

# Absence of Epstein-Barr virus in oesophageal squamous cell carcinoma

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## Abstract

**Aim**—To identify the possible role of Epstein-Barr virus (EBV), in Chinese patients, in the pathogenesis of oesophageal squamous cell carcinoma.

**Methods**—Formalin fixed, paraffin wax embedded tissues from 74 cases of oesophageal squamous cell carcinoma (28 with well differentiated, 27 with moderately differentiated and 18 with poorly differentiated carcinomas) were analysed for EBV using in situ hybridisation for EBV encoded small RNAs.

**Results**—EBV was detected in only a few lymphocytes adjacent to the tumour epithelia in 14 (19%) cases of oesophageal carcinoma. The adjacent, non-pathological oesophageal tissue was EBV negative.

**Conclusions**—EBV does not play a major role in the aetiology of oesophageal squamous cell carcinoma.

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Keywords: Epstein-Barr virus, oesophageal carcinoma, in situ hybridisation, EBER.

Oesophageal cancer is a disease of high incidence and mortality in many regions of the world.<sup>1</sup> In Hong Kong, this disease is the sixth most common cause of death due to cancer. Epidemiological and experimental studies have suggested that some chemical agents, nutritional deficiencies and physical factors are associated with the development of oesophageal cancer<sup>2</sup>; however, they have failed to isolate the definite aetiological agent of oesophageal cancer. There is some evidence that certain micro-organisms, such as several viruses, fungi and bacteria, have an aetiological role in the carcinogenesis of oesophageal cancer. Of these micro-organisms, the relation between human papillomavirus (HPV) and oesophageal cancer has been studied most extensively. Nevertheless, in a study a few years ago we failed to demonstrate HPV using DNA slot blot analysis and in situ hybridisation (ISH) in oesophageal squamous cell carcinomas in Hong Kong Chinese.<sup>4</sup> One possible limitation of that study was that these methods lacked sensitivity as the polymerase chain reaction (PCR) was not used to amplify the DNA. The close proximity of the oesophagus to the nasopharynx raises the possibility that Epstein-Barr virus (EBV) may be involved in the carcinogenesis of oesophageal cancer. EBV is a ubiquitous human herpes virus; over 90% of adults worldwide are seropositive for EBV.<sup>5</sup>

This virus shows tropism for both lymphoid and epithelial cells, and it is classically associated with nasopharyngeal carcinoma and Burkitt's lymphoma. Recent studies have indicated that EBV may be implicated in the pathogenesis of oral hairy leukoplakia in patients with AIDS, lymphoproliferative disorders in immunocompromised patients as well as other lymphoid and epithelial malignancies.<sup>6-8</sup> The EBV related lymphoid malignancies include Hodgkin's disease, T cell lymphomas, nasal lymphomas of T and natural killer cell phenotype, some B cell lymphomas, and lymphomas arising in organ transplant recipients and immunocompromised patients (including those with AIDS). EBV related carcinomas usually resemble nasopharyngeal carcinomas, being undifferentiated and with a prominent lymphoid stroma (lympho-epithelioma-like/undifferentiated carcinoma). They include undifferentiated carcinomas especially those of foregut origin (for example, salivary gland, stomach, thymus, and lung).<sup>8-13</sup>

Recently, EBV was demonstrated in the nuclei of carcinoma cells using ISH in a Japanese patient with undifferentiated carcinoma of the oesophagus.<sup>14</sup> To our knowledge, EBV has not been demonstrated in cases of oesophageal squamous cell carcinoma to date. Hong Kong has a high incidence of oesophageal carcinoma and nasopharyngeal carcinoma in comparison with Western countries<sup>15</sup> and nearly all of the nasopharyngeal carcinomas in Hong Kong are EBV positive. Thus, the aim of this study was to determine whether EBV has a role in the aetiology of squamous cell carcinoma in Hong Kong Chinese.

## Methods

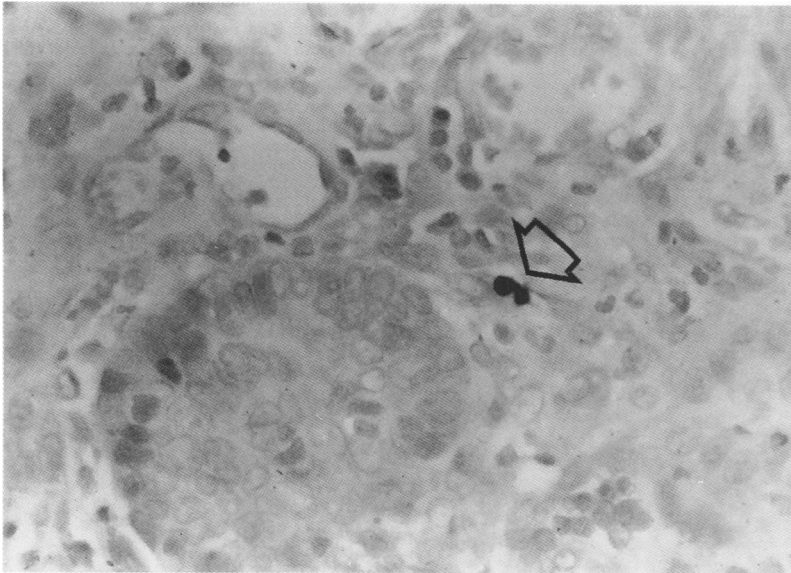
Seventy four cases (62 men and 12 women) of oesophageal squamous cell carcinoma were retrieved from the files of the Department of Pathology at Queen Mary's hospital. The patients had undergone surgery between 1990 and 1993, and ranged in age from 39 to 89 years (mean 63 years). Tissue was excised from the upper portion of the oesophagus in six (8%) cases, from the mid portion in 44 (60%) and from the lower portion in 24 (32%). Haematoxylin and eosin stained sections were reviewed and the diagnosis confirmed. Of the 74 cases, 28 (38%) had well differentiated, 27 (36%) had moderately differentiated and 19 (26%) had poorly differentiated squamous cell carcinoma. The degree of lymphoid infiltration was also noted for each case.

Sections (5 µm) from representative blocks were deparaffinised, rehydrated and pretreated

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EBER-ISH in a case of oesophageal squamous cell carcinoma. Note the positive reaction (arrow) in the nucleus of a lymphocyte in the tumour stroma. (Original magnification  $\times 500$ .)

with proteinase K (Sigma, St Louis, Missouri, USA, P8038). ISH for EBV encoded small RNAs (EBERs) was then performed on these sections using the EVB-ISH kit from Dako (Kyoto, Japan), in RNase-free conditions at room temperature according to the manufacturer's instructions. The fluorescein conjugated EBER oligonucleotide probe was detected using alkaline phosphatase conjugated rabbit anti-fluorescein isothiocyanate antibody (Fab<sub>2</sub> fragment). Nitro blue tetrazolium, together with 5-bromo-4-chloro-3-indolylphosphate (BCIP), was used as substrate and a case of nasopharyngeal carcinoma served as a positive control. The slides were then mounted and examined. Positive staining was regarded as black colour at the site of hybridisation.

### Results

None of the epithelial cells in the 74 cases of oesophageal squamous cell carcinoma was positive for EBV. Strong signals were observed in the nuclei of the tumour cells in the control case of nasopharyngeal carcinoma, demonstrating the specificity of this technique in detecting EBV. Positive signals with the EBER probe were also seen in occasional infiltrating lymphocytes in the tumour stroma (figure) of 14 oesophageal squamous cell carcinomas (five well differentiated, two moderately differentiated and six poorly differentiated). The number of lymphocytes positive for EBV was less than five in all except one case in which 12 lymphocytes were positive. A large portion of these cases were poorly differentiated squamous cell carcinomas (43%) although there was, in fact, no significant correlation between tumour differentiation and the presence of EBV positive lymphoid cells ( $p > 0.1$ ; Fisher's exact test). All cases with EBV positive lymphocytes had a moderate to severe lymphoid infiltrate around the clusters of tumour cells. However, a mild to moderate lymphoid infiltrate was also noted in the stroma of other tumours. In

addition, there was no significant difference between those patients with or without EBER positive lymphocytes with respect to age ( $p = 0.40$ ; Student's *t* test), sex ( $p = 0.44$ ; Fisher's exact test) or tumour site ( $p = 0.99$ ; Fisher's exact test).

### Discussion

EBV DNA has been found in diverse sites of the body in patients without apparent EBV related diseases.<sup>16</sup> It has been detected in our department by PCR in the upper digestive tract—for example, salivary glands, tonsils, nasopharynx, and larynx. The proximity of these organs may suggest that the oesophagus may also harbour EBV. EBV has also been demonstrated in oesophageal epithelial cells in some cases of AIDS associated oesophageal ulceration.<sup>17</sup> In the present study, however, EBV was not detected in normal oesophageal epithelium. EBV, if present, is more likely to be found in infiltrating lymphocytes rather than in epithelial cells. It could also be argued that EBV is present at a level below the detection threshold of ISH.

Mori *et al*<sup>14</sup> detected EBV using ISH in a case of oesophageal undifferentiated carcinoma (lymphoepithelioma-like carcinoma) but in none of 29 cases of squamous cell carcinoma. The absence of EBV RNA in the tumour cells of oesophageal squamous cell carcinomas in the present study also suggests that EBV does not play a significant role in the pathogenesis of this tumour. These findings concur with those of Wong *et al*<sup>18</sup> who studied oesophageal carcinoma cell lines of Chinese origin.

The significance of positive signals in occasional lymphocytes in the stroma of the some cases of oesophageal squamous cell carcinoma remains unclear but may indicate a previous EBV infection. Those positive signals, present in carcinoma rather than normal cells, may reflect the presence of more EBV positive lymphocytes in oesophageal carcinomas and thus a greater chance of picking up EBER positive cells. However, a remote possibility that EBV acts as a cofactor in the carcinogenesis of oesophageal carcinoma cannot be excluded completely. EBV may alter the tissue microenvironment and thus affect the susceptibility of oesophageal epithelial cells to carcinogens.

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