—Case Reports—

Stage IV Epstein-Barr Virus-Associated Early Gastric Cancer and Comparative Analysis of Genetic Alterations in Primary and Metastatic Tumors

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Early gastric cancer (EGC) with synchronous distant metastasis is extremely rare. Here, we report a case of stage IV Epstein-Barr virus (EBV)-associated EGC. A 51-year-old man presented with vague abdominal pain of 3 weeks' duration. Imaging studies revealed enlargement of the left supraclavicular, perigastric, and para-aortic lymph nodes, a huge gastric polypoid mass, and multiple liver masses. Histopathological examination of a biopsy specimen of the supraclavicular lymph node showed poorly differentiated carcinoma expressing EBV in tumor cell nuclei. The gastric mass exhibited tubular adenocarcinoma, which also expressed EBV in tumor cell nuclei. After 3 weeks of palliative chemotherapy with fluoropyrimidine and platinum, the patient died of liver failure. Next-generation sequencing analysis revealed mutation of the *CDKN1B* gene in the metastatic carcinoma and mutations of the *CTNNB1* and *PIK3R1* genes in the gastric carcinoma. In addition to the rare presentation of EBV-associated EGC, this case showed marked morphological and molecular differences between primary and metastatic tumors, which suggests clonal evolution of EBV-associated GC. (J Nippon Med Sch 2020; 87: 350–354)

Key words: EBV, early gastric cancer, stage IV, next-generation sequencing

Introduction

Gastric cancer (GC) is the fourth most common malignant tumor and the second most common cause of cancer death; 900,000 GC cases are reported annually worldwide and more than 700,000 deaths are attributable to the disease[1,2]. Recent development of novel diagnostic procedures has increased the number of early-detected GCs. In Asia, more than 50% of GCs are diagnosed as early gastric cancer (EGC), which is defined as invasive cancer that does not invade beyond the submucosa, regardless of lymph node metastasis. The prognosis for EGC is favorable: the 5-year survival rate is greater than 90% after curative resection[3,4]. However, the rate of lymph node metastasis in EGC invading the submucosa is about 20%, and stage IV EGC constitutes about 0.14% of all GCs[5].

About 10% of GCs are associated with Epstein-Barr virus (EBV) infection[6,7]. EBV-associated gastric cancer (EBVaGC) is a distinct subtype of GC and is distinguished from non-EBVaGC by its characteristic morphology, clinical features, and molecular profiles. Cancer Genome Atlas analyses define EBVaGC as one of the 4 major molecular subtypes of GC[8]. The prognosis for EBVaGC is generally better than that for non-EBVaGC. In patients with EGC, the rate of lymph node metastasis was significantly lower for EBVaGC[9–11].

We recently treated a patient with EBV-associated EGC accompanied by synchronous multiple distant metastases. To our knowledge, this unusual presentation of EBV-associated EGC has not been previously reported. Herein, we discuss this case of stage IV EBV-associated EGC and review the literature on stage IV EGC.

Case Presentation

A 51-year-old man presented with vague abdominal pain of 3 weeks' duration. The patient reported a 10% weight

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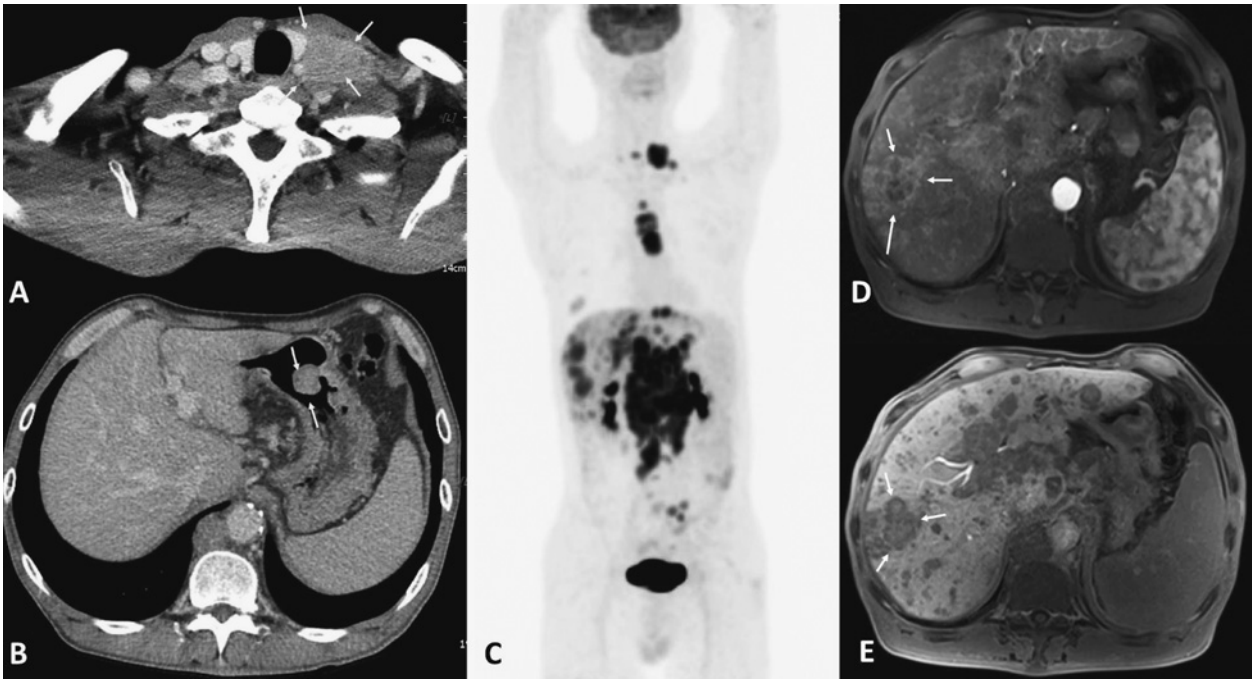

Fig. 1 Imaging findings for the disseminated tumors.
A-B: Computed tomography scanning showed a 3.5-cm left supraclavicular lymph node (A) and 2.2-cm polypoid mass on the stomach (B). C: Positron emission tomography revealed extensive metastatic lymphadenopathies and multiple liver metastases. D-E: Liver dynamic contrast-enhanced magnetic resonance imaging scan showed a 3.8-cm hepatic mass with transient rim enhancement in the arterial phase (D) and hypodensity in the 20-minute delayed phase (E).

loss during the past 6 months. He had a 10-year history of type 2 diabetes mellitus, was a current smoker (smoking history, 30 pack-years), and drank 1 bottle of alcohol 3 times a week. Physical examination revealed a palpable, fixed, non-tender mass at the left supraclavicular area. Abdominal physical examination yielded no specific findings. Blood testing showed elevation of α-fetoprotein (7.61 ng/mL) and liver enzymes (AST 328 U/L, ALT 50 U/L; total bilirubin 1.38 mg/dL). A complete blood count and carbohydrate antigen19-9 and carcinoembryonic antigen levels were within normal ranges.

Computed tomography revealed enlargement of the left supraclavicular, perigastric, and para-aortic lymph nodes, a huge gastric polypoid mass, and multiple liver masses not associated with cirrhosis or splenomegaly. Positron emission tomography showed high uptake in the enlarged lymph nodes and multiple liver masses. Magnetic resonance imaging of the liver masses revealed rim enhancement and hypodensity in the 20-minute delayed phase, which suggests metastasis rather than primary liver cancer (**Fig. 1**).

Histopathological analysis of a biopsy specimen of the supraclavicular lymph node revealed clusters of poorly differentiated tumor cells surrounded by small lymphocytes (**Fig. 2A**). Immunostaining and EBV in situ hybridization were performed for differential diagnosis of metastatic carcinoma, lymphoma, and neuroendocrine carcinoma. Tumor characteristics were most consistent with metastasis of EBV-associated carcinoma (**Table 1**; **Fig. 2B**).

In the search for the primary tumor, clinical evaluation of the nasopharynx was unremarkable. Esophagogastroduodenoscopy revealed a 4-cm polypoid mass in the greater curvature side of the low stomach body, which had been seen in computed tomography (**Fig. 2C**). Analysis of a biopsy specimen of the tumor surface revealed high-grade dysplasia. Endoscopic en-bloc resection was performed for histological evaluation of the gastric mass. The gastric tumor exhibited multifocal well-differentiated adenocarcinoma in the background of high-grade dysplasia (**Fig. 2D**). Some tumor glands invaded the submucosa and lymphovascular space (**Fig. 2E**). All tumor cells exhibited nuclear EBV expression (**Fig. 2F**) and cytokeratin profiles (CK7-/CK20-/CK19+) similar to that of metastatic carcinoma (**Table 1**). In the search for genetic linkages between these tumors, as well as targetable genes, we performed targeted next-generation sequencing (Oncomine Comprehensive Assay v3, Thermofisher Scientific, Waltham, MA, US) of the gastric and metastatic tumors. With a tentative diagnosis of stage IV

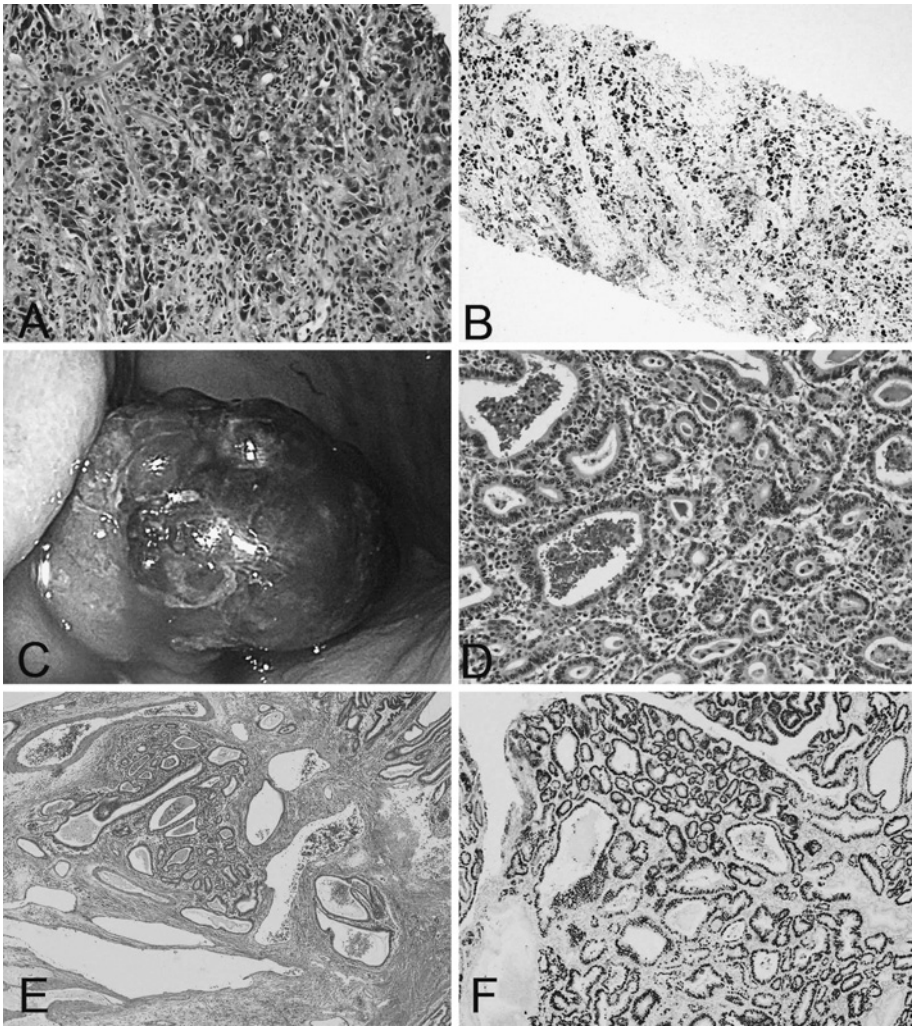

Fig. 2 Histopathological features of metastatic carcinoma and gastric carcinoma.
A-B: The supraclavicular lymph node showed poorly differentiated tumor cells mixed with small lymphocytes (A). Poorly differentiated carcinoma from the lymph node expressed EBV in the nuclei (B). C-F: Endoscopy revealed a large polypoid mass in the stomach (C). The tumors exhibited well-differentiated tubular adenocarcinoma (D) and invasion of the submucosa (E). Adenocarcinoma of the stomach expressed EBV in the nuclei (F).

EBV-associated GC, we administered palliative chemotherapy with fluoropyrimidine and platinum. After 3 weeks of chemotherapy, the patient was unable to tolerate further treatment, because of worsening liver failure (AST 1,145 U/L, ALT 165 U/L, total bilirubin 6.04 mg/dL), and died of the disease.

The results of next-generation sequencing were obtained after the patient's death (**Table 1**). Genetic analyses of both tumor samples were successful, except for copy number alteration analysis of the metastatic carcinoma, which was not possible because of low cellularity. The pathogenic variant detected in the metastatic carcinoma was a c.475+1 G>T splice site mutation of the *CDKN1B* gene (COSM5945858). The pathogenic variants detected in the gastric carcinoma were I35S missense mutation of the *CTNNB1* gene (COSM5674) and *N564D* missense mutation of the *PIK3R1* gene (COSM1220623). There were 5 overlapping variants of uncertain significance in the metastatic carcinoma and gastric carcinoma. Additional genetic analysis of normal gastric mucosa confirmed that these variants were nonpathogenic germline variants. We concluded that the 2 tumors did not share pathogenic somatic mutations.

This study was approved by the institutional research board of Kyung Hee University Hospital (IRB No. 2019-12-070).

Discussion

Stage IV EGC has rarely been reported and makes up only 0.14% of all GCs[5]. Previously reported cases of stage

Table 1 Results of ancillary analysis of metastatic and gastric carcinoma

	Metastatic carcinoma			Gastric carcinoma		
Immunohistochemistry						
CK	+			Not performed		
CK7/CK19/CK20	−/+/−			−/+/−		
Synaptophysin, CD56	−/−			Not performed		
P40	−			Not performed		
CD45/CD30	−/−			Not performed		
EBV in situ hybridization	+, tumor cell nuclei			+, tumor cell nuclei		
Next-generation sequencing	Gene	Variant	Allele ratio	Gene	Variant	Allele ratio
Pathogenic somatic variant	*CDKN1B*	splice site	(15%, 112/706)	*CTNNB1* *PIK3R1*	p.I35S p.N564D	(32%, 576/1,768) (31%, 623/1,997)
Germline variant	*NBN* *ATM* *STK11* *MRE11* *FANCD2*	p.A46T p.H2480R p.P281 L p.I396T p.E622K	(41%, 823/1,997) (54%, 1,079/1,998) (52%, 1,050/1,991) (42%, 32/77) (31%, 31/102)	*NBN* *ATM* *STK11* *MRE11* *FANCD2*	p.A46T p.H2480R p.P281 L p.I396T p.E622K	(47%, 918/2,000) (46%, 952/1,999) (50%, 1,001/1,995) (47%, 954/1,998) (25%, 498/1,997)

IV EGC were associated with localized metastatic tumors, mainly involving the liver, ovary, and distant lymph nodes, that were resectable[5,12–15]. The present metastatic tumors were extensively disseminated, and systemic chemotherapy was the only treatment option. Observation of multiple lymphadenopathies and liver masses suggested lymphoma or metastatic carcinoma. The only possible primary lesion detected-in the stomach-was not initially considered to be the origin of the metastatic tumors, as the mass exhibited a luminal protruding polyp without infiltration of the gastric wall. Regarding the origin of the metastatic carcinoma, GC metastasis is suggested by the overlapping cytokeratin profile (CK7-/CK19+/CK20-), EBV expression, presence of lymphovascular invasion, and absence of other visible primary lesions. In contrast, the marked difference in tumor morphology, non-overlapping genetic mutations, and extent of submucosal invasion by the tumor tend not to support a gastric origin. In our opinion, GC metastasis is most likely, because of the presence of EBV expression in both the GC and metastatic carcinoma.

The present diagnostic approach was largely guided by detection of EBV expression in the metastatic tumors, which is the basis of lymphoma diagnosis. EBV expression is sometimes detected in carcinomas with lymphocyte-rich stroma arising in various organs[16]; most such tumors express EBV in tumor-infiltrating lymphocytes but not in tumor cell nuclei. In contrast, nuclear EBV expression can be detected in EBV-associated nasopharyngeal carcinoma and EBVaGC. Because a poorly differentiated morphology is common in EBV-associated nasopharyngeal carcinoma, we considered the gastric lesion to be a candidate primary tumor, after excluding the nasopharyngeal lesion.

EBVaGC is a morphologically heterogeneous group comprising lymphoepithelioma-like carcinoma, carcinoma with Crohn disease-like lymphoid reaction, and conventional adenocarcinoma[17]. Of these, cases morphologically resembling conventional adenocarcinoma are most unusual and can easily be overlooked if EBV expression is not routinely investigated during pathological examination of GC. In the present case, the morphology of the GC was classified as conventional adenocarcinoma, whereas that of the metastatic carcinoma resembled lymphoepithelioma-like carcinoma, which indicates morphological diversity in the primary and metastatic tumors of EBVaGC.

A few studies found molecular alterations in stage IV EGC. An and colleagues reported 2 cases of stage IV EGC with microsatellite instability[15]. To our knowledge, ours is the first report to include a comprehensive analysis of genetic alterations in stage IV EBV-associated EGC. The mutations in *PIK3R1* and *CTNNB1* in the GC from the present case are common genetic alterations in EBVaGC[8]. However, we observed a different molecular profile in the metastatic tumors, namely, a *CDKN1B* mutation without mutation in *PIK3R1* or *CTNNB1*. We believe that the differences in the molecular profiles and morphologies of the metastatic and primary tumors are best explained by clonal evolution of the primary carcinoma

and subsequent metastasis of aggressive clones.

In summary, this is the first reported case of stage IV EBV-associated EGC. In addition to the rare presentation of EBV-associated EGC, this case exhibited marked morphological and molecular differences between primary and metastatic tumors, which suggests clonal evolution of EBVaGC.

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Conflict of Interest: The authors declare no conflict of interest.

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