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RESEARCH

Human Papillomavirus in Benign Prostatic Hyperplasia and Prostatic Adenocarcinoma Patients

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Abstract The aim of this study was to determine the prevalence of human papillomavirus (HPV) types in tissue and HPV antibodies in prostatic disease. Prostate tissue samples were collected from 51 patients diagnosed with adenocarcinoma and 11 with benign prostatic hyperplasia (BPH). All tissue samples were confirmed by histology. Plasma samples were available for 52 prostate patients. We investigated HPV DNA prevalence by PCR, and PCR positive samples were HPV type determined by sequencing. Prevalence of antibodies against twenty-seven HPV proteins from fourteen different HPV types was assessed in the plasma samples. The HPV DNA prevalence in the tissue samples was 14% (7/51) for prostate cancer samples and 27% (3/11) for BPHs. HPV-18 was the only type detected in tissue samples (10/62). No significant difference in HPV prevalence between the prostate cancer and BPH samples was found. HPV-positive cells were identified in eight of our thirteen prostate tissue slides (3/3 BPH and 5/10 adenocarcinoma) by in situ hybridisation, and the positive cells were found in epithelial cells and peripheral blood cells. Serology data showed no significant increase in levels of antibodies against any of the HPV-18 proteins tested for in prostatic disease patients. Antibodies against HPV-1, HPV-4, HPV-6 and HPV-11 were significantly higher in the group of males with prostatic disease. Our study did not show an association between prostatic disease and either presence of HPV DNA in samples or previous exposure of high-risk HPV.

Keywords Benign prostatic hyperplasia (BPH) · Human papillomavirus (HPV) · Polymerase chain reaction (PCR) · Prostate cancer · Serology

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Introduction

It is well established that human papillomavirus (HPV) is the major cause of cervical cancer, with certain high-risk HPV types involved in the carcinogenesis [1]. Circumstantial evidence suggests that HPV could be associated with prostate cancer, including similarities in the predictive factors and clinical course compared with cervical cancer. Indeed, high-risk HPV types have previously been found

with varying prevalence in prostate cancer as well as in benign prostatic hyperplasia (BPH) and even in normal prostate tissue [2–5]. However these reports have been inconclusive and the link between prostate cancer and HPV is still a matter open to debate.

Against an association between HPV and prostate cancer is the fact that prostate cancer is chiefly an adenocarcinoma while cervical cancer is mainly squamous cell carcinoma. Adenocarcinoma of the cervix comprises only about 10–15% of all cervical carcinomas, and 70% are HPV-18 positive [6, 7]. Squamous cell carcinoma of the prostate is extremely rare, comprising only 0.5–1% of all diagnosed prostate cancers [8]. Previous studies on HPV and prostate cancer have identified HPV DNA with highly varying prevalences [2–5, 9]. These divergent findings can be explained by methodological problems due to different sensitivities of the various assays for detection of HPV DNA [10, 11], a presumably low copy number of HPVs in prostatic specimens [5] and, to some extent, the histological and genetic heterogeneity of prostate tumours [12]. Finally, serological epidemiology has shown no link between the level of HPV-16 or -18 antibodies and the prevalence of prostate cancer [13–15].

Our objective of this study was to investigate the role of HPV in the development of prostate cancer and BPH by analysing prostate tissue for HPV DNA and plasma for antibodies against fourteen different HPV types. We investigated HPV DNA prevalence in prostate tissue and antibodies against fourteen different HPV types in plasma from patients with prostatic adenocarcinoma and BPH. An association between prostatic disease and presence of HPV DNA or previous exposure to HPV was not found in this study.

Materials and Methods

Patients and Samples

We obtained prostate tissue which had been removed as a part of a diagnostic or surgical procedure from patients presenting to the Urology Clinics at the Princess Alexandra Hospital and the Royal Brisbane Hospital in Brisbane, Queensland, Australia between 2004 and 2008. Informed consent was obtained from all patients, and this project was approved by the Princess Alexandra Hospital Human Research Ethics Committee (PAH 2005/060). Altogether 62 prostate tissue samples were collected; 51 from adenocarcinomas and 11 from BPH. All tissue samples were confirmed by histology. The patients' median age was 63 years, with a range of 44–85 years. Data on Gleason score, location, and extension of the tumour were collected. The prostate tissue samples were snap-frozen and stored at −80°C. DNA was then extracted using the DNeasy® tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Blood samples (5 ml) were collected from 52 prostate patients, and the age range for the prostate patients who provided blood samples was 44–85 years, with a median of 62.5 years. From each patient who gave blood, a prostate biopsy was collected and diagnosis confirmed by histology. For each blood sample, the peripheral blood monocytes (PBMCs) were separated from the plasma, and the plasma was stored at −20°C until analysed. Furthermore, paraffin-embedded tissue for in situ hybridisation (ISH) was also available for all patients.

HPV Typing of Tissue Samples

The DNA extracted from tissue samples was tested with PCR for HPV DNA with the general primer-pair FAP59/FAP64 [16], and the HPV-18 type-specific primer pairs HPV18FAP59/HPV18FAP64. The primer sequences for the HPV-18 type specific FAP PCR were HPV18FAP59: 5'-TAACTGTTGGTAATCCATATT -3' and HPV18FAP64: 5'- CCAGTATCTACCATATCACCATC -3', with this primer pair yielding an amplicon of 484 bp. The previously described protocol for FAP59/FAP64 was followed for the two primer pair sets except for the $MgCl_2$ concentration, which was modified to 3.5 mM [16]. The sensitivity of both primer pairs were tested and determined to have a sensitivity of 10 copies of HPV-18 (unpublished data). The amplicons were identified by size determination in UV light, and positive samples were HPV type-determined by cloning and sequencing.

In Situ Hybridization

For the ISH assay, paraffin sections from thirteen of the HPV-positive males (ten HPV-18 positive samples and three HPV DNA negative samples) were cut to a thickness of 5 μm and mounted on Super Frost Plus slides. One extra section per paraffin block was cut and stained with H&E and used to determine specimen quality. The high-risk HPV probe, INFORM® HPV III Family 16 Probe (Ventana Medical System), was used for our ISH assay. This probe detects 16 different high-risk HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68, -73 and -82). The ISH assay was performed according to the manufacturer's guidelines using the Discovery® XT automated system (Ventana Medical System). Paraffin sections of HPV-positive cervical cancer tissue were provided by the manufacturer to run parallel with our samples as a positive control for the assay.

HPV Serology

A fluorescent bead technology-based multiplex serology assay [17] was used for the detection of HPV antibodies,

and was performed at the DFKZ, Germany. We screened for antibodies against the L1 capsid proteins from mucosal HPV types -6, -11, -16, -18, -31, -33, -35, -45, -52, and -58, cutaneous HPV types -1, -4, -8, and -77, the E6 and E7 oncoproteins from HPV-6, -11, -16, -18 and -33, the E1, E2 and E4 proteins from HPV-16, and used CagA from *Helicobacter pylori* and the JC polyomavirus capsid protein VP1 as control antigens. Fifty-three plasma samples from prostate patients were analysed and each patient sample was sex- and age-matched with two healthy control samples [18], as done previously [19]. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using unconditional logistic regression and adjusted for age.

Results

The HPV DNA prevalence in the tissue samples was 14% (7/51) for prostate cancer samples and 27% (3/11) for BPHs. The only HPV type detected in the tissue samples was HPV-18 (10/62). The DNA sequences of the HPV-18 positive samples belonged to different strains of HPV-18. Chi-square analysis was used to test the association between HPV prevalence and the presence of prostate cancer or BPH for the tissue samples (n=62), but no significant difference in HPV prevalence between the prostate cancer and BPH samples was found.

We have also been able to identify HPV-positive cells with in situ hybridisation in eight of our thirteen prostate tissue slides (3/3 BPH and 5/10 adenocarcinoma). This is the first time that HPV has been identified in prostate tissue with in situ hybridisation. Due to very few positive cells and few virus copies per positive cell, we have not been able to produce an image of these results. However, the positive cells were found mostly in epithelial cells but also in peripheral blood cells.

The serology results from our antibody screening are presented in Table 1. Our serology data showed no significantly increased levels of antibodies against any of the HPV-18 proteins tested for in prostatic disease patients, or any correlation between HPV-18 DNA positivity in tissue and detection of HPV-18 antibodies in plasma. However, despite a lack of statistical significance, it is interesting to note the high OR for HPV-18 E7 of 4.16 (95% CI: 0.36–48.0). Moreover, we found that the number of antibodies detected against the mucosal low-risk HPV types HPV-6 and HPV-11 was significantly higher in the prostate patient group than the age-matched healthy controls (HPV-6 OR=3.23, 95% CI: 1.15–9.06 and HPV-11 OR=3.23, 95% CI: 1.15–9.04) [19]. Furthermore, in the prostate patient group we found that L1 antibodies against the cutaneous HPV-1 and HPV-4 were in significantly increased numbers (HPV-1 OR=3.90, 95% CI: 1.50–10.10 and HPV-4 OR=4.98, 95% CI: 2.32–10.70). For the serology assay, there was no difference between prostate

Table 1 Serology results showing antibodies detected in patients with prostatic disease compared to healthy controls

Protein of interest[a]	Prostatic disease patients	Healthy controls	OR (95%CI)
HPV-1 L1	**13/53 (24.5%)**	**8/104 (7.7%)**	**3.90 (1.50–10.1)**
HPV-4 L1	**25/53 (47.2%)**	**16/104 (15.4%)**	**4.98 (2.32–10.7)**
HPV-6 L1	**10/53 (18.9%)**	**7/104 (6.7%)**	**3.23 (1.15–9.06)**
HPV-8 L1	13/53 (25.5%)	21/104 (20.2%)	1.29 (0.58–2.86)
HPV-11 L1	**10/53 (18.9%)**	**7/104 (6.7%)**	**3.23 (1.15–9.04)**
HPV-16 L1	5/53 (9.4%)	4/104 (3.9%)	2.61 (0.67–10.2)
HPV-18 L1	3/53 (5.7%)	4/104 (3.9%)	1.50 (0.32–6.96)
HPV-31 L1	4/53 (7.6%)	8/104 (7.7%)	0.98 (0.28–3.41)
HPV-33 L1	2/53 (3.8%)	1/104 (1.0%)	4.09 (0.36–46.7)
HPV-45 L1	3/53 (5.7%)	6/104 (5.8%)	0.98 (0.23–4.12)
HPV-52 L1	3/53 (5.7%)	8/104 (7.7%)	0.71 (0.18–2.84)
HPV-58 L1	5/53 (9.4%)	5/104 (4.8%)	2.06 (0.57–7.47)
HPV-77 L1	12/53 (22.6%)	15/104 (14.4%)	1.74 (0.75–4.07)
HPV-11 E7	0	1/104 (1.0%)	np
HPV-16 E7	1/53 (1.9%)	2/104 (2.0%)	0.98 (0.09–11.1)
HPV-18 E7	2/53 (3.8%)	1/104 (1.0%)	4.16 (0.36–48.0)
HPV-16 E6	0	1/104 (1.0%)	np
HPV-16 E4	1/53 (1.9%)	1/104 (1.0%)	1.98 (0.12–32.6)
JC VP1	37/53 (69.8%)	72/104 (69.2%)	1.03 (0.50–2.12)
CagA	35/53 (66.0%)	78/104 (75.0%)	0.65 (0.31–1.33)

np no pos cases

bold indicate significant p-values

[a] antigens tested for but not shown in the table had zero positives in both prostatic disease patients and healthy controls

patients and healthy controls for the control antigens (*H. pylori* CagA and JC VP1).

Discussion

The HPV DNA findings in prostate tissue do not necessarily reflect active infection or that the prostatic disease was caused by HPV: they could very well represent transient infection. However, it is interesting that only HPV-18 is detected in the prostate tissue samples, especially since prostate cancer is primarily an adenocarcinoma and 70% of all cervical adenocarcinomas are HPV-18 positive [6]. When comparing the HPV DNA prevalence in prostatic carcinoma with BPH, the difference is not significant. There could be a significant difference in HPV DNA prevalence in healthy prostate tissue compared to cancer and BPH. However, normal prostate tissue biopsies are rarely collected and we lack healthy control tissue for our study.

It can be argued that our high prevalence of HPV-18 could be due to PCR contamination. However, the same person who has analysed the prostate samples have been using the same PCR (with the same PCR reagents and controls) for a skin HPV study and have never detected HPV-18 in the skin samples [20].

It is interesting to note that HPV-18 is a common HPV genotype in Australia and is the dominant genotype identified in high prevalence in breast cancer tissue in patients across Australia [21, 22].

The few positive cells that could be seen with the ISH technique were located in the epithelial layer of the prostate and in blood cells. The probe we used is currently the most sensitive, and has a sensitivity of least 10 copies per cell. One explanation for our weakly positive ISH analysis could be that the HPV copy number does not to exceed 10 copies per cell. Others have faced the same problem of finding HPV DNA in prostate tissue with PCR, but being unable to detect HPV with ISH in the same tissue samples [4]. Laser-assisted microdissection followed by single cell PCR may help in clarifying this issue.

While detection of HPV DNA in tissue shows that the virus is currently present in a subset of cases, HPV capsid (L1) protein serology reflects past exposure. Detection of antibodies against the oncoproteins E6 and E7 from high-risk HPV types has previously been shown in HPV-associated cancers [19]. Our statistical analyses for positivity to the oncoproteins E6 or E7 from any of the high-risk HPV types showed no significance alone or after stratification by pathology or Gleason score. There was an increased risk of prostatic disease in patients with antibodies to HPV-18 E7, but this did not correlate with HPV-18 in the tissue or blood samples and was not significant. However, the limitation with serological analysis is that it reflects general exposure to HPV and cannot distinguish HPV infections within specific anatomical sites.

Antibodies against HPV-6 and HPV-11 were significantly higher in the prostate patients than in the healthy controls, but we did not identify HPV-6 or HPV-11 in any of our tissue or peripheral blood samples. Previous studies have found positive associations between risk of prostate cancer and sexual activity (age at first intercourse and number of sexual partners) and sexually transmitted diseases [23, 24], even though the results vary and do not always show higher antibody levels and previous infection with HPV as a risk factor for prostate cancer [24]. The higher levels of antibodies against HPV-6 and -11 in the males with prostatic disease might reflect and strengthen the evidence that there is a link between prostatic disease and sexual activity. Interestingly, we found that antibodies against HPV-1 and -4 are significantly higher in the group of males with prostatic disease. These two HPV types were not identified by PCR in this study and are skin HPV types. This is an interesting finding, but difficult to link to development of BPH or prostate cancer.

In summary, our study with a limited number of samples did not show an association between prostatic disease and either presence of HPV DNA in samples or previous exposure of high-risk HPV from our serology data. A larger study is required on both prostate cancer patients and BPH patients to further deduce whether specific HPV types contribute to the initiation or formation of prostate cancer.

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Conflicting Interests None.

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Ethical approval This project was approved by the Princess Alexandra Hospital Human Research Ethics Committee (PAH 2005/060).

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