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Case report

Gastric adenocarcinoma with differentiation to sarcomatous components associated with monoclonal Epstein-Barr virus infection and LMP-1 expression

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Abstract. A case of gastric adenocarcinoma with sarcomatous differentiation in a 65-year-old male was investigated for possible association with the Epstein-Barr virus (EBV). The presence of EBV-DNA could be proven by the polymerase chain reaction (PCR), and EBERs signals were detected in tumour nuclei, strongly in sarcomatous areas and weakly elsewhere. Monoclonal EBV infection was evident in terms of a single band of lymphocyte determined membrane antigen demonstrated by the PCR method. Latent membrane protein 1 was strongly positive in cells of sarcomatous components but very weakly positive in carcinoma components. EBV-determined nuclear antigen-2 was absent in both. This case of adenocarcinoma suggests that EBV plays an important role in tumorigenesis, contributing in particular to sarcomatous differentiation.

Key words: Gastric carcinoma – Epstein-Barr virus – Sarcoma – LMP-1 – LYDMA

Introduction

Recent reports have revealed a high incidence of association between poorly differentiated gastric adenocarcinomas with lymphoid infiltration, a special type of gastric carcinoma histologically resembling lymphoepithelioma in the nasopharynx, and monoclonal Epstein-Barr virus (EBV) infection (Burka et al. 1990; Shibata et al. 1991 b). Although at a lower incidence (16%), even common-type gastric adenocarcinomas demonstrate EBV infection (Shibata and Weiss 1992), although the gastric epithelium has been thought to lack an EBV receptor (Purtilo et al. 1992).

While sarcomatous differentiation is not rare in carcinomas of the breast, oesophagus and lower urinary tract (Gersell and Katzenstein 1981; Linder et al. 1987; Roy et al. 1987), gastric carcinomas seldom exhibit such a feature and, to our knowledge, only 41 cases have been accepted as true "carcinosarcoma" to date (Cho et al. 1990).

In the present report we document a case of gastric carcinoma differentiating to sarcomatous components, associated with monoclonal EBV infection. It is of potential interest in shedding light on the role of EBV in tumorigenesis, and also in differentiating carcinomatous and sarcomatous phenotypes.

Case report

A 65-year-old male came to the hospital because of sudden haematemesis. A gastroendoscopic biopsy revealed gastric carcinoma. His past history included diabetes mellitus presenting in the past year. Laboratory data were within normal limits, except for increased blood urea nitrogen, a decrease in serum total protein and an alpha fetoprotein value of 11.8 ng/ml. No examination for antibodies to EBV was performed. After a total gastrectomy, he remains free from relapse 20 months later.

Materials and methods

The material available for study consisted of the resected specimen of the primary tumour. The tissue had been formalin-fixed and paraffin-embedded. Sections were stained with haematoxylin and eosin for routine histopathological assessment.

Routine immunohistochemical examinations were carried out on 4-μm sections of formalin-fixed, paraffin-embedded tissue, in all cases using the avidin-biotin-peroxidase complex method.

Antibodies directed against the following antigens were utilized: carcinoembryonic antigen (CEA; Dako; dilution 1:1000), epithelial membrane antigen (Lipshaw; dilution 1:10), cytokeratin (AE1/AE3, ICN; dilution 1:300), carbohydrate antigen 19-9 (Compagnie Oris Industrie, France; dilution 1:50), carbohydrate antigen 125 (Compagnie Oris Industrie; dilution 1:10), desmin (Lipshaw; dilution 1:500) and vimentin (Amersham; dilution 1:40). In addition, monoclonal antibodies to one of the major latent membrane proteins (LMP)-1 (Dakopatts, Calif., USA) and EBV-determined nuclear antigens (EBNA)-2 (Dakopatts) were used to provide histological localization of EBV employing an alkaline phosphatase anti-alkaline phosphatase (APAAP) Kit System 40 (Dako). Fixed nasopharyngeal lymphoepithelioma tissue served

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as a positive control and EBV-negative non-specific lymphadenitis tissue served as a negative control.

A fluorescein isothiocyanate (FITC) conjugated EBV- (EBERs) oligonucleotides probe (Dakopatts) was used for conventional RNA in situ hybridization (ISH) with 4-μm-thick sections of formalin-fixed and paraffin-embedded tissue. Detection was with an APAAP kit, System 40 (Dakopatts) after immunoreaction with anti-FITC antibody. The same positive and negative controls were used as in the routine immunohistochemical examination.

For the polymerase chain reaction (PCR) DNA was extracted from formalin-fixed, paraffin-embedded tissues cut into 10-μm-thick sections by proteolysis and phenol/chloroform/isoamyl alcohol extraction as previously described (Goelz et al. 1985). The target EBV sequence was a 240 base pair (bp) piece in the internal repeat 3 (IR3) and primers were designed according to the publication of Wright et al. (1991). To ensure that adequate DNA had been available for amplification, PCR of a single-copy of the HER2 gene giving rise to a sequence 241 bp in length was also performed (Wright et al. 1991). EBV lymphocyte-determined membrane antigen (LYDMA) gene composed of variable numbers of 33 bp tandem repeats was investigated to ascertain the monoclonality of EBV infection in tumour cells using the primers reported by Shibata et al. (1991).

PCR procedures were performed by means of the methods previously described (Shibata et al. 1991a; Wright et al. 1991).

DNA extracted from formalin-fixed, paraffin-embedded Raji cells was used as a positive control and DNA from non-specific lymphadenitis as a negative control.

Results

Examinations of the surgical specimen obtained at total gastrectomy revealed a round, ulcerated irregular tumour measuring 5.5 cm in maximum diameter, located in the lower part of the body on the lesser curvature. No visceral metastasis was evident. Histologically, the tumour was found composed of collision of both carcinomatous and sarcomatous components that invaded the main muscle coats of the gastric wall. The cells of sarcomatous components contained spindle-shaped, large pleomorphic nuclei with coarse chromatin, inconspicuous nucleoli and scanty cytoplasm (Fig. 1a). The carcinoma consisted of irregularly shaped atypical glands and was diagnosed as a well-differentiated adenocarcinoma (Fig. 1b). Nests of cancer cells were surrounded by intersecting fascicles composed of plasma cells and small lymphocytes.

Immunohistochemical results are summarized in Table 1. Sarcomatous components reacted to desmin and weakly to epithelial antibodies. LMP-1 binding was strongly positive in sarcomatous areas but very weak in the carcinoma. EBNA2 was negative in both.

ISH demonstrated clear EBERs signals, strongly expressed in tumour nuclei of sarcomatous components (Fig. 2a) and somewhat weaker in the carcinoma nuclei (Fig. 2b). They were completely absent in both the lymphoid stroma and normal gastric mucosa.

PCR products gave visible single bands for HER2 and EBV-IR3 (241 and 240 bp in length, respectively) and LYDMA (about 230 bp in length Fig. 3).

Discussion

Histopathological features of gastric carcinosarcomas are usually exemplified by mixtures of well-differentiated

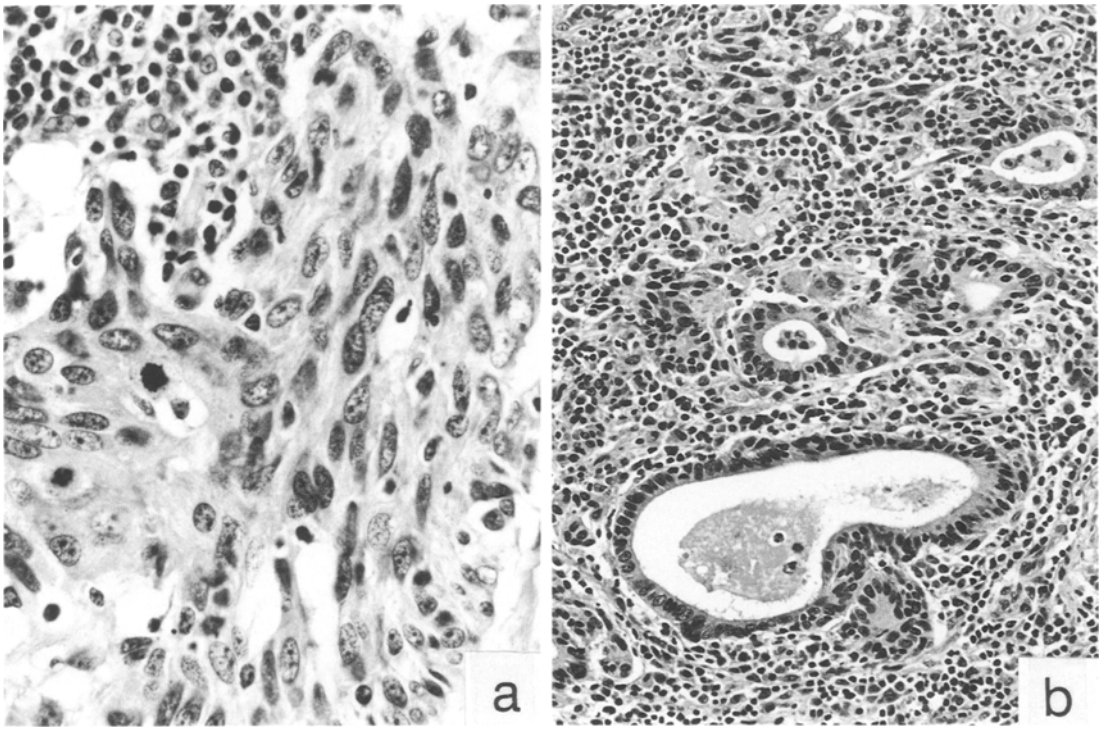

Fig. 1a, b. Microscopic photographs of H & E staining. **a** The cells of sarcomatous components consisted of spindle-shaped large pleomorphic nuclei with coarse chromatin and inconspicuous nucleoli, and scanty cytoplasm, ×200. **b** The areas of carcinoma were composed of irregularly shaped atypical glands surrounded by lymphoid stroma, ×100

Table 1. The results of immunohistochemical examination

	Carcinomatous components	Sarcomatous components
Desmin	−	++
Vimenin	−	−
CEA	+++	+
EMA	++	+
Keratin	++	+
CA19-9	+++	−
AE-1	++	+
LMP-1	±	++
EBNA-2	−	−

±, Very weak-; +, low; ++, intermediate; +++, high antigenetic; −, no antigen detectable

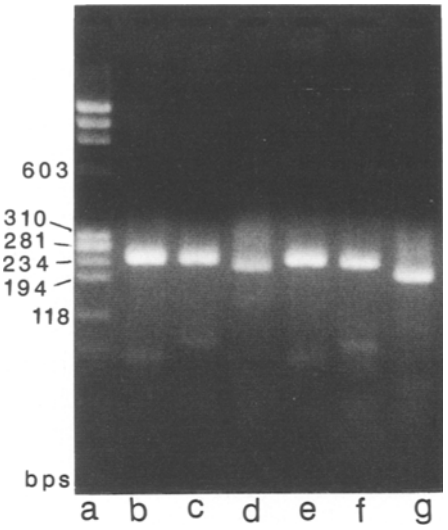

Fig. 3. Polymerase chain reaction results for HER2 gene, EBV-IR3 region and LYDMA gene in Raji cells as a positive control and the present case. Clearly visible single bands for HER2, EBV-IR3, 241 and 240 bp in length, respectively, and LYDMA about 230 bps in length. *Lane a*, marker; *lanes b–d*, HER2, EBV-IR3 and LYDMA of Raji cells; *lanes e–g*, HER2, EBV-IR3 and LYDMA of the present case

adenocarcinoma and spindle cell sarcoma elements (Cho et al. 1990). The underlying cause of this biphasic pattern with simultaneous expression of both epithelial and non-epithelial phenotypes within the same lesion is unclear. However, recent results have led to the hypothesis that sarcomatous components represent a kind of metaplastic alteration (Tanimura and Furuta 1967; Hanada et al. 1985; Weidner and Zekan 1986). Supportive evidence includes: the presence of cells histologically intermediate between the two elements (Tanimura and Furuta 1967); positive expression of immunohistochemical markers of epithelial differentiation including CEA in sarcomatous cells (Hanada et al. 1985; Weidner and Zekan 1986); and the presence of epithelial features in sarcomatous cells under the transmission electron microscope (TEM; Hanada et al. 1985). In the present case, the gastric tumour was made up of well-differentiated adenocarcinoma and sarcomatous components, with spindle-shaped atypical cells, being positive for desmin in contrast to

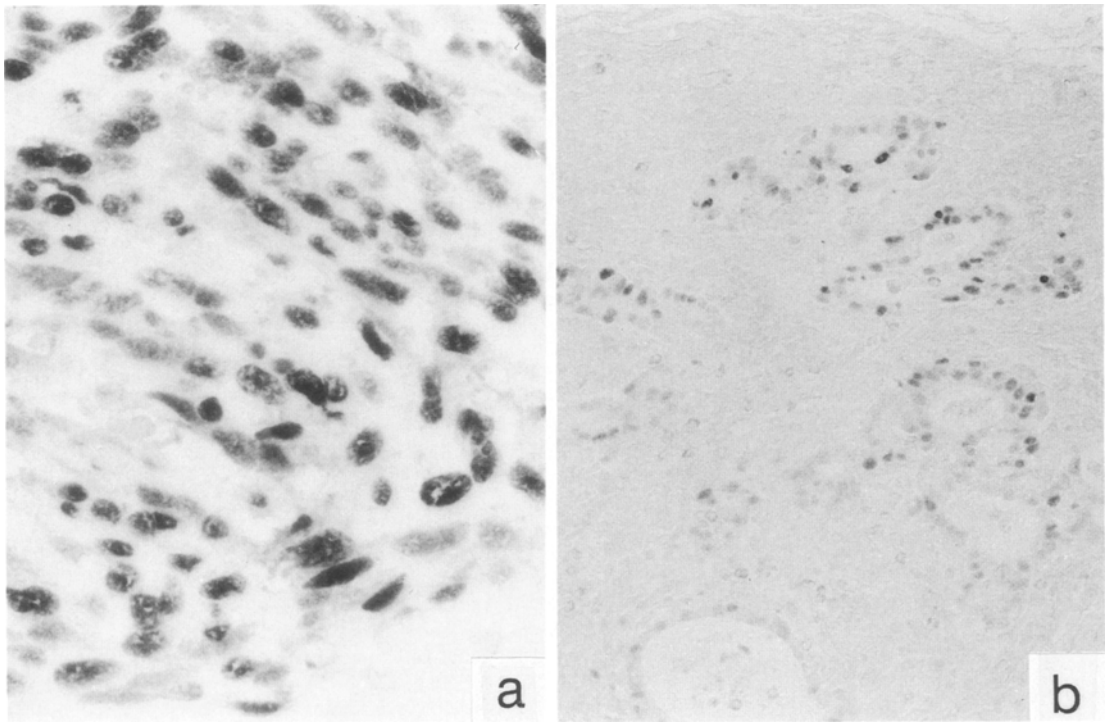

Fig. 2a, b. In situ hybridization results using (EBERs) RNA probe. **a** Clear EBERs signals strongly expressed in tumour nuclei of sarcomatous components, ×200. **b** Somewhat weaker in carcinomatous nuclei, ×100

the weakly positive epithelial elements. The reason that well-differentiated adenocarcinoma rather than moderately or poorly differentiated adenocarcinoma predominates as the epithelial component in gastric carcinosarcoma cases is unknown (Dundas et al. 1988; Cho et al. 1990), but our case is similar to the majority of those reported. While no unequivocal proof could be gained from TEM of formalin-fixed paraffin-embedded tissue precluding an absolute diagnosis of carcinosarcoma, we use the term "sarcomatous component", since this seems appropriate from the results of immunohistochemical and histological examination.

Application of PCR for detection of the EBV-IR3 sequence revealed the presence of EBV. In addition, RNA ISH also demonstrated abundant EBERs signals strictly localized in tumour nuclei, especially within the sarcomatous component. It has been generally considered that EBV infects only B-lymphocytes and oral epithelium with expression of the EBV receptor (C3d CR2 or CD21) on the target cell surface (Fingeroth et al. 1984; Young et al. 1986) being a necessary condition for EBV infection. Such a receptor may also be present on cells of gastric epithelium or carcinoma, to judge by the present and previous findings, permitting intrusion (Young et al. 1989).

The EBV genome has terminal repeats (TR) composed of 1–6 units of homologous direct tandems of approximately 500 bp. As the number of TR is distinct among individual EBV species it is theoretically possible to distinguish whether EBV infection is monoclonal or polyclonal by means of investigation of the TR size (Rabb-Traub and Phym 1986). However, preservation is not optimal during the processes of formalin fixation and paraffin embedding. The EBV-LYDMA gene, composed of a variable number of tandem 33 bp repeats, for similar reasons, demonstrates sizes of this heterogeneous region characteristic for each EBV species, and does not suffer from the same disadvantages for estimating monoclonality of EBV infection (Fennewald et al. 1984; Hennessy et al. 1984). Thus its short length means better preservation, and since we could not obtain fresh tumour tissue, LYDMA was employed. Since a single LYDMA band was evident, monoclonality of EBV infection was proved in the present study.

LMP and EBNA-2 are putative viral oncogenes, that is to say, they have close relations to the in vitro immortalization process in B-lymphocytes (Hammerschmidt and Sugden 1989). LMP expression is dependent on cellular factors which are present only in activated cells (Allday et al. 1989). LMP induces the cells to proliferate and to produce soluble growth factors which enhance cellular proliferation (Crawford 1992). EBNA-2 is an essential molecule in lymphocyte transformation by EBV (Cohen et al. 1989) and induces CD23, whose extracellular site acts as a growth factor (Purtilo 1980). In the present case, LMP-1 were clearly positive in sarcomatous components but very weak in epithelial components, while EBNA-2 was negative.

In conclusion, the question of a causal role for EBV in the development of this tumour remains unanswered. However, it seems likely that EBV is closely related to tumorigenesis because of the monoclonal infection and positive LMP-1 expression, with some role in sarcomatous differentiation. The latter is evidenced by the differential between sarcomatous and carcinomatous components for both EBERs and LMP-1.

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