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Association of human herpesvirus type 6 DNA with human bladder cancer

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Abstract

We examined the presence of human herpesvirus type 6 (HHV6) DNA in a series of 74 bladder carcinomas from a Mediterranean population to elucidate their possible role as cofactor in the development of bladder cancer with or without associated human papillomavirus (HPV) infection. HHV-6 type B DNA was present in 5 men (6.8%) out of the 74 tumors investigated; two of them had associated HPV-16 DNA in the same specimen. In one case that had associated urothelial carcinoma in situ, both HHV-6B and HPV-16 DNA were present. In conclusion, the low incidence of HHV-6B in bladder cancer and the ubiquitous nature of HHV-6 infection are more consistent with a bystander role rather than cofactor in the oncogenesis of bladder cancer.

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Keywords: Bladder cancer; Carcinogenesis; Human herpesvirus type 6; Human papillomavirus

1. Introduction

The role of DNA viruses in the oncogenesis of bladder urothelial tumors remains controversial and most reports concentrate on human papillomavirus (HPV) [1–10]. In general DNA viruses as pathogenic factors in cases of urothelial bladder carcinoma have received little attention; and to our knowledge no previous studies on human herpesvirus type 6 (HHV-6) incidence in bladder tumors have been reported. HHV-6 might act as oncogenic cofactor of HPV infected bladder cancer and evidences suggest that HHV-6 contain in vitro transforming genes which are retained in both transformed and tumor-derived cell

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lines [1,3–5,7,11]. As reported, the current working hypothesis is that HPV is necessary, but not sufficient, for the development of premalignant and malignant lesions of the genital tract [3,12,13]. Recent studies have shown that HHV-6 can replicate in vitro in human epithelial cells including cervical epithelial cells and that molecular clones of HHV-6 can transactivate HPV promoters in vitro thus enhancing the expression of the viral oncoproteins E6 and E7 [3,13,14]. These findings open a way of investigating the possible association between HHV-6 and HPV not only in cervical cancer but in other epithelial tumors including urothelial bladder carcinomas [12].

The aim of our study was to investigate the presence of HHV-6 DNA in carcinomas of the urinary bladder from a Mediterranean-based population and explore their possible role as cofactor of bladder cancers with or without associated HPV infection.

2. Material and methods

The study series included 74 tumor samples and 22 non-malignant bladder specimens (normal urothelium $n=10$; chronic non-specific cystitis $n=12$) that were retrieved from Reina Sofia University Hospital (Cordoba, Spain) files. Specimens obtained by transurethral resection or cystectomy were formalin-fixed and paraffin-embedded following standard procedure. The mean age of patients was 61.8±7.3 (range 43–78). Six (6.2%) patients were female. The tumors were classified according to WHO (World Health Organization) grading criteria and clinically staged following the TNM (Tumor, Node, Metastasis) 2002 revision [15,16]. They included 38 (51.3%) low grade superficial (stage Ta or T1), and 36 (48.7%) high grade (stages T2–T4); 17 (23%) had associated urothelial carcinoma in situ. Representative paraffin blocks were selected based on the amount of tumor present and additional studies included the assessment of p53 status by immunohistochemistry and the Streptavidin–biotin peroxidase method (LSAB staining kit, DakoCytomation, Denmark). P53 was then quantitated using the grid-counting method to establish a labeling index (LI) (percentage of positive nuclei in 1000 cells). A LI of ≥10% was considered positive for p53 over-expression.

2.1. DNA extraction and PCR analysis for HPV and HHV-6

Three to four 10 μm tissue sections from each block were collected into microcentrifuge tubes and paraffin was removed twice with xylene and washed once with 0.5 ml of 100% ethanol to remove the solvent. The samples were incubated for 6 h at 55 °C in 300 μl of digestion buffer (50 mM Tris pH 8.5; 1 mM EDTA; 0.5% Tween 20 and 200 μg ml of proteinase K). The solution was heated at 95 °C for 10 min to inactivate proteinase K. DNA was then extracted twice with phenol–chloroform and the aqueous phase precipitated with 95% ethanol at −20 °C overnight. The quality of the DNA was assessed by PCR amplification of human β-globin DNA fragment of 536 bp with KM29 (5′-GGTTGGCCAATCTACTCCCAGG-3′) and RS42 (5′-GCTCACTCAGTGTGGCAAAG-3′) primers, which demonstrated the absence of inhibitors and the integrity of processed DNA. Specimens were further tested with the L1 consensus HPV primers MY11 (5′-GCCCAGGGACATAACAATGG-3′) and MY09 (5′-CGTCCCAGGGGATACTGATC-3′) and PCR products were visualized on ethidium bromide stained agarose gels.

Positive samples for generic HPV infection were amplified with HPV 6, 11, 16 and 18 specific primers pairs resulting in amplification of HPV 16 DNA fragment of 152 bp with the primers HPV964 (5′-AATGCTAGTGCTTATGCAGC-3′) and the primer HPV965 (5′-ATTTACTGCAACATTGGGTAC-3′). PCR was performed mixing 5–10 μl of purified DNA with a solution containing 2 mM magnesium chloride, 50 mM potassium chloride, 10 mM Tris–HCl, pH 8.3, 0.01% gelatine, 200 μM dNTP, 2.5 U of Taq DNA polymerase and 2 μM of the primers. All the reactions were carried out with an automated DNA thermal cycler (Perkin-Elmer, Norwalk, CT). Cycling parameters were 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, for 40 cycles, followed by a final extension at 72 °C for 7 min. Amplified DNA fragments were hybridised with specific [^{32}P]ATP end-labeled probe HPV966 (5′-GCAAACCACCTATAGGGGAACACTGGGGCA-3′). The complexes

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