

RECTUM CANCER AND HUMAN PAPILOMAVIRUS INFECTIONS

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ABSTRACT

Human Papillomavirus (HPV) infections have been implicated in anogenital neoplasia in both sexes. In this study, HPV has been shown to be associated with rectal adenocarcinomas.

We used a consensus primer polymerase chain reaction DNA amplification methods for the detection and typing of human papillomavirus in 14 patients with rectum cancers. The cases were further tested by restriction endonuclease enzyme analysis for HPV types 6, 11, 16, 18, 31, 39, 51 and 58.

By polymerase chain reaction, human papillomavirus 18 DNA was demonstrated in two of 14 rectum cancer cases. No HPV DNA amplification was detected in the other patients.

These data indicate that an association exists between human papillomavirus and rectum cancer. The reason(s) why HPV is associated with adenocarcinoma of the rectum, despite presence of HPV in genital cancers, requires further study.

Key Words: Human papillomavirus, rectal adenocarcinoma

ÖZET

REKTUM KANSERİ VE İNSAN PAPILOMA VİRÜS İNFEKSİYONU

İnsan papillomavirus (İPV) enfeksiyonları, her iki cinsten anogenital neoplazilerde görülmektedir. Bu çalışmada, İPV'nün rektal adenokarsinomalarla ilişkisi gösterilmiştir. Rektum kanserli 14 hastada insan papillomavirüsünün belirlenmesi ve tiplendirilmesi için ortak primerli polimeraz zincir reaksiyonu metodu kullanılmıştır. Örnekler, restriksiyon enzim analizi kullanılarak İPV tipleri 6, 11, 16, 18, 31, 39, 51 ve 58 için test edilmiştir. Polimeraz zincir reaksiyonu ile 14 rektum kanserli hastanın ikisinde İPV 18 DNA'sı bulunmuştur. Diğer hastalarda İPV DNA'sı belirlenmemiştir. Bu çalışmada insan papillomavirüsü ile rektum kanser arasında bir ilişkinin olduğuna işaret edilmiştir. Genital kanserlerde İPV'nin varlığına rağmen, İPV'nin rektum adenokarsinomuna nasıl sebep olduğuna dair ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: İnsan papillomavirüsü, rektal adenokarsinoma

INTRODUCTION

Human papillomavirus has a well known, association with dysplasia and carcinoma of the uterine cervix and vulva (1-4). In recent

years, several studies have established a similar link between HPV infections and anal and colon carcinoma (5-8). Adenocarcinoma of the colon and rectum is one of the most prevalent of all internal malignancies. Affec-

ting both men and women, it competes with cancer of the breast and lung for incidence and mortality. The causes of the rectum carcinoma remain unknown. Despite associations of viral infections with other neoplasms for over three quarters of a century, a viral causative agent for rectum cancer has not been proposed. A few investigators have attempted to establish a link between this virus and adenocarcinoma [one notable exception being cervical adenocarcinoma, in which HPV is found in 36% to 64% of cases (9,10). Recently, however, it has been reported that HPV antigen or viral DNA is present in a large proportion of colonic adenocarcinomas studied by immunohisto-chemistry or in situ hybridization (11,12). The human papillomavirus seems a likely candidate for such an association because of its known associations with other neoplasms of the perineum and elsewhere.

Human papillomaviruses include at least 70 genotypes, of which HPV types 6, 11, 16, 18, 31, 33 and 35 are isolated most commonly from anogenital lesions. In the anogenital tract, HPV types 6 and 11 are associated mostly with benign condylomas; whereas types 16, 18, 31, 33 and 35 are associated more commonly with dysplasia and carcinoma (2,3,13). The HPV genome consists of a closed, circular, double-stranded DNA molecule containing a total of eight open reading frames. These include six early region genes (E1, E2, E4, E5, E6 and E7) and two late region genes (L1 and L2). The E6 and E7 open reading frames are conserved highly in HPV types 16- and 18-associated cervical carcinomas (14-16). In addition, these open reading frames appear to be necessary for maintenance of the transformed state (16).

This study describes the amplification of HPV DNA sequences in adenocarcinomas of rectum by the polymerase chain reaction (PCR) and thus adds to the growing list of malignancies associated with infection by this virus.

PATIENTS AND METHODS

All rectal biopsy samples were obtained from the Department of Gastroenterology, Gazi University Hospital, Ankara. The study group comprised of 14 patients with eleven adenocarcinoma, two nonspecific ulceration of rectitis, and one malign lymphoma, histopathologically. Tumor DNAs extracted as descri-

bed (17) from 14 rectum cancers were subjected to PCR amplification for HPVs as described by Manos et al (4). Briefly, Cetus HPV L1 consensus primers MY09 and MY11 (Perkin-Elmer Cetus, Norwalk, CT) which amplify an approximately 450-bp region of a large number of HPVs (types 6, 11, 16, 18, 31, 33, 35, 39, 40, 45, 51-59) and β -globin primers GH20 and PC04 (Perkin-Elmer Cetus, Norwalk, CT), which amplify a 268-bp region of the β -globin gene were employed simultaneously in the amplification reaction (18). The β -globin primers served as an internal control to test the suitability of tumor DNA for amplification and were used at a lower concentration than the HPV L1 consensus primers (0.05 μ M vs. 0.5 μ M). The reaction mixture contained the two sets of primers, 10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.01 % gelatin, 200 μ M of each dNTP, about 1 μ g of template DNA, and 2.5 units of Taq polymerase in a 50 μ l vol. Amplification was performed in a Cetus thermocycler (Perkin-Elmer Cetus Corp.) with thermocycle step parameters of 95 °C for 30 seconds, 55 °C for 30 seconds, and 37 °C for 1 minute, for 30 cycles. In addition to patient samples, HPV positive control (HPV-18 present on plasmid) and negative control (no target DNA in mix) tested for human papillomavirus by polymerase chain reaction. Ten microliters of PCR product was separated by electrophoresis using 3 % agarose gel (Sigma, A7431) containing ethidium bromide (5 μ g/25 ml) and was visualized by UV illumination.

Analysis of the L1 amplified regions of HPV by restriction endonuclease digestion was carried out using three restriction enzymes separately-Hae III, BstN I and Dde I; the predicted sizes of the digestion fragments are changed for the different types of HPV. For example, BstN I should yield 284-, 38- and 130-bp fragments from type 16, 113- and 342-bp fragments from type 18, 322- and 127-bp fragments from type 58 and 4-, 146- and 305-bp fragments from type 39, while Hae III should generate 217-, 108- and 124-bp fragments from type 6, 379- and 73-bp fragments from type 51 and 328- and 124-bp fragments from type 31. The fragments of HPV types 6 and 11 by Hae III were same; but the fragments of HPV 6 digested with Dde I was different than that of HPV 11: HPV 6 (382, 67

bp); HPV 11 (2, 447 bp). A volume of 12 µl of each amplified sample was added to the corresponding restriction enzyme cocktail under conditions specified by the manufacturer. Digested fragments were loaded on to a 3% or 4 % agarose gel, electrophoresed in TBE buffer (0.045 M Tris-borate, 0.001 M EDTA).

RESULTS

There were eight male and 6 female patients. The patients ranged in age from 26 to 85 years,

with a mean age of 48 years. Rectal bleeding, hemorrhoidal prolapse, and anorectal pain on combinations were the presenting symptoms in 13, 5 and 5 patients, respectively. Among the 14 patients examined, eleven had adenocarcinoma, two had nonspecific ulceration of rectitis and one had malign lymphoma. Selected clinical data and HPV results for the 14 patients are shown in Table 1.

Table 1. Selected clinical data and HPV results of 14 patients with rectum tumor

Patient No	Age(yr)/Sex	Symptoms	Site/Histology	HPV results
1	39/M	Bleeding	Rectum, nonspecific ulceration of rectitis	-
2	45/F	Bleeding	Rectum, adenocarcinoma	-
3	33/F	Bleeding	Rectum, adenocarcinoma	-
4	39/M	Bleeding,pain	Rectum, adenocarcinoma	-
5	40/F	Bleeding,pain,prolapse	Rectum,nonspecific ulceration of rectitis	-
6	26/M	Bleeding,pain,prolapse	Rectum, adenocarcinoma	-
7	61/M	ND	Rectum, malign lymphoma	-
8	31/M	Bleeding,pain,prolapse	Rectum, adenocarcinoma	-
9	85/F	Bleeding,pain,prolapse	Rectum, adenocarcinoma	-
10	63/M	Bleeding	Rectum, adenocarcinoma	+ HPV 18
11	55/M	Bleeding,prolapse	Rectum, adenocarcinoma	+ HPV 18
12	56/M	Bleeding	Rectum, adenocarcinoma	-
13	55/F	Bleeding	Rectum, adenocarcinoma	-
14	46/F	Bleeding	Rectum, adenocarcinoma, CIN I of cervix	-

HPV: human papillomavirus; M: male; F: female; +: positive; -: negative; ND: no data available

β-globin sequences were consistently amplified from all 14 tumor DNA specimens, indicating that the tumor DNAs were suitable for PCR. Amplified HPV DNA was found in 2 of 11 adenocarcinomas. Further analysis with Hae III and BstN I gave fragments of the expected size, demonstrating that the amplified DNA was HPV 18. Amplified HPV DNA was not detected in the other patients. Patient who had CIN I of the cervix was found negative for HPV DNA in the cervical specimen.

DISCUSSION

The carcinogenic potential of the papillomavirus has been previously demonstrated (19). The mechanism by which oncogenesis occurs is not known, but retroviruses and DNA viruses are known to activate protooncogenesis

(20). This is probably a multistep process with dietary (21,22) and genetic interactions (23, 24). Considerable evidence supports a strong association and possibly a causal relationship between HPV and both benign and malignant lesions of the urogenital tract and the head and neck region (13,25). The links between HPV infection and both anal squamous cell carcinoma and colon neoplasia have also been firmly established (8,26).

Using polymerase chain reaction methods in this study, we detected HPV DNA in 2 of 14 rectum cases and identified HPV type 18. HPV type 16 is most commonly associated with anal squamous all carcinomas, whereas HPV type 18 predominates in adenocarcinomas (7,26-28). Thus, HPV type 18 may also be associated with rectal adenocarcinoma.

Risk factors for anorectal HPV infection are ill defined. None of the 14 patients whose histories were known had evidence of human immunodeficiency virus infection and immunosuppression as an additional risk factor for the development of anorectal neoplasia (29). Proto-oncogene abnormalities have also been identified in colon neoplasia, including the c-ras and c-myc oncogenes (30). Hybridization techniques have demonstrated the c-myc oncogene and HPV DNA (HPV 16 and HPV 18 DNA) in human colorectal tumors (31). Evidence that HPV and c-myc oncogene may play a role in the carcinogenesis of colorectal cancers adds strength to the argument of multistage carcinogenesis.

Moreover, human papillomavirus may alter cell-cycle regulation in infected cells by several mechanisms. The HPV E6 and E7 genes bind the p53 and retinoblastoma tumor-suppressor gene products respectively (32,33). This specific binding may play a role in the ability of the E6 and E7 genes to promote increased proliferation.

The virus's association with cancers of the colon or the rectum remains less clear. Although the current data suggests a high degree of concordance between HPV infection and tumors of the human colon, additional cases with the rectum adenocarcinoma will be required to support this association.

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