Absence of Epstein-Barr virus in esophageal squamous cell carcinoma

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SUMMARY. It is strongly suspected that the Epstein-Barr virus (EBV) plays a role in the genesis of nasopharyngeal and gastric carcinoma. The aim of this study was to search for such a connection between esophageal squamous cell carcinoma (ESCC) and EBV. We investigated 104 surgically resected esophageal cancers using in situ hybridization (ISH) for EBV-encoded RNA (EBER). We found no EBER-positive cancer cells in any tests, although there were five samples in which EBER-positive tumor-infiltrating lymphocytes (TILs) were found. We conclude from this study that EBV is not associated with ESCC.

KEY WORDS: Epstein-Barr virus, esophageal squamous cell carcinoma, etiology.

INTRODUCTION

The prevalence of esophageal carcinoma differs in different parts of the world, with the highest risk areas in China and Iran.1,2 In Thailand, the highest incidence is in the south in Songkla province with the age-standardized incidence rate of 6/100,000 males.3 Most have squamous cell carcinoma, as in other parts of Asia, in contrast to adenocarcinoma which is more common in Western countries. Alcohol drinking and tobacco smoking are associated risk factors.2,4 Other etiologies are discussed, including Epstein-Barr virus (EBV) infection.

EBV is a lymphocryptovirus belonging to the gamma-herpes viriane family. Its primary appearance is as an etiologic agent of infectious mononucleosis, which is a self-limiting disease, although EBV infection has also been implicated in the development of a variety of malignancies, including nasopharyngeal carcinoma, gastric carcinoma, and lymphoma.7-10 An association between EBV and esophageal squamous cell carcinoma (ESCC) is also suspected: there are both negative and positive studies concerning this and the theory is still controversial.11-20 We analyze the association of EBV and ESCC in one institution in Thailand.

MATERIALS AND METHODS

Surgical specimens were collected from 104 patients with esophageal squamous cell carcinoma who underwent esophagectomy between January, 1998 and December, 2003 at Prince of Songkla University Hospital.

In situ Hybridization

An in situ hybridization (ISH) study for EBV mRNA was performed on formalin-fixed, paraffin-embedded tissue using the Epstein-Barr virus Probe ISH Kit (Novocastra Laboratories, UK). The EBV probe is hybridized to abundantly expressed Epstein-Barr virus encoded RNA (EBER) transcripts which are concentrated in the nuclei of latently infected cells, as previously described.21 The ISH procedure steps followed the manufacturer’s manual. Briefly, tissue sections of 5 microns were deparaffinized with xylene, rehydrated in 99% ethanol, 95% ethanol and graded water, respectively and digested with proteinase K (7 mg/mL in 50 mmol/L Tris/HCl, pH 7.6) for 30 min at 37°C. After dehydration and air-drying, fluorescein-labeled oligonucleotide cocktail probes were applied to the sections for 2 h at 37°C, then blocked with normal rabbit serum. Rabbit F(ab') anti-FITC/AP was added for 30 min followed by overnight incubation of the enzyme substrate solution (BCIP/NBT/Levamisole). The slides were washed in running tap water and mounted with glycerol buffer. Appropriate positive and negative controls were run in every batch tested.
A known EBV-positive Burkitt’s lymphoma was used as a positive control, and non-probed samples from each specimen as a negative control.

**RESULTS**

The 104 patients ranged in age from 43 to 76 years (median age, 63 years); 82 of these were male, and 22 female. The EBER-ISH did not show the presence of EBV in tumor cells in any cases. The EBER signal was present in only a few of the tumor-infiltrating lymphocytes (TILs) surrounding the carcinoma in five cases. Less than 5% of the total lymphocyte background were positive-stained cells.

**DISCUSSION**

There are many studies concerning the association between the EBV and ESCC. There are two popular and accepted techniques to identify the EBV–ISH for EBER expression and PCR for EBV DNA.

All of the positive reports used the PCR technique. Jenkins et al. found EBV present in 8.3% of the cases (5/60) they studied in the USA. Sedaghat et al. found EBV in 42.8% of cases (12/28) from Iran. Wang et al. found EBV in 35% (11/31) of cases from Taiwan and Awerkiew et al. found EBV in 35% (8/23) of cases from Germany. In contrast, the negative reports used the ISH and/or the PCR technique. There are three reports from Japan; Yanai et al. found all of their 36 samples were negative by ISH and reported 16.7% (6/36) EBER-positivity in TILs. Mizobuchi et al. found all of their 41 surgical specimens and the 12 cell lines were negative by PCR. Hong et al. found all of their 30 cell lines were negative by PCR. The results were similar in two reports from China; Chang et al. found none of the 103 carcinomas tested were ISH positive, and Wang et al. reported all of their 51 samples were negative by both techniques, with a further three cases of EBV presenting in TIL. Cho et al. from Korea found none of their 142 cases were positive by ISH and EBER-positive TIL was found in eight of 142 cases (5.6%).

Our data of ESCC revealed no EBER signal, which would be found in the nuclei of latently infected cells. Moreover, we found EBV-positive TILs in five ESCC cases using ISH. TILs have been considered a manifestation of a host immune response to inflammation or cancer cells. These observations were similar to the previous studies.

We propose that the EBV identified in ESCC by PCR may be derived from contaminated TILs, and the EBV reservoir lymphocytes present in tumors as in other organs in the body. We could come to no other conclusion from our data but that EBV does not play a role in the etiology of ESCC.

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**References**