Epstein-Barr Virus-associated Gastric Adenocarcinoma among Japanese Americans in Hawaii

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Abstract

This study confirms the observation that some gastric adenocarcinomas contain Epstein-Barr viral (EBV) sequences in their carcinoma cells. EBV sequences were detected by polymerase chain reaction and in situ hybridization in the tumors of 19 of 187 (10.2%) Japanese-American men and women living in Hawaii. The EBV-associated gastric cancers were more frequently present in men than in women: 14 of 99 (14.3%) men versus 5 of 88 (5.7%) women (P = 0.046). EBV type A was found in 17 of the 19 EBV-associated cancers, a finding consistent with the type A predominance in Japanese populations. Intestinal and diffuse-type tumors were both EBV-positive, and moderate to marked inflammation was usually present. The virus was not found in adjacent normal nonneoplastic mucosal cells or in mucosa showing intestinal metaplasia. EBV-associated tumors were found at stages 1 or 2 in 53% of cases, compared with 36% of the EBV-negative cancers (P = 0.13). The presence of EBV did not appear to influence survival. The relatively high incidence of gastric cancer compared to other EBV-associated tumors makes EBV-associated gastric cancer potentially one of the most common EBV-related tumors in the United States.

Introduction

Many human tumors are linked to viral infection. EBV was the first virus associated with human neoplasia. EBV has been found in the tumor cells of nasopharyngeal carcinoma (1, 2) and other lymphoproliferative tumors (3, 4). Intestinal and diffuse-type tumors were both EBV-positive, and moderate to marked inflammation was usually present. The virus was not found in adjacent normal nonneoplastic mucosal cells or in mucosa showing intestinal metaplasia. EBV-associated tumors were found at stages 1 or 2 in 53% of cases, compared with 36% of the EBV-negative cancers (P = 0.13). The presence of EBV did not appear to influence survival. The relatively high incidence of gastric cancer compared to other EBV-associated tumors makes EBV-associated gastric cancer potentially one of the most common EBV-related tumors in the United States.

Materials and Methods

Patients. Gastric cancers from 187 patients were analyzed. There were 99 men from the Japan-Hawaii Cancer Study, a long-term, well-characterized cohort of Japanese-American men living in Hawaii (12). The men were born in the years 1900 through 1919, had registered for military service in Honolulu in the years 1940-1942, and were subject to periodic examinations beginning in 1965. Gastric cancers from 88 Japanese-American women living in Hawaii were also examined. Tumor sections obtained from gastrectomy specimens were examined microscopically, and the cancers were classified by one of us (G. N. V.) according to the criteria of Lauren (13). For this presentation, the intestinal-mixed-other type will be referred to as the intestinal type (14). The tumors were staged according to the American Joint Committee Manual for Staging of Cancer (15). In addition, the corresponding formalin-fixed paraffin-embedded gastric cancer specimens were examined as below for the presence of EBV sequences. Clinical data were obtained from the Hawaii Tumor Registry. Fisher’s exact test with one-sided P values and the Mantel-Cox test were used for statistical analysis.

Polymerase Chain Reaction. DNA was extracted from the formalin-fixed, paraffin-embedded tissue sections from each cancer specimen and examined for EBV sequences as previously described (11). A PCR assay against the EBNA1 EBV gene was used to screen all specimens. The EBV-positive and -negative specimens were all positive for the human p-globin sequence, verifying the suitability of the extracted DNA for PCR. The EBV-positive specimens were further amplified with primers which amplify a polymorphic region of EBNA2 which differs between types A and B EBV variants (16).

In Situ Hybridization. ISH was performed on the EBV PCR-positive specimens using a biotin-labeled EBER1 oligomer probe as previously described (11). In addition, 30 EBV PCR-negative cancers were subjected to ISH and were uniformly negative except for rare positive signals in scattered lymphocytes. A gastric cancer was consid-
EBV and Gastric Adenocarcinoma

Table 1  Comparison of EBV-positive and EBV-negative gastric adenocarcinoma

<table>
<thead>
<tr>
<th></th>
<th>EBV-negative</th>
<th>EBV-positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>168 (89.8%)</td>
<td>19 (10.2%)</td>
<td>187</td>
</tr>
<tr>
<td>Male</td>
<td>85 (85.9%)</td>
<td>14 (14.3%)</td>
<td>99</td>
</tr>
<tr>
<td>Female</td>
<td>83 (94.3%)</td>
<td>5 (15.7%)</td>
<td>88</td>
</tr>
<tr>
<td>Mean age</td>
<td>69.1</td>
<td>69.5</td>
<td>69.2</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52 (31.0%)</td>
<td>7 (36.8%)</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>8 (4.8%)</td>
<td>3 (15.8%)</td>
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<tr>
<td>3</td>
<td>84 (50.0%)</td>
<td>7 (36.8%)</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>24 (14.3%)</td>
<td>2 (10.5%)</td>
<td>26</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>119 (70.8%)</td>
<td>17 (89.5%)</td>
<td>136</td>
</tr>
<tr>
<td>Diffuse</td>
<td>49 (29.2%)</td>
<td>2 (10.5%)</td>
<td>51</td>
</tr>
</tbody>
</table>

ered EBV associated if it was EBV PCR positive and demonstrated labeling of the tumor cells by ISH.

Results

EBV was detected by PCR and ISH in 19 of the 187 (10.2%) specimens (Table 1). EBV type A was detected in 17 of the cases, and Type B was detected in one female case. The EBV in one tumor could not be typed as A or B. No mixed infections were detected. Grossly, the EBV-associated cancers were present in the antrum or corpus, and were polypoid or ulcerated. The tumors were often very large with an average size, measured as a cross-product of two dimensions, of 47 cm². Intestinal metaplasia was present in all specimens. In two patients, double primary gastric cancers were present. Both tumors were EBV-associated in one patient (Case 1), while only the larger tumor was EBV-associated in the other patient (Case 2). Another patient (Case 3) had a metachronous colon adenocarcinoma that was EBV-negative.

The EBV sequences were specifically identified within the carcinoma cells and evenly labeled by the ISH assay (Fig. 1). The EBV EBER1 signals were localized to the nucleus as previously reported (17). Tumor cells were EBV-negative by ISH in 11 cases in which the sections were weakly EBV-positive by PCR. In these cases, scattered lymphocytes were EBV-positive by ISH. Similarly, scattered lymphocytes in some EBV-associated gastric adenocarcinomas were EBV-positive by ISH. There was no apparent relationship between the EBV-positivity of these lymphocytes and the tumor. There was a sharp demarcation between the EBV-positive neoplastic and the EBV-negative normal epithelium. The surrounding intestinal metaplasia was EBV-negative. However, some

![Fig. 1](image-url)  
EBV-associated gastric adenocarcinoma of the intestinal type. A, ISH for the EBV EBER1 RNA showing the junction between the EBV-negative normal mucosal with intestinal metaplasia and the EBV-positive gastric adenocarcinoma cells. × 20. B, higher power of the junction between the tumor and intestinal metaplasia. H&E, × 100. C, corresponding ISH demonstrating EBV-positivity is restricted to the adenocarcinoma cells. × 40. D, higher power demonstrating the expected localization of EBER1 RNA to the nuclei. × 400.
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Dysplastic cells present at variable distances from the primary tumor were positive for EBV (Fig. 2). It is uncertain whether these dysplastic cells represent extensions of the primary EBV-associated cancer or residual EBV-infected dysplastic epithelium.

There were few morphological features which distinguished between the EBV-positive and -negative carcinomas. The majority (89.5%) of the EBV-associated tumors were of the intestinal type, but this proportion was not significantly greater than the frequency (70.8%) of intestinal-type EBV-negative tumors \( (P = 0.065) \). The EBV-associated gastric cancers tended to have a greater infiltration with acute and chronic inflammation, especially at the tumor margins, but this was not specific, since some EBV-negative cases had similar infiltrations. This diffuse, moderate to intense heterotypic leukocytosis characterized all but one EBV-associated cancer, although only three of the EBV tumors fell into the category of gastric cancer with intense lymphoid infiltration \( (18) \) (Table 2). The exceptional tumor was a Stage 4 intestinal cancer (Case 3) with focal areas of intense neutrophilic infiltration. The deep margins of most of the EBV-associated tumors were rounded and well defined. Some of the deepest aggregates of tumor cells were more poorly differentiated and resembled lymphoepithelial-like carcinomas with transformation of the glandular architecture into islands surrounded by lymphocytes (Fig. 3).

EBV-associated gastric cancers were significantly \( (P = 0.046) \) more common in men (14.3%) compared to women (5.7%). The mean ages between the two groups were similar. There was no apparent change in the incidence of EBV-associated gastric cancer in this group of patients over the period from 1972 to 1991 (data not shown). The EBV-associated tumors were detected at earlier stages (53%, Stages 1 and 2) compared to EBV-negative tumors (36%, Stages 1 and 2), but this difference was not found to be statistically significant \( (P = 0.132) \). Similarly, there was no significant survival difference between the EBV-positive and -negative male cases, al-

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**Table 2** Characteristics of EBV-associated gastric adenocarcinoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Age</th>
<th>Sex</th>
<th>Type</th>
<th>Stage</th>
<th>Survival</th>
<th>Size</th>
<th>Location</th>
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<tr>
<td>1</td>
<td>62</td>
<td>M</td>
<td>I</td>
<td>1</td>
<td>3</td>
<td>16A</td>
<td>C</td>
<td>Both tumors EBV-positive</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>M</td>
<td>I</td>
<td>2</td>
<td>45D</td>
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<td>C</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>M</td>
<td>I</td>
<td>4</td>
<td>40D</td>
<td>11 x 9</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>M</td>
<td>I</td>
<td>3</td>
<td>34A</td>
<td>5.5 x 4</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>M</td>
<td>I</td>
<td>2</td>
<td>147A</td>
<td>6.5 x 4</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>M</td>
<td>I</td>
<td>4</td>
<td>6D</td>
<td>5.5 x 4.5</td>
<td>C &amp; A</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>74</td>
<td>M</td>
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</tr>
<tr>
<td>8</td>
<td>69</td>
<td>M</td>
<td>I</td>
<td>1</td>
<td>66A</td>
<td>4.5 x 2</td>
<td>C &amp; A</td>
<td></td>
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<tr>
<td>9</td>
<td>70</td>
<td>M</td>
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<td>1</td>
<td>29A</td>
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<td>C &amp; A</td>
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<td>67</td>
<td>M</td>
<td>D</td>
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<td>32D</td>
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<td>C &amp; A</td>
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<td>11</td>
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<td>13</td>
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<td></td>
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<tr>
<td>18</td>
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<td>F</td>
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<td>1</td>
<td>90A</td>
<td>2 x 2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>81</td>
<td>F</td>
<td>I</td>
<td>1</td>
<td>119A</td>
<td>5 x 5</td>
<td>A</td>
<td></td>
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</table>

* A, intestinal type; D, diffuse type.
* Survival in months from time of gastrectomy. A, alive; D, dead.
* C, corpus; A, antrum.

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Fig. 2. A, island of dysplastic gastric epithelium isolated from the main tumor by approximate two similar fields of view. H&E, x 40. B, ISH demonstrating EBV-positivity, x 40.
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The female cases. Theme were insufficient follow-up intervals for analysis of (P though there was a tendency (P = 0.26) for relatively longer survival of the stage 3 and 4 EBV-positive cancers. There were insufficient follow-up intervals for analysis of the female cases.

Discussion
This study confirms our previous finding (11) that the carcinoma cells of some typical gastric adenocarcinomas uniformly contain EBV sequences. Since there are marked geographic variations in the frequency of gastric cancer (19), we sought to compare the frequencies of EBV-associated gastric cancer between Los Angeles (11) and Hawaii. The Hawaii Japanese population, from which the patients of this study are derived, experience gastric cancer rates that are intermediate between those of indigenous Japanese and the lower frequency of Caucasians in the United States (19, 20). The frequency of the EBV association, however, was not significantly different between the two populations (P = 0.084). When the two studies are combined, 41 of 325 (12.6%) gastric cancers are EBV associated. It is also unlikely that EBV-associated gastric cancer is responsible for the excess numbers of gastric cancers observed in this population since EBV was identified in both diffuse and intestinal types of cancers, while the intestinal-type tumors primarily account for the excess numbers of cancers in high-risk populations (19). Both studies showed a male predominance for EBV-associated gastric cancer, although the male:female ratio in Hawaii (2.5:1) is lower than that in Los Angeles (7:1). The basis for the difference is unclear but could be ethnic since individuals of Japanese ancestry constituted only 5% of the subjects in the original study. The relative incidence of EBV-associated gastric adenocarcinoma remained low and did not change significantly from 1972 to 1991 in this population or in other populations.4

Transformation to gastric carcinoma may occur over decades since childhood exposures or alterations persist as risk factors in adulthood (19). The histological changes of severe atrophic gastritis and intestinal metaplasia are precursor lesions to gastric cancer and precede malignant transformation by many years (21). Therefore, it is unlikely that the EBV infection of gastric epithelium played a role in the very first stages of gastric carcinogenesis since EBV sequences were not detected in either non-neoplastic epithelium or in areas of the mucosa showing intestinal metaplasia.

The timing and selection factors that determine whether a gastric cancer will be infected with EBV are unclear. It may be that alterations in some cancers allow the virus to infect and persist as a “passenger” in the transformed cells. Alternatively, the EBV infection, as hypothesized for other EBV-associated tumors, may precede and directly contribute to the transformation that accounts for the development of a subset of gastric cancers. The available data do not conclusively distinguish between these two roles. The detection of EBV in all tumor cells, however, suggests that infection precedes clonal expansion. Further study is necessary to clarify these issues.

The presence of EBV in the tumor cells did not yield statistically significant differences in tumor stage or clinical outcome. This may have resulted from the small numbers of subjects available for analysis. There were more early stage (1 and 2) cases among the subjects with infected cancers, and those of later stage (3 and 4) tended to show longer survival than patients with uninfected cancers. If these features are retained after the accumulation of a larger number of cases it may be attributed, in part, to a favorable host response against tumor-associated antigens. There is a wide range of host inflammatory responses generated by EBV infection. The most intense lymphoid infiltration is present in lymphoepithelioma-like carcinomas which resemble nasopharyngeal lymphoepitheliomas (8). The majority of these rare and poorly differentiated cancers are EBV associated. The EBV-associated typical gastric adenocarcinomas in this study exhibited less inflammation and were clearly not lymphoepithelial-like carcinomas. Some deeper portions of these adenocarcinomas, however, exhibited isolated tumor cell islands surrounded by more intense inflammation (Fig. 3).

The EBV infecting the gastric tumor cells appears analogous to the virus in other types of tumors since they were homologous at the EBER1, EBNA1, and EBNA2 loci. The EBV-associated gastric cancers were predominately

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*Fig. 1. A, junction between better differentiated adenocarcinoma (left) and more poorly differentiated adenocarcinoma (right) with more intense lymphoid infiltration. H&E, × 100. B, ISH of corresponding section demonstrates EBV positivity of both portions of the tumor.*

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4 D. Shibata, personal observation.
type A, similar to the pattern seen in nasopharyngeal carcinoma and Hodgkin’s disease (22, 23). In contrast, African Burkitt’s lymphoma and human immunodeficiency virus-associated non-Hodgkin’s lymphoma contain both EBV types A and B (24, 25). Although the prevalence of EBV type A or B in this population was not tested, type A predominates in Japan (26).

The detection of EBV in only a portion of histologically identical tumors is similar to some EBV-associated tumors (Hodgkin’s disease, 30–50%; and sporadic Burkitt’s lymphoma, 10–20%) (9, 27) whereas the majority of nasopharyngeal carcinoma, endemic (African) Burkitt’s lymphoma, and undifferentiated gastric cancer with intense lymphoid stroma are EBV associated (1, 2, 8). The exact role of EBV in the pathogenesis of these tumors is as yet unknown. The relatively high incidence of gastric cancer compared to other EBV-associated neoplasms makes EBV-associated gastric cancer potentially one of the most common EBV-related tumors in the United States.

Acknowledgments

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References


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