Simultaneous EBV-Positive Lymphoepithelioma-like Carcinoma and EBV-Negative Intestinal-Type Adenocarcinoma in a Patient With Helicobacter pylori-Associated Chronic Gastritis

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Abstract

We report the case of a 72-year-old man with 2 simultaneous gastric carcinomas. The larger, ulcerated mass in the antrum was a conventional infiltrating intestinal-type adenocarcinoma. The associated antral-type mucosa showed moderate chronic gastritis, foci with complete and incomplete intestinal metaplasia, and mild to moderate Helicobacter pylori infection. The second, smaller tumor was found within fundic-type mucosa and was a lymphoepithelioma-like carcinoma associated with Epstein-Barr virus (EBV) infection shown by the EBV-encoded small RNA (EBER) test. The EBER test result was negative in the intestinal type adenocarcinoma. To our knowledge, this is the first report of simultaneous gastric carcinomas with 2 different morphologic phenotypes, in which only one tumor was associated with EBV infection, while the second tumor was related to H pylori–associated chronic gastritis. Our report demonstrates 2 different but simultaneous etiologic pathways of gastric carcinogenesis in the same patient.

Gastric carcinoma is approached conceptually as an infectious disease, often related to Helicobacter pylori infection.1-3 A significant relative risk of developing malignancy in patients with H pylori infection was noted only for intestinal types of adenocarcinoma in a Scandinavian study4 and for intestinal and diffuse types of gastric adenocarcinoma in a US study.5 Lymphoepithelioma-like carcinoma (LELC) is a rare type of gastric carcinoma.6-11 It has been described to have a favorable prognosis compared with that of ordinary gastric adenocarcinoma and has been suggested to be closely associated with the Epstein-Barr virus (EBV). More than 80% of LELCs have been found to be related to EBV infection, compared with only about 6% of diffuse and 7% of intestinal-type adenocarcinomas.12,13

Multiple gastric carcinomas are rare. They were reported in 2.6% of 970 patients with gastric carcinoma in a study by Tokunaga et al.13 The occurrence of both LELC and nonlymphoepithelial carcinoma of the stomach that developed synchronously or metachronously is rare and was reported in only 4 Japanese cases by Matsunou et al.12 In that study, 4 non-LELC intramucosal adenocarcinomas that developed synchronous or metachronous LELC showed hybridization signals with EBV-encoded small RNAs. We describe a Scandinavian patient with H pylori–associated chronic gastritis and simultaneous EBV-negative intestinal-type adenocarcinoma and EBV-positive LELC of the stomach.

Case Report

A 72-year-old man with a 3-month history of epigastric pain had anemia (hemoglobin concentration, 9 g/dL [90 g/L])
and a positive fecal occult blood test result. Upper gastrointestinal endoscopy revealed a large ulcerated tumor of the antrum. Endoscopic biopsy of the tumor showed moderately differentiated adenocarcinoma. The patient underwent distal partial gastrectomy. Multiple liver metastases and enlarged regional lymph nodes were noted at operation and sampled for histologic evaluation.

Materials and Methods

Immunohistochemical Analysis

For immunohistochemical analysis, paraffin blocks were cut at 4 to 6 µm, dried overnight at 60°C, and deparaffinized in xylene. Subsequently, sections were rehydrated through graded alcohols in water. Heat-induced epitope retrieval was achieved by boiling sections in EDTA buffer, pH 8.9, in a microwave oven at 1,000 W for 20 minutes (4 times for 5 minutes each). After boiling, sections were permitted to cool at room temperature for 20 minutes, rinsed thoroughly with water, and placed in Tris-buffered saline (TBS) for 5 minutes. Endogenous peroxidase was blocked with Peroxidase Block solution provided in the EnVision kit (DakoCytomation, Glostrup, Denmark) for 5 minutes, and slides were rinsed and washed with TBS. The primary antibodies were incubated for 30 minutes at room temperature. The list of the antibodies and their sources and dilutions used for staining are given in Table I. Immunostaining was performed using the EnVision method according to the manufacturer’s instructions.

In Situ Hybridization

Sections (thickness, 4 µm) were cut from paraffin-embedded blocks of formalin-fixed tissue samples, dewaxed in xylene, and rehydrated in serially graded ethanol and then digested with Proteinase K (3 mg/L) for 30 minutes at 37°C, hybridized for 2 hours at 37°C. The DNA probes were fluorescein-conjugated EBV-encoded small RNA (EBER) oligonucleotides. Fluorescein-conjugated immunoglobulin κ and λ light chain oligonucleotides were used as positive internal controls to demonstrate messenger RNA preservation. The detection was accomplished by using anti–fluorescein isothiocyanate antibodies and 3,3’-diaminobenzidine tetrahydrochloride was used as the chromogen. All reagents were purchased from DakoCytomation.

Gene Rearrangement Studies

An immunoglobulin heavy chain gene rearrangement study (IgH-CDR3) was performed as described previously. A T-cell receptor γ gene rearrangement study used a previously described method with the following primer modifications: Vγ11 (Vγ1-8), 5’-TCT GGR GTC TAT TAC TGT GC-3’; Vγ9, 5’-CCA CTC TCA CCA TTC AC-3’; Vγ10-11, 5’-CAA GTY CGT AGA GAA AGA AGA-3’; and JγII, 5’-CAA GTY CGT AGA GAA AGA AGA-3’.

Results

Gross Features

The specimen consisted of distal stomach composed predominantly of pylorus and antrum with a small segment of the corpus at the proximal resection margin and the duodenal bulb at the distal resection margin. A large, centrally ulcerated antral tumor involved predominantly the posterior wall with extension into the lesser curvature. The greatest tumor diameter was 10 cm. Three centimeters proximal to this tumor and extending to the proximal resection margin was a focal thickening of the mucosal folds; the greatest diameter was 1.5 cm. No other lesions were noted macroscopically. The specimen was fixed overnight, and sections were processed routinely for histologic examination.

<table>
<thead>
<tr>
<th>Antibody (Clone)</th>
<th>Dilution</th>
<th>Source</th>
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<tbody>
<tr>
<td>AE1/AE3 (AE1, AE3)</td>
<td>1:20</td>
<td>DakoCytomation, Glostrup, Denmark</td>
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<td>Cytokeratin 20 (IT-KS20.8)</td>
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<td>Progen Biotechnik, Heidelberg, Germany</td>
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<td>Cytokeratin 7 (OV-TL12/30)</td>
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<td>Cytokeratin 5/6 (D5/16B4)</td>
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<td>Boehringer, Mannheim, Germany</td>
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<tr>
<td>Helicobacter pylori (polyclonal)</td>
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<td>Novocastra Laboratories, Newcastle upon Tyne, England</td>
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<td>CD3 (PS-1)</td>
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<td>CD4 (4B-12)</td>
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<td>CD21 (2G9)</td>
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<td>CD45 (2B11/PD726)</td>
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<tr>
<td>Immunoglobulin light chain λ (polyclonal)</td>
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<td>DakoCytomation</td>
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Microscopic Features

Sections of the larger antral tumor showed an ulcerated, moderately differentiated, intestinal-type adenocarcinoma with papillary and tubular architectural features. The tumor extended distally into the pylorus and proximally was limited within the antral-type mucosa. The surrounding gastric mucosa showed moderate inflammation and moderate to marked incomplete and focal complete intestinal metaplasia. No definite evidence of atrophy involving antral-type mucosa was noted. Focal, mild to moderate infection by *H pylori* of mucosa adjacent to the tumor was identified by immunohistochemical analysis Image 1.

Sections of the smaller mucosal lesion at the proximal resection margin showed complete obliteration of the mucosal architecture by an intensely cellular, predominantly lymphoid lesion with pushing margins toward the submucosa. Closer examination revealed inconspicuous epithelioid tumor cells arranged in loose cords and aggregates. The epithelioid cell nuclei were oval with vesicular chromatin and prominent nucleoli. Mitotic figures were not noted. A dense lymphocytic infiltrate was represented by small lymphocytes, abundant plasma cells, and occasional eosinophils Image 2 and Image 3. Both eosinophils and plasma cells were more numerous at the interface of LELC and benign gastric mucosa, while small lymphocytes

*Image 1* A, Intestinal-type adenocarcinoma (H&E, ×100). B, Adenocarcinoma cells are completely negative for Epstein-Barr virus–encoded small RNA (×400). C, Antral-type gastric mucosa with chronic inflammation and intestinal metaplasia (H&E, ×20). D, Abundant *Helicobacter pylori* organisms demonstrated immunohistochemically (by the EnVision method [see text]) in benign antral mucosa (×400).
appeared confined to the tumor. Occasional lymphoid aggregates, some with germinal centers, were present. This tumor was diagnosed as an LELC. The surrounding mucosa showed moderate to marked chronic inflammation and focal complete intestinal metaplasia intermixed with areas of mildly to moderately atrophic fundic-type gastric mucosa.

The needle biopsy sample from the liver and 2 of 4 lymph nodes from the major curvature showed metastatic intestinal-type adenocarcinoma.

EBER in situ hybridization signals were observed uniformly in the nuclei and nucleoli but not the cytoplasm of the epithelial cells of the LELC. Adjacent nonneoplastic gastric mucosa and lymphocytes in LELC and throughout gastric tissue were completely negative. The intestinal-type adenocarcinoma also was completely negative.

While both carcinomas expressed AE1/AE3 pancytokeratins, cytokeratin (CK) 8, CK20, and CK7, and did not express CK5/6, the expression of CK8 and CK20 was much weaker in the LELC than in the intestinal-type adenocarcinoma. Both the LELC and the adenocarcinoma were negative for CD21.

The lymphocyte infiltrate in the lymphoepithelioma-like gastric carcinoma expressed CD45, with the great majority of cells also expressing CD3. The CD4/CD8 ratio was 1:1.5. CD20+ cells were rare. Plasma cells were polytypic with a κ/λ ratio of 2:1.
Cytokeratin 8 was expressed weakly in LELC (EnVision method). Poorly differentiated carcinoma cell clusters separated with intervening non-desmoplastic stroma infiltrated uniformly with abundant lymphocytes and plasma cells (cytokeratin 8, ×400). Only malignant epithelial cells in LELC expressed Epstein-Barr virus-encoded small RNA (×400).

High-power views of lymphoepithelioma-like carcinoma. Carcinoma cells are found in small clusters and cords (H&E, ×400). Immunostaining (EnVision method [see text]) greatly facilitated the detection of malignant cells (AE1/AE3, ×400). Unstained malignant cells were highlighted by the EnVision method (CD45, ×400).
Molecular analysis showed polyclonal patterns for T-cell receptor γ and IgH chain gene rearrangement.

Discussion

The coexistence of intestinal-type adenocarcinoma and LELC in the same patient is an extremely uncommon event. To the best of our knowledge, only 4 cases of in situ non-LELC that developed synchronous or metachronous LELC have been described to date. In these patients, all 4 of the non-LELCs were EBV-positive, and 10 of 11 subsequently identified LELC cancerous lesions also showed EBV positivity.12 In a Japanese study, Toku-naga at al13 described 4 cases with double carcinomas. Two cases with double carcinomas were positive in both, and another 2 double cases were positive in one and negative in the other. There is, however, no reference to types of these multiple simultaneous carcinomas or the H pylori status of the gastric mucosa. Herein, we report a case of simultaneous small fundic EBV-positive LELC and large pyloric EBV-negative intestinal-type gastric adenocarcinoma in the background of H pylori-associated chronic gastritis.

The highest rate of EBV infection in typical gastric adenocarcinoma was detected in a US study by using polymerase chain reaction and in situ hybridization techniques16 and was 16%. In the large Japanese study involving 999 gastric carcinomas, EBV infection was reported in 6.9% of all gastric carcinomas.13 However, EBV involvement was seen in almost all of the LELCs.13 Similarly, in a study by Wu and coauthors,17 EBV was detected in 100% of LELCs and 14% of common gastric carcinomas. Compared with EBV-negative gastric carcinoma, gastric LELC tended to have a relatively higher frequency of proximal location, diffuse histologic subtype, p53 overexpression, and reduced E-cadherin expression but a lower frequency of lymph node metastasis, previous H pylori infection, and c-erb-b2 overexpression.17 The identification of EBV-associated synchronous multicentric cancers of both LELC and non-LELC types suggests that EBV infection might be an early event in the induction process of these tumors.12

Distinct clinicopathologic and genetic pathways exist in gastric LELC, in which EBV might have a more important role than H pylori infection.17 Although H pylori infection is considered to be universally associated with the development of a precursor lesion for intestinal-type adenocarcinoma,18,19 it probably has no role in the development of LELC. Wu and coauthors17 found H pylori seropositivity in 36% of the LELCs, but they found it in 68% of patients with non-LELC gastric carcinoma.

The association of EBV with some epithelial neoplasms has been reported to depend on ethnic and/or regional background.20,21 To the best of our knowledge, our case is the first case in which EBV-related LELC gastric carcinoma has been documented in a patient of Scandinavian (Norwegian) origin.

The carcinogenic potential of infectious agents linked to protracted chronic inflammatory conditions is well recognized. Both H pylori and EBV have prominent roles driving different paths in the carcinogenesis of gastric carcinoma, which is illustrated clearly in our case.

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References