

Epstein-Barr virus (EBV) and gastric carcinoma in Malaysian patients

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Abstract

Epstein-Barr virus (EBV) has consistently been detected in the tumour cells of nasopharyngeal carcinoma and lymphoepithelial-like carcinoma of the salivary glands, and have occasionally been found in similar tumours at other sites. Moreover, recent studies from various parts of the world including the Orient have shown about 10% of gastric carcinomas to be EBV-associated. We studied 50 gastric carcinomas from Malaysia to investigate its association with EBV in the Malaysian population. They comprised 37 intestinal and 13 diffuse type carcinomas from 32 male and 18 female patients, age range from 29 to 86 years with an ethnic distribution of Malay: Chinese: Indian with the ratio of 4: 27: 19. EBV gene and gene-expression were examined in sections of formalin-fixed, paraffin-embedded tissue using commercially available probes for detecting EBV encoded RNAs (EBERs) by in situ hybridization and monoclonal antibodies to EBV latent membrane protein-1 (LMP-1) by standard immunohistochemistry. Five of 50 gastric carcinomas showed EBER intranuclear positivity in all tumour cells but no cases expressed LMP-1. The EBV-associated cases were classified as intestinal type in 4 and diffuse type in one case and all were histologically unremarkable. EBV-positive tumours were found in 3 Chinese and 2 Indian patients with none in the small Malay group. Four EBV-positive tumours were in male patients, with age-range of 65 to 86 years. We conclude that our findings of about 10% of Malaysian gastric carcinomas being EBV-associated is in line with the results from other parts of the world and from other ethnic groups.

Key words: Epstein-Barr virus, gastric carcinoma

INTRODUCTION

Epstein-Barr virus (EBV) was originally discovered by Epstein and associates in 1964 with the ultrastructural demonstration of Herpes-like virus particles in cultured Burkitt lymphoma tumour cells.¹ EBV is the primary aetiological agent of infectious mononucleosis, and has been linked to an increasing number of neoplastic conditions including Burkitt lymphomas, other B and T cell non-Hodgkin and Hodgkin lymphomas, particularly in immunodeficiency-related conditions as well as epithelial neoplasms occurring notably in the nasopharynx.^{2,3,4} Most nasopharyngeal carcinomas are undifferentiated and accompanied by intense lymphoid infiltration (hence the term lymphoepithelioma) and this tumour was the first epithelial malignancy shown to have consistent association with EBV. A similarly strong association of EBV with lymphoepithelial-like carcinoma of the salivary glands have been demonstrated in both the Eskimo and Oriental

populations,^{5,6} and also most cases of lymphoepithelial-like carcinoma of the thymus and lung have been EBV positive.⁷ The association of EBV with undifferentiated lymphoepithelioma-like carcinoma^{8,9,10,11,12} and adenocarcinoma^{13,14,15,16} of the stomach has also been demonstrated recently.

Many such tumours arising in the stomach have been examined for the presence of EBV. In a proportion of these, the virus was detected by in-situ hybridization (ISH) for EBV encoded RNA (EBER), and in all these studies EBV was localized to the neoplastic cells.⁹ DNA extracts of 20 tumours have also been examined by Southern blot hybridization leading to the detection of a single terminal repeat restriction fragment.¹² Such data provide strong evidence that these tumours represent clonal expansions of single EBV-infected cells.

Shibata and Weiss⁹ were the first to report on EBV infection in gastric adenocarcinoma of the intestinal and diffuse type without prominent

lymphoid infiltration. By using EBER ISH, infected tumour cells were identified in 22 (16 %) of 138 cases. Adjacent dysplastic epithelium was also found to harbour the virus which, however, was not detected in the surrounding lymphocytes, normal stromal cells, intestinal metaplastic epithelium or normal gastric mucosa.

Depending on the analysis of unselected and selected cases, the incidence of EBV in gastric carcinoma has ranged from <2% to 18%.^{12,14,15,16} There are some regional differences in the incidence of EBV positive gastric carcinoma, with the highest in the USA (16%) and Germany (18%), and the lowest (4 %) in China. Other countries have included Korea (14 %), Japan (7 %), Taiwan (11 %), Hong Kong (7 %), Russia (8 %) and Egypt (13 %).¹⁷

MATERIAL AND METHODS

The cases were selected from the archives of the Pathology Department, Hospital Ipoh. They were consecutive gastrectomy specimens for gastric carcinoma from 1994 till 2000 and no cases were selectively left out unless the specimens were inadequate for ISH study. The histology was reviewed and the demographic information obtained from the histopathology request forms.

EBER was detected by ISH of sections of formalin-fixed, paraffin-embedded tissue using the EBV Probe ISH Kit from Novocastra Laboratories (Newcastle, UK) using the manufacturers instructions. EBV latent membrane protein-1 (LMP-1) was detected by standard immunohistochemistry using the CS.1-4 monoclonal antibodies (Dako, Glostrup, Denmark) following microwave pretreatment with citrate buffer. Lymph nodes with EBV-positive Hodgkin lymphoma were used as positive control.

RESULTS

A summary of our data are listed in Table 1. The patients' ages ranged from 29 to 86 years with a racial distribution ratio of Malay: Chinese : Indian of 4: 27: 19. The male to female ratio was 1: 1.8. 37 cases had histological features of intestinal and 13 of diffuse type. No lymphoepithelial-like carcinoma was found, however, one tumour of interest showed in addition focally lymphoepithelial-like differentiation.

Five of 50 (10%) cases of the gastric adenocarcinomas (Table 1) showed uniform

EBER positivity in all the tumour cell nuclei but were LMP-1 negative (Fig. 1). The surrounding lymphocytes (present in some cases) were EBV negative.

TABLE 1: Summary of EBV associated gastric carcinoma

	Number examined	No. EBV positive	% positive
Gastric carcinoma	50	5	10
Female	18	1	5.6
Male	32	4	12.5
Intestinal	37	4	10.8
Diffuse	13	1	7.7

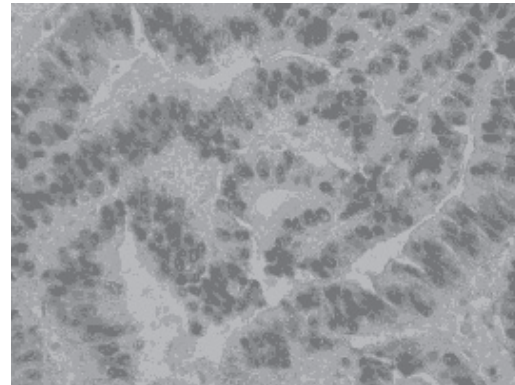


FIG. 1: Expression of EBV encoded RNA (EBER) in intestinal type gastric carcinoma detected by in situ hybridization × 40.

EBV positive tumours were found predominantly in males. The male to female ratio was 4:1. Three of the patients were Chinese, 2 Indians and one Malay. Four EBV positive tumours were of intestinal and one of diffuse type. The intestinal type carcinoma that showed a focus of lymphoepithelial-like differentiation was negative for EBV.

DISCUSSION

The gastric lymphoepithelial-like carcinomas that resemble nasopharyngeal carcinomas are commonly positive for EBV.^{8,10} However, tumour cells in both intestinal and diffuse type gastric carcinoma have also shown the presence of EBV in some cases. Thus EBV-associated gastric carcinomas have shown no distinct histopathological features. There was no

lymphoepithelial-like gastric carcinoma in this series, and the only intestinal type with focal lymphoepithelial-like carcinoma was negative for EBV. Most of our gastric tumours lacked intense lymphoid stroma. Occasionally, mild lymphoid infiltration was present but this was negative for EBV. Gastric carcinoma has a higher incidence in males and EBV-associated gastric carcinomas have predominantly been found in men, a finding also evident in our study. However, the aetiology of this sex bias is unknown.

The biological importance of EBV genomes in gastric carcinoma is debatable. The presence of the EBV genome and its expression in gastric carcinoma tumour cells raises the possibility that EBV may contribute to the neoplastic transformation. However, it is currently unknown if EBV infection of the gastric epithelial cells occur before or after malignant transformation. EBV infection might occur after neoplastic transformation has induced aberrant expression of EBV receptors. Alternatively, the gastric EBV infection might occur before and contribute to transformation. Changes associated with risk for gastric carcinoma in benign gastric mucosa may induce the expression of an EBV receptor and therefore allow infection to precede transformation. We conclude from this study that EBV is associated with about 10% of Malaysian gastric carcinoma, which is a finding in line with the results from other parts of the world and from other ethnic groups. EBV positive cases were found in otherwise unremarkable cases of intestinal type and diffuse type gastric carcinoma.

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