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ABSENCE OF CYTOMEGALOVIRUS, EPSTEIN-BARR VIRUS, AND PAPILLOMAVIRUS DNA FROM ADENOMA AND ADENOCARCINOMA OF THE COLON

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Summary. — Biopsy specimens from 13 patients with adenocarcinoma of the colon and from 10 patients with endoscopic polypectomies for colon adenoma were examined for the presence of the DNA of cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human papillomavirus (HPV) types 2, 6, 16 and 18. The specific activities of viral DNA probes obtained by nick-translation ranged from 10^7 to 10^8 cpm/μg DNA. By Southern blot hybridization with an estimated sensitivity of 10 pg virus DNA whi chcorresponded to 0.05 virus genome equivalents per cell we failed to detect any virus DNA in the biopsy material tested.

Key words: hybridization; cytomegalovirus; Epstein-Barr virus; human papillomaviruses; adenocarcinoma and adenoma of the colon

Introduction

It is well established that human cytomegalovirus (CMV) is the causative agent in a variety of non-malignant diseases (Weller, 1971; Michelson-Fiske, 1977). Some evidence has accumulated that it is also associated with several neoplastic diseases of man including paraganglioma (Heine *et al.*, 1971), prostatic cancer (Laychock *et al.*, 1978), and Kaposi's sarcoma (Giraldo *et al.*, 1972, 1975, 1978). Several lines of evidence linked CMV with adenocarcinoma of the colon. The presence of CMV was reported in clinical materials of patients suffering from chronic intestinal diseases, such as ulcerative colitis (Powell *et al.*, 1961; Levine *et al.*, 1964) which are associated with a markedly increased incidence of colon cancer (de Dombal *et al.*, 1966; Farmer *et al.*, 1973). CMV was isolated from cell cultures derived from adenocarcinoma of the colon (Hashiro *et al.*, 1979) and CMV DNA was reported to be present in the diseased bowel of patients with ulcerative colitis, familial polyposis, and carcinoma of the colon (Roche and Huang, 1977; Roche *et al.* 1981; Huang and Roche, 1978) as well as in macroscopically normal tissue

adjacent to the tumour and in nonmalignant diseases (Roche *et al.*, 1981) indicating that CMV is frequently present in the colon tissue.

The possible involvement of CMV in tumour pathogenesis is supported by its capability to transform hamster (Albrecht and Rapp, 1973; Boldogh *et al.*, 1978) and human cells (Rapp *et al.*, 1975; Géder *et al.*, 1976, 1977). In addition, CMV resembles other oncogenic viruses in its ability to stimulate the production of enzymes involved in DNA synthesis (Huang, 1975; Isom, 1979; Tanaka *et al.*, 1978; Závada *et al.*, 1976), as well as host cell proteins, DNA and RNA synthesis (Ben-Porat and Kaplan, 1973; St. Jeor *et al.*, 1974; Tanaka *et al.*, 1975, 1978). The CMV DNA transforming region localized within a 2.9 kb fragment between map units 0.123 and 0.140 was identified by transfection with cloned CMV DNA fragments (Nelson *et al.*, 1982; 1984).

In our previous study we failed to detect CMV DNA in any of 7 colon biopsies from adenocarcinomas (Břicháček *et al.*, 1980) using the whole CMV DNA as a probe in reassociation kinetics test with an estimated sensitivity of 0.3 genome equivalents per cell. In this communication we present our results obtained by Southern-blot hybridization. In addition to the CMV DNA probe, we also used DNA probes from Epstein-Barr virus (EBV) and human papillomavirus (HPV) types 2, 6, 16 and 18.

Materials and Methods

Patients and biopsies. Clinical materials were obtained from the Department of Surgery, Faculty of Medical Hygiene, Charles University, Prague (adenocarcinoma of colon) and from the Department of Internal Medicine and Laboratory of Gastroenterology, Polyclinics of Charles University, Prague (colon adenoma, endoscopic polypectomy was carried out by the method of high frequency diathermocoagulation). Prior to surgery no patient had been given immunosuppressive medication, but all had received phtalylsulphathiazol (Ftalazol, Spofa, Prague) for 5 days (6 g/day) and neomycin for 2 days (2 g/day). Materials for hybridization were taken at surgery; portions of the same material were submitted for histological investigation. The diagnosis of carcinoma of the sigmoid colon was confirmed by histology in 13 patients; in the remaining 10 patients colon adenoma was diagnosed. The resected specimens were placed into tissue culture medium and were immediately transported on ice to the laboratory. They were stored at −70 °C until examined.

Preparation of biopsy DNA and blot hybridization. After thawing the specimens were homogenized and treated with 1 mg of pronase per ml in the presence of 1 % SDS at 37 °C for 12 hr. The biopsy DNA was further purified as described by Bartsch *et al.* (1987). DNA probes consisted of equimolar mixtures of the following plasmids: CMV — cM-5002, cM-1015, cM-5007, and cM-5009 (Rüger *et al.*, 1984), kindly provided by B. Fleckenstein (Institut für klinische Virologie der Universität Erlangen — Nürnberg, F.R.G.); EBV — cM B-14, cM 301—99, cM 302—21, cM 302—23, and pM 966—20 (Polack *et al.*, 1984), kindly provided by G. Bornkamm (Institut für Virologie, Freiburg, F.R.G.); and HPV — HPV 2, HPV 6, HPV 16, and HPV 18 (De Villiers *et al.*, 1981; Gissmann *et al.*, 1982; Dürst *et al.*, 1983; Boshart *et al.*, 1984), kindly provided by H. zur Hausen (Deutsche Krebsforschungszentrum, Heidelberg, F.R.G.). After cleavage with the respective endonuclease, HPV inserts were separated from the vector part of the plasmid by two runs of electrophoresis in 0.8 % agarose gel. Virus inserts were extracted from the agarose by electroelution. CMV and EBV plasmids and HPV inserts were labelled by ^{32}P-dCTP (110 TBq/mmol; Izinta, Budapest, Hungary) by nick-translation. Blot hybridization was performed as described by Bartsch *et al.* (1987) except that hybridization temperature for HPV DNA was 57 °C (T_m − 40 °C, low stringency) and the last washing in 0.2 × SSC was carried out at 32.6 °C (T_m − 40 °C); after one week exposure onto an X-ray film the same blot was washed in 0.2 × SSC at 47.6 °C (T_m −

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25 °C, high stringency). For CMV the hybridization and washing temperatures were 72 °C and 53.6 °C, respectively (high stringency). Total amount of 0.6 ng of cleaved plasmid containing 0.4—0.5 ng of respective virus insert was assayed on each blot as a positive control. Taking into account that about 10 pg of virus DNA could be detected in 4 μg of cellular DNA as revealed by reconstruction experiment, the estimated sensitivity of our detection system was 0.05 virus genome equivalents per cell.

Results

Hybridization of biopsy DNA specimens cleaved with *EcoRI* blotted to nitrocellulose and probed independently with CMV and HPV DNA at low and high stringencies is shown in Figs. 1, 2, and 3. Only CMV plasmids lacking homology with cellular DNA (Rüger *et al.*, 1984) were employed as probes. We failed to detect any specific hybridization signal in the DNA samples isolated from neoplastic or from adjacent healthy control tissue. Furthermore, no hybridization signal was detected when the same set of DNA samples was assayed with a DNA probe consisting of a mixture of labelled EBV plasmids (not shown).

Under low stringency conditions, the mixture of ^{32}P-labelled HPV 2, 6, 16 and 18 DNA inserts hybridized with all human biopsy DNAs without any signs of specificity (Fig. 2), resembling the distribution of cleaved and electrophoresed and ethidium bromide stained DNA just prior to blotting (not shown), and it also hybridized to some extent with type-different HPV 30 DNA (Fig. 2). The lack of a specific hybridization pattern under low stringency condition together with the positive hybridization with non-homologous HPV 30 DNA do not support a notion that another HPV type not included in our probe is present in a significant amount in the tested specimens.

Taken together, in hybridization tests with an estimated sensitivity of 0.05 virus genome equivalents per cell we failed to detect the DNA of CMV, EBV, and HPVs in any of the specimens obtained either from 13 adenocarcinoma of the colon or from 10 colon adenoma patients.

Discussion

Controversial data on the presence of CMV DNA in adenocarcinoma of the colon have been reported. They have covered the whole spectrum of possibilities from a total failure to prove the presence of CMV DNA in adenocarcinoma (Břicháček *et al.*, 1980; Hart *et al.*, 1982) to its demonstration in neoplastic as well as in healthy control tissues (Roche *et al.*, 1981). It is not easy to understand these controversies. The outcome of the tests could be influenced by a variety of factors such as differences in the reassociation techniques used, the nature of the CMV probes used, and the possible presence of sequences homologous to human DNA in these probes (Rüger *et al.*, 1984). Geografical and ethnic factors might also be involved. Our interest in the presence of EBV in carcinoma of the colon stems from a certain degree of similarity between sigmoid part of the colon and Waldeyer's ring in which association of EBV with epithelial cells has been proved (Břicháček *et al.*,

1984). It is typical for both of these localities that in their early embryonic development the three germinal layers (entoderm, ectoderm, and mesoderm) are closely associated. Close contact of lymphocytes with epithelial cells is especially relevant in this respect. The use of HPV DNA probes was inspired by the recently demonstrated association of papillomaviruses with several human malignancies, namely with carcinomas of the larynx and lower genital tract, including the perianal region (reviewed by Gissmann, 1984). It should also be noted that bovine papillomaviruses are associated with cancers of the alimentary canal in cattle (Jarrett, 1980).

The present results do not lend any support to the hypothesis that either CMV, EBV or HPV were involved in the aetiology of carcinomas and polyposes of human colon.

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Legends to figures (Plates XXXVIII—XL):

Fig. 1. Southern blot hybridization of *EcoRI* cleaved human DNA (4 μg) from colon adenoma (a—j), colon carcinoma (k—w) and adjacent healthy tissue (n′, o′, p′, q′, r′, v′, w′) biopsies with ^{32}P-dCTP labelled CMV DNA probe under high stringency conditions. Following CMV plasmids in total amount of 0.6 ng were used as positive controls: *HindIII* cleaved cM-1015 (A), cM-5007 (C), cM-5009 (D), and *EcoRI* cleaved cM-5002 (B).

Fig. 2. Southern blot hybridization of *EcoRI* cleaved human DNA 4 μg) from colon adenoma a—j), colon carcinoma k—w) and adjacent healthy tissue n′, o′, p′, q′, r′, v′, w′), biopsies with ^{32}P-dCTP labelled HPV DNA probe under low stringency conditions. Following HPV plasmid in total amount of 0.6 ng were used as a positive controls: *EcoRI* cleaved HPV 2 A) and HPV 18 D), *BamHI* cleaved HPV 6 (B), HPV 16 (C) and HPV 30 (E).

Fig. 3. Southern blot hybridization of *EcoRI* cleaved human DNA (4 μg) from colon adenoma (a—j), colon carcinoma (k—w) and adjacent healthy tissue (n′, o′, p′, q′, r′, v′, w′) biopsies with ^{32}P-dCTP labelled HPV DNA probe under low stringency conditions and subsequently washed under high stringency conditions. Following HPV plasmids in total amount of 0.6 ng were used as positive controls: *EcoRI* cleaved HPV 2 (A) and HPV 18 (D), *BamHI* cleaved HPV 6 (B), HPV 16 (C) and HPV 30 (E).