Short Communication

Epstein-Barr Virus Involvement in Gastric Cancer: Biomarker for Lymph Node Metastasis[1]

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Abstract

EBV involvement in gastric cancer is characterized by episomal monoclonality, high antibody titers, EBV encoded small RNA and EBV nuclear antigen 1 expression in all tumor cells, and in the intramucosal stage, by a unique morphology. EBV involvement varies by population (~7% of gastric cancers in Japan and >15% in Western countries), sex, histological type, and tumor location. The present study compares frequency of lymph node metastasis (LNM) between 170 EBV-positive and 1590 EBV-negative gastric cancer cases in Japan by level of invasiveness. Frequency of LNM increased with increasing depth of invasiveness but was consistently and significantly greater for EBV-negative cases ($P = 0.0018$). In particular, there were no instances of LNM among 75 EBV-positive cases as compared with 53 among 562 EBV-negative cases restricted to the mucosa and submucosa (odds ratio, 0; 95% confidence limits, 0–0.20). The finding suggests that genetic control of metastasis may differ between EBV-related and other gastric cancers. Also, the possibility that EBV-positive, noninvasive gastric cancers may not require lymph node dissection suggests that routine assay of biopsy specimens for EBV involvement could be important in populations, like that of Japan, where early gastric cancers are seen frequently.

Introduction

EBV involvement, as determined by EBER[3] *in situ* hybridization, occurs in ~7% of gastric cancers in Japan and >15% in Western countries (1). Characteristics of EBV-associated gastric cancers include episomal monoclonality, high antibody titers, and EBER and EBV nuclear antigen 1 expression in all tumor cells (2). EBV involvement is more frequent among males and in tumors located in the upper part of the stomach and in gastric remnant cancer. EBV-positive early intramucosal lesions are characterized by a unique histology (3). EBV-positive gastric carcinomas typically show intensive CD8-positive lymphocytic infiltration in the tumor nests (4). EBV involvement in gastric cancer was first observed in tumors with lymphoepithelioma-like histology. This type of rare cancer was reported previously and was known to have a better prognosis than other gastric cancers (5). Here we present evidence that EBV involvement is negatively associated with lymph node metastasis.

Materials and Methods

In a previous study (1), we determined EBV involvement in a total of 1848 gastric cancers from 1795 consecutive gastrectomy cases filed in the pathology departments of nine different hospitals in Japan. Over one-half (56%) of the cases were from Kagoshima, and the rest were approximately evenly distributed among eight other cities. Paraffin blocks made from the main tumors were used for H&E and *in situ* hybridization studies. Paraffin sections were deparaffinized, rehydrated, predigested with Pronase, dehydrated, and hybridized overnight at 37°C with a concentration of 0.5 μg of digoxigenin-labeled probe as reported previously (1). After washing by 0.5× SSC, hybridization was detected by an antidigoxigenin antibody-alkaline phosphatase conjugate (Boehringer-Mannheim) as suggested by the manufacturer. Lymph node sections from a patient with infectious mononucleosis were used for positive control, and a sense probe for EBER-1 was used for negative control for each procedure.

For the present report, we reviewed lymph node metastasis in 170 EBV-positive and 1590 EBV-negative gastrectomy cases with lymph node dissection. Gastric cancer cases were classified by increasing depth of invasion, as follows: cancer cells restricted to the mucosa and invasion in the submucosal layer, muscular layer, or subserosa. For cases with multiple gastric cancers, the analysis was based on the most deeply invasive lesion.

Results

As expected, LNM increased with increasing tumor depth ($P < 0.0001$ for nonhomogeneity; all Ps are adjusted for other factors) and varied by histology ($P = 0.0004$). Metastasis was strongly, and negatively, associated with EBV involvement ($P = 0.0018$); 53 of 170 EBV-positive cases (31%) had LNM as compared with 764 of 1590 EBV-negative cases (48%). This negative association was observed at all four levels of tumor depth, among which metastasis rates varied widely (Table 1).

Discussion

Our findings suggest that testing biopsy specimens for EBV involvement before surgery would provide valuable information about the appropriateness of conservative therapies such as

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[3] The abbreviations used are: EBER, EBV encoded small RNA; LNM, lymph node metastasis.

Table 1 LNM by EBV involvement and depth of invasion

Depth	EBV-negative		EBV-positive		Total	
	Frequency	(%)	Frequency	(%)	Frequency	(%)
Mucosa	5/284	(1.8)	0/30	(0)	5/314	(1.6)
Submucosa	48/278	(17.3)	0/45	(0)	48/323	(14.9)
Muscular layer	69/185	(37.3)	5/17	(29.4)	74/202	(36.6)
Serosa	642/843	(76.2)	48/78	(61.5)	690/921	(74.9)
Total	764/1590	(48.1)	53/170	(31.2)	817/1760	(46.4)

endoscopic strip resection, laparoscopic resection, and local resection without lymph node dissection in cases of intramucosal cancer and invasive cancer restricted to the submucosa, even when the lesion is ulcerated. Further investigation of this possibility by clinical trial may be appropriate. Early gastric cancers, with limited invasion, are seen fairly frequently in Japan, where gastric cancer rates are very high and large-scale screening programs are in place. We found no instances of LNM among 75 EBV-positive cases with early cancers as compared with 53 among 562 EBV-negative cases (odds ratio, 0; 95% confidence limits, 0–0.20; adjusted for tumor depth and histology).

For more deeply invasive cancers, the frequency of LNM was fairly high, even among EBV-positive cases (29 and 62% for muscular layer and serosa, respectively), which suggests that a decision on lymph node resection probably would not depend upon whether the cancer was positive or negative for EBV. A statistically significant difference was apparent, nevertheless (odds ratio, 0.54; 95% confidence limits, 0.34–0.88).

Overall, our results suggest that genetic control of metastasis may differ between EBV-positive and EBV-negative gastric cancers. That possibility warrants further study using more probing investigative techniques.

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