The International Journal of Biological Markers, Vol. 22 no. 4, pp. 245-251

Prevalence of BK virus and human papillomavirus in human prostate cancer

V. Balis[1], G. Sourvinos[1], N. Soulitzis[1], E. Giannikaki[2], F. Sofras[3], D.A. Spandidos[1]

[1]Laboratory of Clinical Virology, Faculty of Medicine, University of Crete, Heraklion, Crete
[2]Department of Pathology, University Hospital of Crete, Heraklion, Crete
[3]Department of Urology, University Hospital of Crete, Heraklion, Crete - Greece

ABSTRACT: Polyomaviruses such as the BK virus (BKV), JC virus (JCV) and SV40, as well as the human papillomaviruses (HPV) are frequently detected throughout human populations, causing subclinical persistent infections and inducing oncogenesis in human and other cell lines. To test the involvement of these viruses in prostate tumorigenesis, we investigated the prevalence of BKV, JCV and HPV in a series of human prostatic malignancies. Forty-two samples of diagnosed prostatic malignancies were tested using standard polymerase chain reaction (PCR) protocols. Differentiation between BKV and JCV among the polyomavirus-positive samples was achieved after sequencing analysis of the PCR products. Reconstitution of BKV in vitro was performed and indirect immunofluorescence for the large T-antigen of the virus was applied to confirm the production of progeny virus. Detection and typing of HPV was carried out by PCR. The overall prevalence of polyomaviruses was 19% in the prostate cancer cases. Sequencing analysis of the polyomavirus-positive specimens revealed the presence of BKV in all samples. Reconstitution of the BKV from the BKV-positive prostate samples was successfully achieved in cell culture and progeny viral particles were obtained, confirming the presence of the virus in the human biopsies. HPV was detected in 4.8% of the samples, however, no HPV-11, HPV-16, HPV-18 or HPV-33 types were identified. BKV was frequently detected and could play a relevant role in the development and progression of human prostate cancer, whereas HPV does not seem to be implicated in this type of human neoplasia. (Int J Biol Markers 2007; 22: 245-51)

Key words: Polyomavirus, BK virus, HPV, PCR, Prostate cancer, Large T-antigen

INTRODUCTION

The Polyomaviridae family including the human BK virus (BKV), JC virus (JCV) and simian virus (SV40) as well as the human papillomaviruses (HPV) are common pathogens of the urinary tract and they can readily be associated with the pathogenesis of urinary tract malignancies such as prostate cancer. The polyoma genetic material consists of a circular double-stranded DNA molecule in the order of 5 kb (1). Its genomes contain an early region coding for the T-antigens (large T and small t), an early and a late region coding for the late expression proteins (agno plus capsid proteins), as well as a noncoding regulatory region (2, 3). Based on the structure of the noncoding regulatory region, JCV can be classified into 2 forms: the archetype and rearranged variants. Archetypal JCV contains a single copy of the promoter and enhancer. BKV and JCV share approximately 72% nucleotide homology with each other and 70% nucleotide homology with SV40.

BKV was first identified in 1971 in a urine sample after a renal transplant (4). BKV has been detected in human tissues such as the brain (5), pancreatic islets and prostate (3, 6). Among other cases, the detection of this specific viral family was possible in immunosuppressed patients either due to immunosuppressive treatment or AIDS infection, as well as in patients, and especially children, who had undergone transplants or were immunodeficient. The types of disease or cases where polyomaviruses are found to be present vary greatly. Examples are renal allografts, bone marrow transplants, AIDS (HIV-1) infections (7), Kaposi's sarcoma, brain tumors, pancreatic islets, kidney, bladder and prostate malignancies (3, 6), hemorrhagic cystitis (8), nephritis, nephropathy (9) and hepatic dysfunction.

Epidemiological studies performed in many countries have shown a peak in seroprevalence in early childhood of 83% in the United Kingdom, 60% in Finland, 71% in Germany, 83% in Italy, and 69% in the United States (10). Such investigations in population cohorts which included both healthy individuals and cancer patients have revealed high titers of antibodies against polyomaviruses and an increased frequency even in normal population samples (10-12). Despite its high seroprevalence and its remarkable tropism for specific types of tissues, BKV's oncogenic capacity has not yet been extensively examined (12, 13).

0393-6155/245-07$15.00/0

Prostate cancer is one of the most common male malignancies in Western countries. Despite the considerable progress in the discovery of genes involved in prostate cancer, the basic molecular mechanisms regulating its development and progression are still very poorly understood (14). The biological characteristics of human prostate cancer, such as its multifocal nature and the lack of a clear molecular association, have led to the assumption that an external or infectious agent could be involved. Previous studies have attempted to correlate oncogenic viruses such as HPV with prostate cancer (15, 16). Several of these investigations showed a clear cancer association with HPV, and others reported that HPV was equally prevalent in benign prostatic hypertrophy (BPH) and even in normal prostate tissue. However, other studies failed to detect HPV in any prostate tissues (15, 17). Furthermore, a study in Nordic cohorts did not support an association between the serological markers of HPV-16, HPV-18, and HPV-33 infections and the risk of prostate cancer (18).

In contrast to HPV, the literature regarding the prevalence of polyomaviruses in prostate cancer is rather limited, even though persistent infection of the urinary tract from this viral family is well established. An immunofluorescence study by Shah et al reported no evidence of large T-antigen expression of primary cultures from prostate carcinoma (12). In contrast, Monini et al were able to isolate a new BKV strain (URO-1) from prostate and other urogenital tumors (19). More recent studies detected BKV sequences by solution PCR and in situ hybridization both in prostate tumors and in benign and atrophic prostate lesions (3, 6). In the present study, we examined a series of patients diagnosed with and treated for prostate cancer in order to assess the prevalence of BKV, JCV and HPV in prostate tumors.

MATERIALS AND METHODS

Patients

Prostate tissue specimens were obtained from patients undergoing surgical treatment for prostate cancer at the Department of Urology, University Hospital of Crete, Greece after the hospital's bioethics review by the human subjects committee. A total of 42 specimens were used in this study: 22 were archival specimens (formalin-fixed, paraffin-embedded tissue) and 20 were fresh frozen specimens. After histological identification on hematoxylin and eosin-stained slides, only cancerous prostate tissues were selected and processed for DNA analysis. Before DNA extraction from fresh frozen samples, histological sections were prepared to determine the proportion of cancer cells microscopically. Clinical and epidemiological data of patients were retrieved from their medical records and are listed in Table I. Informed consent was obtained from all patients participating in the study.

DNA extraction

DNA was extracted from all prostate samples by lysis and proteinase K digestion in 50 mM Tris pH 8.0, 100 mM NaCl, 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% sodium dodecyl sulfate (SDS). After overnight incubation at 37°C, the samples were extracted with phenol chloroform and precipitated with ethanol. DNA concentration and purity was determined by spectrophotometry at λ=260/280nm. The extraction process was performed in an area that was BKV free while great care was taken during the tissue sectioning procedure in order to avoid any contamination. Sectioning of the tissues was carried out using a clean microtome and a separate new blade in each case, as well as clean gloves and forceps. The sections were placed in autoclaved DNAse-free microtubes for the DNA isolation procedure.

Solution PCR

The *VP1* gene which is common in both BKV and JCV was amplified by solution PCR using the forward primer VP_f (5' AGT GGA TGG GCA GCC TAT GTA 3') and the reverse primer VP_r (5' TCC AGG GGA CCC AGA TAT GA 3'), yielding a 95 bp product. The existence of several variations in the nucleotide sequence of the amplified region allowed differentiation between BKV and JCV. The PCR program commenced with incubation at 50°C for 2 minutes followed by a first denaturation step at 95°C for 3 minutes, and then 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds. The final step was 72°C for 5 minutes. The reaction mix in all PCR reactions consisted of PCR buffer 1×, 0.5mM MgCl2, 0.05mM of each dNTP, 0.3mM of each primer

TABLE I - CLINICOPATHOLOGICAL CHARACTERISTICS OF THE STUDY SAMPLES

	Prostate Cancer (%)
Cases, n.	42
Age (mean ± SD, years)	68.4 ± 5.5
PSA blood levels (mean ± SD, ng/ml)	12.3 ± 10.2
TNM	
T1-T3a	28 (66.7)
T3b-T4	14 (33.3)
Gleason score	
2-6	16 (38.1)
7-10	26 (61.9)

and 0.65 units Taq DNA polymerase (Invitrogen) and 100 ng/μL DNA. The plasmid pB-VP1 containing the *VP1* gene was used as positive control. All positive PCR products for the *VP1* gene were subjected to direct sequencing and the DNA sequences were aligned for the differentiation between the polyomaviruses. To ensure that our PCR assay was sensitive enough to detect relatively low levels of viral DNA, serial 10-fold dilutions of the plasmid DNA with a known number of copies (4×10^6 copies/mL) were amplified by PCR. The sensitivity of all PCR assays, and thus the sensitivity of detection of BKV, reached 200 copies/mL (Fig. 1B). HPV DNA was detected using the standard GP5+/GP6+ primers while specific primers were used for typing the HPV-positive samples. Finally, beta-2 microglobulin (β2m) was used as a reference gene to ensure the presence of amplifiable DNA in all the samples. PCR products were visualized on 2% agarose ethidium bromide-stained gels using a UV transilluminator.

Cell culture, transfection and BKV rescue

Vero cells were grown in Glasgow modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. The cells (0.8×10^6) were seeded in 10-cm^2 plates and transfected using the Lipofectamine Plus reagent (Gibco BRL) using 10 μg total DNA extracted from BKV-positive prostate samples. In order to monitor the production of BKV cultivation infectious extracellular virions and the presence of viral DNA, half of the culture medium was harvested once a week and replaced by fresh growth medium. The cultures were kept this way up to 40 days post-transfection. The production of infectious extracellular BKV was analyzed by immunofluorescence after seeding 100 μL of harvest 40 days post-transfection onto Vero cells grown on coverslips in 24-cm^2 wells. Following adsorption, the cells were washed once and fed the complete medium. Three days post-infection, immunofluorescent staining for BKV was performed. According to this assay, infectious BKV particles were detectable 35 days post-transfection.

Immunofluorescence

Vero cells were seeded at 1×10^5 cells per well in 24-well Linbro multiwell plates containing 1 coverslip and infected the following day with the collected supernatant from the BKV-positive culture of Vero cells. Three days post-infection, the cells were fixed for 10 minutes with formaldehyde (5% v/v of the 30% stock solution in PBS containing 2% sucrose), washed 3 times with PBS, then permeabilized for 5 minutes in a PBS solution containing 0.5% Nonidet P-40 and 10% sucrose. The BKV anti-TAg antibody was a mouse monoclonal antibody (MA B8505, Chemicon, USA) which was diluted in PBS containing 1% FBS (1:200 dilution). After incubation at room temperature for 1 hour, the coverslips were washed 3 times with PBS plus 1% FBS and subsequently incubated with fluorescein (FITC)-conjugated anti-IgG (1:200 dilution). After a further 45-minute incubation, the coverslips were washed 3 times with PBS plus 1% FBS, air-dried and finally mounted using Fluoromount-G (Southern Biotech) containing DAPI. Cell samples were examined using a Leica fluorescence microscope.

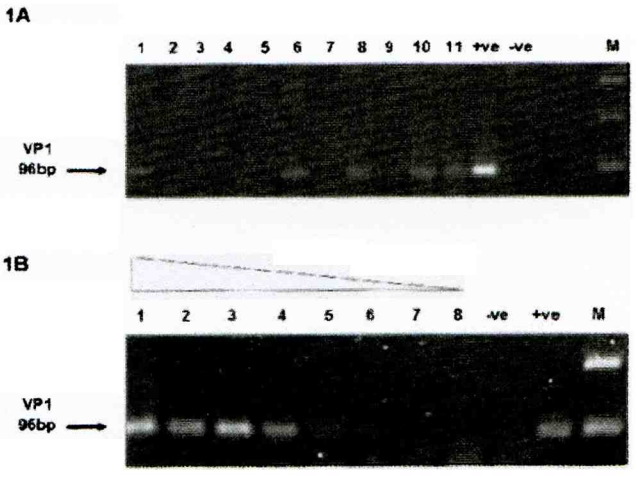

Fig. 1 - *A. Detection of polyoma viral sequences in human prostate samples. Lanes 1, 6, 8 and 11: positive specimens for the VP1 gene. Lanes 2-4, 7, 9: negative cases. +ve: positive control, -ve: blank. M: 100 bp DNA ladder. B. Assay determining the sensitivity of the PCR for the VP1 gene. Serial dilutions of the positive control were amplified by PCR until the PCR signal was undetectable (lane 7).*

Statistical analysis

The association of the BKV status with continuous variables of the patients' clinical data (age, PSA) was examined using Student's *t*-test (after examining the equality of variance with Levene's test) or its nonparametric equivalents, the Mann-Whitney *U* and Kruskal-Wallis *H* tests. Additionally, the chi-square (χ^2) test, using Fisher's exact test when indicated by the analysis, was used to examine BKV status in correlation with the categorical data (TMN, Gleason score) or after the stratification of continuous variables. All statistical analyses were 2-sided and performed with SPSS 11.5 (SPSS, Chicago, IL). Statistical significance was set at the 95% level (p value <0.05).

RESULTS AND DISCUSSION

Forty-two patients with prostate cancer were examined for the presence of BKV, JCV and HPV. Their ages ranged from 55 to 75 years (mean ± SD: 68.4 ± 5.5). The histopathological features of the patients are shown in

	BKV/JCV *VP1* Variable Sequence	Viral strains
BKV	5' **AGGTAGAAGAGGTTAGGGTGTTTGATGGCACAG** 3'	wt BKV (Dunlop)
JCV	5' **AGGTAGA**G**GAGGTTAG**A**GTGTTTGA**G**GG**G**ACAG** 3'	Isolates: JCV SA27_03 genotype 2, UZ-18,UZ-10
JCV	5' **AGGTAGA**G**GAGGTTAG**A**GT**T**TTTGA**G**GG**G**ACAG** 3'	Isolates: JCV USA2, PE1-PE23
Samples (N=5)	5' **AGGTAGAAGAGGTTAGGGTGTTTGATGGCACAG** 3'	wt BKV (Dunlop)
Samples (N=2)	5' **AGGTAGAAGAGGT**C**AGGGTGTTTGATGGCACAG** 3'	Isolates: BKV archetypal strain MT, TW-2
Samples (N=1)	5' **AGGTAGG**A**GAGGT**C**AGGGTGTTTGATGGCACAG** 3'	Isolate: BKV CAP-h2

Fig. 2 - *VP1 variable sequence between BKV and JCV and alignment with the detected sequences in polyomavirus-positive prostate samples. Sequences in bold are common among BKV and JCV whereas roman characters represent variations between the 2 viruses, allowing differentiation between them.*

Table I. The prevalence of polyoma-positive samples was 19% as detected by PCR. Representative examples of polyomavirus- and HPV-positive samples are shown in Figure 1A. Both sets of samples, i.e., frozen and paraffin-embedded tissues, showed high yields in DNA recovery and thus no difference in the detection of viral DNA was observed. Although the *VP1* gene is common in both BKV and JCV, several nucleotide variations within the amplified region have allowed the differentiation between the 2 viruses. All polyomavirus-positive PCR products were subjected to direct sequencing and the DNA sequences were aligned with published viral sequences. The results of this analysis are presented in Figure 2. Five samples were infected by the wild-type BKV (Dunlop), 2 samples contained sequences belonging to the isolates of the BKV archetypal strain, MT and TW-2, while the last polyomavirus-positive specimen contained viral sequences from the isolate BKV CAP-h2. None of the prostate samples were infected with JCV. Additional correlation between the clinical data of the patients and the findings from the molecular analysis did not show any statistically significant association.

The presence of BKV was also confirmed after the successful reconstitution of the virus from the human samples. In particular, total DNA from the wild-type BKV (Dunlop)-infected prostate specimens was transfected into Vero cells. In order to monitor the production of BKV cultivation infectious extracellular virions and the presence of viral DNA, half of the culture medium was harvested once a week and replaced by fresh growth medium. Evidence of a cytopathic effect in the transfected cells became obvious 5 weeks later (Fig. 3A, B). The production of infectious extracellular BKV was analyzed by seeding 10 µL harvest 40 days post-transfection onto Vero cells grown in 24-well plates containing coverslips. Two days post-infection, indirect immunofluorescence staining for the BKV large T-antigen was performed and infectious BKV was detectable, confirming the presence of BK viral genomes in the human prostate samples (Fig. 3C, D). The proportion of BKV-positive cells for immunofluorescence did not exceed 5%, possibly reflecting either low transfection efficiency or the small amounts of infectious particles produced. DNA was extracted from the 40 days post-transfection supernatant and the presence of the *VP1* gene was confirmed by PCR (data not shown).

Considering that BKV is ubiquitous in the human population, an oncogenic potential has been hypothesized for the virus (20, 21). The presence of BKV sequences in tumors has been reported in several studies (22, 23). There are contradictory reports on the presence of BKV DNA in urinary tract tumors: the authors of one study detected BKV DNA using PCR in 31 of 52 samples, whereas other authors were unable to find it (3, 6). Zambrano et al (3) detected BKV in 4 of 30 fresh tissue prostate samples and were unsuccessful with archival specimens, and Das et al (6) observed the virus in atrophic lesions in prostate tumors. One of the most important proteins for BKV is the replicational regulatory protein, the large T-antigen that binds onto the tumor suppressor proteins p53 and pRb1, inhibiting their functions and leading to a variety of transforming effects. Given the low frequency of either *TP53* or *RB1* mutations in prostate cancer, it was intriguing to investigate the prevalence of BKV in prostate tumor samples. Our analysis revealed a considerable prevalence of BKV infection in prostate cancer samples. The inclusion of archival prostate tumors in our cohort of samples did not affect the PCR analysis, perhaps due to the amplification of a small PCR product compared to previous studies. The extraction of good quality DNA from the human samples allowed the reconstitution of the wild-type BKV virus in cell culture. The same procedure was not carried out for the other strains, although they were detected by PCR, due to the technical difficulties in cultivating them in vitro. Our findings provide solid evidence that the prostate could be a preferable site for the establishment of BKV infections inducing cell transformation, possibly due to the inactivation of the major tumor suppressor proteins, p53 and pRb1.

A number of previous studies have shown positive associations between the risk of prostate cancer and indices of sexual activity and sexually transmitted diseases. One potential etiological factor of interest for prostate cancer, in light of the associations with sexual practices, is exposure to HPV. As HPV-16 and HPV-18 are transmitted sexually, and sexual factors have been linked to an

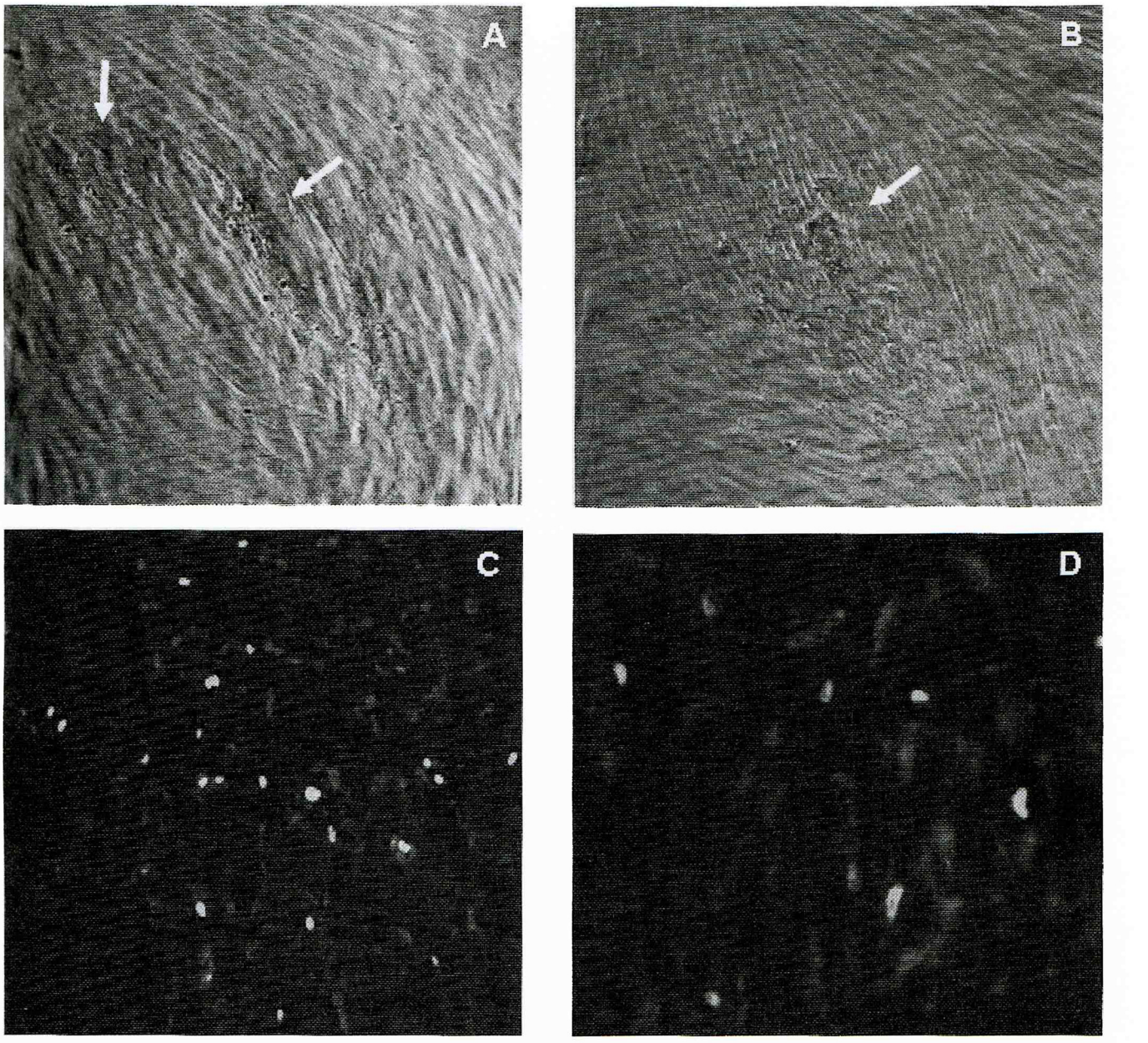

Fig. 3 - *Reconstitution of BKV from human prostate samples in cell culture. A cytopathic effect was detected 40 days post-transfection with total DNA extracted from prostate cancer samples (panels A, B). Indirect immunofluorescence analysis demonstrated the expression of the BKV large T-antigen in cells infected with the 40 days post-transfection supernatant (white). DAPI staining was used to determine the proportion of infected cells: panel C: magnification ×100; panel D: magnification ×200.*

elevated relative risk of prostate cancer, it has been hypothesized that HPV could also be a risk factor for prostate cancer. The prevalence of HPV in the same series of prostate specimens was also examined. PCR analysis using the standard GP5+/GP6+ primers revealed the presence of HPV DNA in only 2 samples (4.8%) (Fig. 4). Further attempts for the typing of these HPV-positive samples using specific primers for HPV-11, -16, -18 and -33 were not successful, suggesting that other HPV types should be implicated.

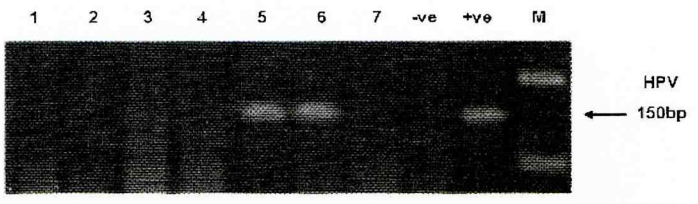

Fig. 4 - *Detection of HPV in human prostate samples. Lanes 5 and 6: positive specimens. Lanes 1-4, 7: negative cases. +ve: positive control, -ve: blank. M: 100 bp DNA ladder.*

No clear positive association between HPV and prostate cancer has been established so far. Initial studies of the possible relationship between HPV and prostate cancer involved attempts to detect HPV DNA in tumor tissue from prostate cancer patients compared with prostate tissue obtained from men with benign conditions. Among 20 investigations, only 2 found an association between tissue evidence of prior HPV infection and prostate cancer. Many of the studies were limited by small sample sizes and used different methods for the detection of HPV. A limited number of serological studies of antibodies to HPV-16 and HPV-18 have also yielded inconsistent results. Two nested case-control studies observed increased relative risks for prostate cancer associated with HPV-16 or HPV-18 antibodies, whereas no associations were observed in 3 other case-control studies.

The presence of the BKV genome shows that the virus is an inhabitant of the prostate and it could be suggestive of its involvement in the development of prostate cancer. These observations, which are in agreement with previous studies, emphasize the need for further investigation into the pathways that might be regulated by BKV, which could, in turn, lead to an improved risk assessment for prostate cancer. We found no evidence to support the hypothesis that HPV infection is related to the development of prostate cancer.

ACKNOWLEDGMENTS

The authors would like to thank Dr Yok L. Kwong, Department of Medicine, Queen Mary Hospital, Hong Kong for providing us with the plasmid pB-VP1.

This study was funded by the University Hospital of Crete, Crete, Greece.

ABBREVIATIONS

BKV: BK virus
JCV: JC virus
HPV: human papillomavirus
PCR: polymerase chain reaction

Address for correspondence:
Prof. D.A. Spandidos
Laboratory of Virology
Medical School, University of Crete
P.O. Box 1527
Heraklion 710 03, Crete, Greece
e-mail: spandidos@spandidos.gr

REFERENCES

1. Gorrill T, Feliciano M, Mukerjee R, Sawaya BE, Khalili K, White MK. Activation of early gene transcription in polyomavirus BK by human immunodeficiency virus type 1 Tat. J Gen Virol 2006; 87: 1557-66.
2. Tognon M, Casalone R, Martini F, et al. Large T antigen coding sequences of two DNA tumor viruses, BK and SV40, and nonrandom chromosome changes in two glioblastoma cell lines. Cancer Genet Cytogenet 1996; 90: 17-23.
3. Zambrano A, Kalantari M, Simoneau A, Jensen JL, Villarreal LP. Detection of human polyomaviruses and papillomaviruses in prostatic tissue reveals the prostate as a habitat for multiple viral infections. Prostate 2002, 53, 263-76.
4. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet 1971; 1: 1253-7.
5. Croul S, Otte J, Khalili K. Brain tumors and polyomaviruses. J Neurovirol 2003; 9: 173-82.
6. Das D, Shah RB, Imperiale MJ. Detection and expression of human BK virus sequences in neoplastic prostate tissues. Oncogene 2004; 23: 7031-46.
7. Cubukcu-Dimopulo O, Greco A, Kumar A, Karluk D, Mittal K, Jagirdar J. BK virus infection in AIDS. Am J Surg Pathol 2000; 24: 145-9.
8. Vogeli TA, Peinemann F, Burdach S, Ackermann R. Urological treatment and clinical course of BK polyomavirus-associated hemorrhagic cystitis in children after bone marrow transplantation. Eur Urol 1999; 36: 252-7.
9. Nickeleit V, Klimkait T, Binet IF, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 2000; 342: 1309-15.
10. Reploeg MD, Storch GA, Clifford DB. Bk virus: a clinical review. Clin Infect Dis 2001; 33: 191-202.
11. Vago L, Cinque P, Sala E, et al. JCV-DNA and BKV-DNA in the CNS tissue and CSF of AIDS patients and normal subjects. Study of 41 cases and review of the literature. J Acquir Immune Defic Syndr Hum Retrovirol 1996; 12: 139-46.
12. Shah KV, Daniel RW, Warszawski RM. High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents of Maryland. J Infect Dis 1973; 128: 784-7.
13. Pal A, Sirota L, Maudru T, Peden K, Lewis AM, Jr. Real-

time, quantitative PCR assays for the detection of virus-specific DNA in samples with mixed populations of polyomaviruses. J Virol Methods 2006; 135: 32-42.
14. Yang XJ, Wu CL, Woda BA, et al. Expression of alpha-Methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. Am J Surg Pathol 2002; 26: 921-5.
15. Effert PJ, Frye RA, Neubauer A, Liu ET, Walther PJ. Human papillomavirus types 16 and 18 are not involved in human prostate carcinogenesis: analysis of archival human prostate cancer specimens by differential polymerase chain reaction. J Urol 1992; 147: 192-6.
16. Ibrahim GK, Gravitt PE, Dittrich KL, et al. Detection of human papillomavirus in the prostate by polymerase chain reaction and in situ hybridization. J Urol 1992; 148: 1822-6.
17. Serfling U, Ciancio G, Zhu WY, Leonardi C, Penneys NS. Human papillomavirus and herpes virus DNA are not detected in benign and malignant prostatic tissue using the polymerase chain reaction. J Urol 1992; 148: 192-4.
18. Korodi Z, Dillner J, Jellum E, et al. Human papillomavirus 16, 18, and 33 infections and risk of prostate cancer: a Nordic nested case-control study. Cancer Epidemiol Biomarkers Prev 2005; 14: 2952-5.
19. Monini P, Rotola A, Di Luca D, et al. DNA rearrangements impairing BK virus productive infection in urinary tract tumors. Virology 1995; 214: 273-9.
20. Imperiale MJ, Pass HI, Sanda MG. Prospects for an SV40 vaccine. Semin Cancer Biol 2001; 11: 81-5.
21. Tognon M, Corallini A, Martini F, Negrini M, Barbanti-Brodano G. Oncogenic transformation by BK virus and association with human tumors. Oncogene 2003; 22: 5192-200.
22. Geetha D, Tong BC, Racusen L, Markowitz JS, Westra WH. Bladder carcinoma in a transplant recipient: evidence to implicate the BK human polyomavirus as a causal transforming agent. Transplantation 2002; 73: 1933-6.
23. Jorgensen GE, Johnsen JI, Ponthan F, Kogner P, Flaegstad T, Traavik T. Human polyomavirus BK (BKV) and neuroblastoma: mechanisms of oncogenic action and possible strategy for novel treatment. Med Pediatr Oncol 2000; 35: 593-6.

Received: May 4, 2007
Accepted: September 18, 2007