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Epstein-Barr virus (EBV) infection and p53 protein expression in gastric carcinoma

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Abstract

In the presented studies p53 protein expression was evaluated in samples of gastric carcinoma originating from 32 selected adult patients (with documented diagnosis of adenocarcinoma of the stomach and without the presence of Helicobacter pylori infection). Among the patients 14 individuals carried EBV-positive gastric carcinoma (group 1) while the 18 remaining patients carried EBV-negative gastric carcinoma (group 2). EBV infection was detected testing the tissue material for the presence of EBER by RNA in situ hybridization (ISH) and testing sera of the patients for EBV-specific antibodies. Expression of p53 protein was analysed using immunohistochemistry. Presence of p53 protein was noted in 9 (64.3%) cases of EBV-positive gastric cancer (group 1) and in 10 (55.5%) cases of EBV-negative gastric cancer (group 2). No significant differences were detected in the frequencies of p53 protein expression in the two studied groups. The results permit to conclude that abnormalities in p53 in gastric cancer are independent of EBV infection, even if EBV may participate in development of the tumour.

Keywords: Epstein-Barr virus; p53 protein; Gastric carcinoma; Tumorigenesis

1. Introduction

Strict relationship between Epstein-Barr virus and various epithelioid diseases has already been well documented (Anagnostopoulos and Hummel, 1996). Involvement of EBV has been demonstrated in the etiopathogenesis of not only the endemically manifested nasopharyngeal carcinoma (NPC) but also of other, non-endemic epithelial malignancies, including gastric carcinoma (Roux and Joab, 1998; Labrecque et al., 1995; Szkaradkiewicz et al., 2002, 2004; Takada, 2000). It is estimated that EBV infection can be demonstrated in about 10% of gastric carcinomas. Nevertheless, the role of EBV in neoplastic transformation of gastric epithelial cells remains unclarified. Present data indicate that EBV infection may protect tumour cells from apoptosis inducing over-expression of the anti-apoptotic bcl-2 gene (Kume et al., 1999). On the other hand, the absence of the growth-inhibitory effect of p53 tumour suppressor gene, due to its inactivating mutations demonstrated in gastric carcinoma, seem to play a basic role in tumorigenesis (Poremba et al., 1995).

Aiming to analyse the role of EBV in the oncogenic pathway, in this study we investigated p53 expression in gastric carcinoma in patients with or without EBV infection.

2. Materials and methods

2.1. Patients

Study subjects were selected from patients who had had a first diagnosis of stomach cancer. The material qualified for the studies (sera and fragments of tumour tissue) originated from 32 patients, in whom the cancer diagnosis was verified by analysis of hematoxylin and eosin (H&E) stained preparations (Watanabe et al., 1990). Moreover, the presence of *Helicobacter pylori* in the studied material was evaluated using the biopsy urease test (Lencomm) and Genta staining method with silver impregnation of histological sections (Genta et al., 1994). In all the selected cases the presence of advanced moderately differentiated adenocarcinoma (G2) of the stomach was confirmed and presence of *Helicobacter pylori* was excluded. In parallel, in

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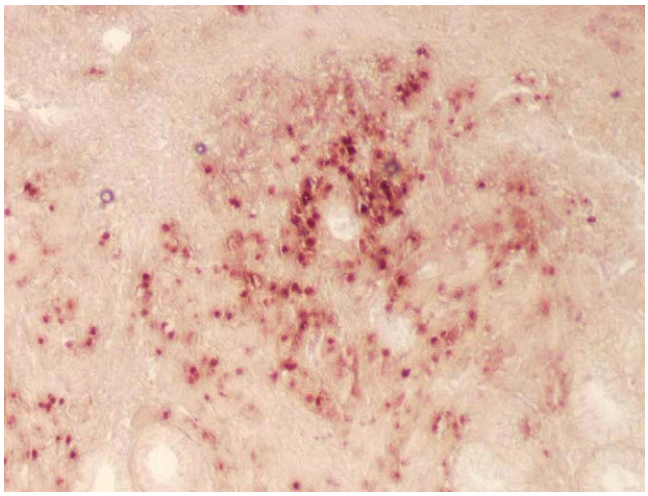

Fig. 1. Gastric adenocarcinoma with individual cell nuclei with EBER.

selected sections detection of EBER was conducted using in situ hybridization (ISH). The results of EBER tests in the material of gastric cancer in the selected 32 patients permitted to distinguish 14 patients (aging 42 to 67 years; 10 men and 4 women) with documented presence of EBER particles in the nuclei of gastric carcinoma cells (Fig. 1), forming the group of EBV-positive gastric carcinoma patients (group 1) and 18 patients (aging 45 to 69 years; 13 men and 5 women) without EBER particles in the studied material (Fig. 2), forming the group of EBV-negative gastric carcinoma patients (group 2).

2.2. Detection of EBER (EBER 1 and EBER 2) in tissue material

EBV DNA product in the form of untranslated RNA (EBER 1 and EBER 2) particles was detected in tissue material using in situ hybridization (ISH) (Howe and Steitz, 1986). The tissue material was fixed in formalin and embedded in paraffin. It originated from samples of gastric cancer (adenocarcinoma) obtained in the 32 patients. Five μm thick sections were deparaffinised and digested with proteinase K for 30 min at 37 °C, and washed in DEPC. This was followed by inactivation of proteinase K in 0.4% PFD solution for 20 min at 4 °C. The hybridisation was performed using a fluorescein-labelled RNA probe of 15 nucleotides in length (PNA Probe/FITC; DakoCytomation) for 15 h at 37 °C. After a thorough washing in SWS solution (DakoCytomation) the product was detected using FITC/AP-specific antibodies. BCIP/NBT (PNA ISH Detection kit; DakoCytomation) was used as a substrate.

Fig. 2. EBER-negative gastric adenocarcinoma.

2.3. Immunohistochemical analysis of p53 protein

Five μm thick sections from the same tumor blocks, used for EBER detection, were immunohistochemically analysed for the presence of p53 protein. Following removal of paraffin, rehydration and blocking of endogenous peroxidase activity with 3% H_2O_2 in distilled water, the tissue was incubated in 10 mM citrate buffer (pH 6.0) in a microwave oven (1000 W) for 15 min. Subsequently, the tissue was rinsed with phosphate-buffered saline (PBS) and treated with the primary antibody, DO-7 mouse anti-human p53 protein (DakoCytomation) in 1:50 dilution, employing 30 min incubation at room temperature. For visualisation of the reaction Universal LSAB^{TM} (labelled streptavidin-biotin) + HRP (horseradish peroxidase) kit (DakoCytomation) was used. Finally, the site of immunoprecipitate formation was detected by applying diaminobenzidine (DAB; Sigma). PBS was substituted for primary antibodies as a negative control. As a positive control, a section of colorectal cancer with high p53 expression was used. All slides were scored according to number of cells stained per 1000 carcinoma cells. The section was considered p53 positive when at least 10% cell nuclei were stained. The expression was scored as none (<10% stained cells), weak (+, 10–30%), moderate (++, 40–70%) or high (+++, >70% stained cells).

2.4. Determination of serum EBV-specific antibodies

EBV-specific antibodies were quantitated by ELISA. Sera of studied patients were tested employing kits for antibodies directed to the early antigen, IgG anti-EA (ETI-EA-G; DiaSorin), antibodies directed to viral capsid antigen, IgM anti-VCA (ETI-VCA-M; DiaSorin), IgG anti-VCA (ETI-VCA-G; DiaSorin) and antibodies reactive with EBV nuclear antigen, IgG anti-EBNA (ETI-EBNA-G; DiaSorin). Absorbance was read at $\lambda = 450/630$ nm, using the Behring Microstrip Reader. Results were expressed in AU/ml, where AU corresponded to an arbitrary unit established by comparison with an antibody standard. Values $\geq$20 AU/ml in the above described tests were considered positive.

2.5. Data analysis

Differences in frequencies of p53 positive results were compared with Fisher's exact test. Differences between distributions of EBV-specific antibodies in the studied groups of patients were compared by nonparametric Mann–Whitney test. *P*-values higher than 0.05 were considered non-significant.