelium, dysplastic cells and normal gastric mucosa. Finally low frequency of Epstein-Barr virus in gastric carcinoma suggests that EBV does not play any important role in the pathogenesis of gastric carcinoma.

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