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Human papillomavirus type 16 associated with multifocal transitional cell carcinomas of the bladder in two transplanted patients

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Abstract This report describes two cases of rapidly progressive, multifocal transitional cell carcinomas of the bladder that developed in two patients after renal and cardiac transplantation, respectively. In both cases human papillomavirus (HPV) type 16 DNA was detected using the polymerase chain reaction DNA amplification method. To our knowledge, this HPV type has not been previously described in multifocal bladder transitional cell carcinoma in transplanted patients. Our findings suggest that HPV may play a major role in the development of rapidly progressive, multifocal transitional cell carcinoma in immunosuppressed patients.

Key words Human papillomavirus, transitional cell carcinoma
Organ transplantation

Introduction

Transitional cell carcinoma of the urinary tract is not uncommon, but its etiology remains unclear and several risk factors (e.g., cigarette smoking, industrial exposure, parasitic infection, etc.) have been incriminated in its pathogenesis [4]. During the past several years, there has been increasing speculation that immunosuppression in transplant patients activates human carcinomas caused by human papillomavirus (HPV) and, in particular, squamous cell carcinomas in the ano-genital regions and skin [1, 19]. We report two cases of multiple bladder cancers that developed in two patients after renal and cardiac transplantation, respectively, in whom it was possible to demonstrate HPV type 16 DNA using the polymerase chain reaction (PCR) DNA amplification method. the relationship between papillomavirus, immunosuppression, and the development of transitional carcinoma is discussed.

Case reports

Case 1

A 48-year-old male with a history of heavy smoking started regular dialysis treatment for end-stage renal failure due to chronic pyelonephritis in December 1985. In January 1991, a cadaveric renal transplantation was performed under OKT3 prophylaxis. Main-

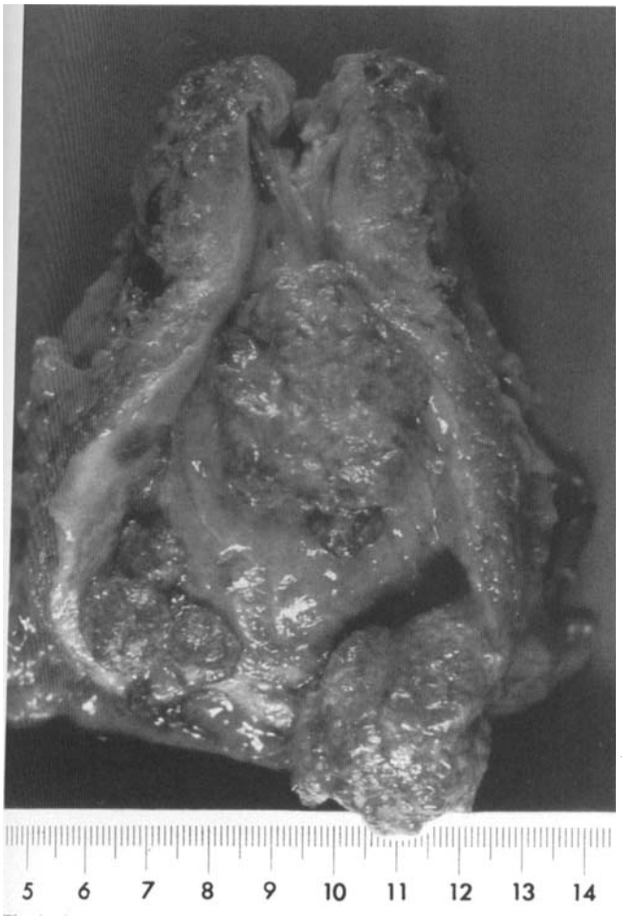

Fig. 1 Cystectomy specimen showing multiple papillomatous tumors involving large portions of the bladder

tenance immunosuppression consisted of 12.5 mg methylprednisolone, 75 mg azathioprine, and 250 mg cyclosporin A daily. In February 1992, a cystourethroscopy was performed because of macroscopic hematuria. This revealed a few large papillary tumor that was partially resected. Histology showed a well-differentiated transitional cell carcinoma with invasion of the inner muscularis, and a radical cystourethrectomy was performed. At that time, immunosuppression was withdrawn and worsening graft function required hemodialysis. Grade 2 transitional cell carcinoma involved all portions of the surgical specimen (Fig. 1) and a pathological stage of T2NOMO was assigned. The patient is doing well 12 months postoperatively.

Case 2

A 59-year-old mate received a heart transplant in August 1991 for hypertensive cardiomyopathy. He was evaluated in January 1992, for repeated hematuria. At that time, he was treated with prednisolone (30 mg/day), azathioprine (150 mg/day), and cyclosporin (400 mg/day). At cystoscopy and biopsy, a multiple, moderately differentiated infiltrating carcinoma was identified. Evaluation was negative for metastasis and total cystectomy and construction of a vesicosigmoidocutaneous conduit were performed. Histological examination of the surgical specimen showed a multifocal invasive grade 2 transitional cell carcinoma (G2pT3bNOMO) and a well-differentiated adenocarcinoma of the prostate, without capsule invasion (G1pT2a). Five months postoperatively multiple metastases were noted and the patient died.

The presence of HPV DNA was studied using the polymerase chain reaction (PCR) DNA amplification method from fresh-frozen tissues, as previously described with some modifications [17]. Selected specimens included the multiple transitional cell carcinomas of each patient: three specimens for patient 1 and two specimens for patient 2. Positive control DNA (pBr 322 containing genomic HPV 16) and a negative control corresponding to normal urothelium of each patient were also included.

Fresh-frozen tissues were cut, homogenized, and then resuspended in TE (10 mM TRIS, 100 mM EDTA, 100 mM NaCl, pH 8) containing SDS and 500 µg/ml proteinase K (Boehringer). Samples were vortexed for 2–3 min and incubated for 16 h at 50 °C. This step was followed by DNA purification (gene clean Kit-ozyme).

The oligonucleotide primers used in the study were synthesized by Dr. A. Bollen (Centre de Recherche Industrielle, Free University of Brussels) and were selected from the E7 region of the HPV genome (Table 1).

The DNA was suspended in 20 µl of water. Half of this solution was engaged in PCR in a final volume of 20 µl containing: 50 mM KCl, 10 mM TRIS HCl pH 8.4, 1.5 mM $MgCl_2$, 0.01 % BSA, 0.05 % W1, 0.25 mM of each DNTP, 20 pmol of each primer, and 1 U TAQ polymerase (BRL). Samples were covered with two drops of mineral oil (Sigma) and subjected to 30 cycles of amplification at the following temperature and time: denaturation at 95 °C for 1 min, annealing at 64 °C for 1 min, and extinction at 72 °C for 2 min.

After amplification, half of the material was run on a 4 % nusieve, 1 % agarose gel in TBE, stained with ethidium bromide, and photographed under UV light. All tumor specimens of each patient analyzed exhibited a band corresponding to the expected 127 base pairs, characteristic of HPV type 16 (Fig. 2). No band specific for

Table 1 Specific primers for the enzymatic amplification of HPV DNA fragments

Primers	Position of first nucleotide	Sequence	Length of the amplified segment	
HPV 6B	562–584 785–764	TGTATTAGACCTGCAACCTCCAG TTCCCAACAGAAGCTGTTGCAC	224	bp
HPV 11	562–583 767–746	AGTACTAGACCTGCAGCCTCCT GTAGTTGTCTGATGTCTCCCGTC	206	bp
HPV 16	694–713 820–798	GCAGAACCGGACAGAGCCCA GTGTGCCCATTAACAGGTCTTCC	127	bp
HPV 18	740–759 848–828	GCCCGACGAGCCGAACCACA GGAATGCTCGAAGGTCGTCTG	109	bp

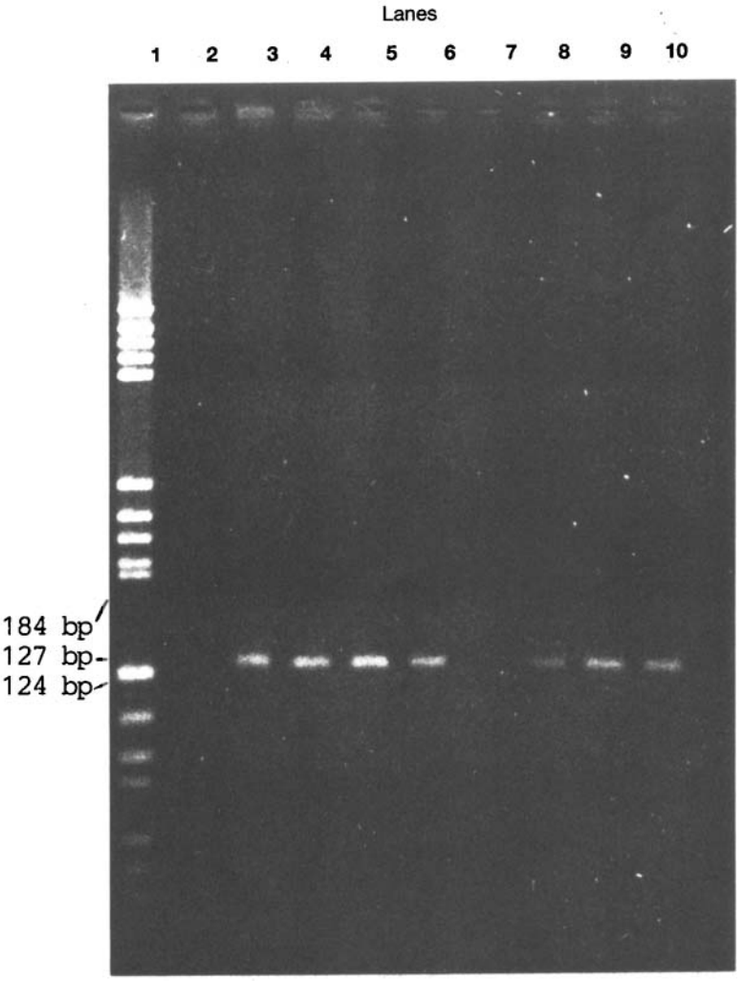

Fig. 2 Agarose gel electrophoresis of products amplified using HPV types 6B, 11, 16, and 18 primers. PCR was performed as described in the text. The reaction mixture was subjected to 4 % nusieve, 1 % agarose electrophoresis and then visualized by ethidium bromide staining under UV fluorescence. The number on the ordinate refers to base pairs. *Lane 1* molecular DNA weight marker V pBr 322-DNA HAE III, *Lane 2* negative control (patient 1; normal urothelium), *Lanes 3–5* multiple transitional cell carcinomas – HPV 16-positive (patient 1), *Lane 6* positive control (pBr 322 containing genomic HPV 16; patient 1), *Lane 7* negative control (patient 2; normal urothelium), *Lanes 8–9* multiple transitional cell carcinomas – HPV 16-positive (patient 2), *Lane 10* positive control (pBr 322 containing genomic HPV 16; patient 2)

HPV 6B, 11, or 18 was detectable. The normal urothelium and prostatic tissue were also negative.

Discussion

Organ transplant recipients who receive immunosuppressive drugs are at an increase risk of developing some virally induced tumors. The majority of these tumors are lymphomas associated with Epstein-Barr virus infection or malignant lesions of the skin and mucosal epithelium caused by HPV infection [4, 11]. HPV-linked skin tumors are the most frequent and occur in 40 % of grafted patients up to 20 years post-transplantation in the Netherlands [9]. In the same way, HPV-linked carcinoma of the genital tract is twice as common in graft recipients as in nonimmunosuppressed controls, but no clear-cut relation ship with the duration of the graft and quality of the immunosuppressive treatment can be established [6]. HPV is usually classified as low-risk or high-risk. Low-risk HPV types (e. g., HPV 1, 2, 3, 4, 6, and 11) are regularly encountered in warts, condylomata, and low-grade, squamous intraepithelial lesions. In contrast, high-risk types (e. g., HPV 16, 18, 31, 33, and 35) are associated with high-grade dysplasia in situ or invasive carcinomas [3, 8, 15].

Cancer of the bladder in cattle has been associated with the bovine papillomavirus (BPV) [18]. Recently, there have been a few reports suggesting some correlation between the HPV infection and benign or malignant lesions of the urinary tract in humans. HPV types 6–11 DNA have previously been described in benign condylomata of the urethra or bladder but also in transitional cell carcinomas. Therefore, HPV types 16 or 18 are generally associated with malignant tumors [2, 5, 10, 14, 20, 21]. The prevalence of HPV infection associated with transitional carcinoma in the general population seems exceptional [7]. Indeed, in a preliminary study of 73 transitional carcinomas presenting in nonimmunosuppressed patients, testing for HPV 6, 11, 16, 18, 31, 33,

and 35 were all negative for HPV sequences [16]. In these two cases, HPV type 16 DNA was found only in the multifocal tumor specimens and not in normal, adjacent urothelium or prostatic tissue; this constitutes strong evidence linking HPV with bladder cancer. In addition, tumor specimens were obtained by open surgery and not by transurethral resection. Thus, potential contamination by HPV-colonized urethral mucosa can be ruled out.

It seems that the expression of HPV types 16 and 18 by bladder cancer is significantly correlated with poor survival and a high rate of recurrence, and that there is no particular relationship with histologic grading or staging. For some authors this may represent an objective factor for prognostic determination [12]. Our study confirms these findings; indeed, the progression of bladder carcinoma was particularly rapid in patient 2, who died 5 months after the tumor was diagnosed.

Thus, for the urologist, the development of bladder carcinoma in organ transplant recipients raises special problems, and the surgical management will depend upon whether the allograft is to be retained or sacrificed [11]. Many factors must be weighed before making this decision, and determination of the HPV type in transitional cell carcinoma may provide the clinician with an important prognostic parameter. However, rigorous investigations are needed in the future to determine the exact role of HPV in the development of transitional carcinomas and to provide a guideline for the prevention and/or management of transplant patients with HPV-positive bladder cancer, particularly of the more virulent type.

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