Short Communication

No link between viral findings in the prostate and subsequent cancer development

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In an investigation of 201 prostate tissue samples from patients with benign prostate hyperplasia that later progressed to prostate cancer and 201 matched controls that did not, there were no differences in the prevalence of adenovirus, herpesvirus, papilloma virus, polyoma virus and Candida albicans DNA.

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Mutations in genes associated with the immune defence have been identified in hereditary prostate cancer, indicating that infection and/or inflammation of the prostate may be important mediators for the development of prostate cancer (Palapattu et al, 2005; Sun et al, 2005). Moreover, population studies have revealed an increased relative risk for development of prostate cancer in men with a prior history of sexually transmitted infections (Dennis and Dawson, 2002). These findings support the hypothesis that an infectious agent can be a potential cofactor in prostate cancer development. Human papilloma virus (HPV), Epstein–Barr virus (EBV) and the polyoma viruses JCV and BKV represent viruses with proven linkage to different human cancers and have been traced in prostate cancer tissues (Grinstein et al, 2002; Zambrano et al, 2002). To further evaluate if a viral infection could contribute to prostate cancer development, we conducted a case–control study of 402 patients with benign prostate hyperplasia (BPH), of which 201 later progressed to prostate cancer. We examined whether the presence of genetic traces of EBV, herpes simplex virus (HSV) 1 and 2, cytomegalovirus (CMV), adenovirus, HPV, polyoma viruses BKV and JCV and Candida albicans in the prostate correlate with histological inflammation and subsequent prostate cancer diagnosis.

MATERIALS AND METHODS

A case–control study of 402 archival prostate tissue samples obtained during transurethral resection of the prostate (TURP) collected at the Department of Pathology at the University Hospital of Northern Sweden, Umeå was conducted as described previously (Alexeyev et al, 2006; Bergh et al, 2006). Briefly, tissues were obtained from men with BPH (median age 64, range 51 – 71), fixed in formalin, paraffin-embedded and stored at room temperature until tested. A total of 201 men developed prostate cancer at least 6 months after the TURP. For each case, a control was randomly selected from a cohort of patients that did not develop prostate cancer. The case–control pairs were matched for year of birth, residence and year of TURP. Histological inflammation was graded as mild or severe as described (Alexeyev et al, 2006). DNA from prostate tissue was purified and checked for integrity as described (Alexeyev et al, 2006). Nested PCR assays were used for all the assays except HPV and C. albicans PCRs. Primers and PCR protocols for adenovirus (Allard et al, 2001), CMV (Brytting et al, 1991), EBV (Meyohas et al, 1996), HSV1 and 2 (Aurelius et al, 1991) and HPV (de Roda Husman et al, 1995) were used with minor modifications. Primers for the polyoma viruses JCV and BKV and C. albicans were designed according to published sequence information (Table 1). To verify the positive PCR findings, PCR products were purified with QIAquick Purification Kit protocol (Qiagen®, Hilden, Germany) and directly sequenced in the ABI PRISM 3700 DNA ANALYSER (AME Bioscience, Toroed, Norway) using the Big Dye™ Terminator Cycle Sequencing kit 1.1 (Applied Biosystems, Forster City, CA, USA). Histological inflammation in prostate tissue was graded as described earlier (Alexeyev et al, 2006). Fisher exact test was used for statistical analysis.

RESULTS

Out of 402 samples tested, 352 (87.6%) were positive for the human β-globin gene. These samples were considered to have sufficient DNA quality and were therefore used for subsequent analysis in viral and fungal PCRs. Of the 352 samples tested, 31 (8.8%) were positive for EBV and 10 (2.8%) for JCV. No other viral DNAs were detected. Of 240 samples that were available for C. albicans-specific PCR, two were (0.8%) positive. We then assessed whether the
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samples positive for the inflammation and prostate cancer development. Only archival infection could precede and, possibly, contribute to prostate the present study, it had a potential to evaluate if viral/fungal microbial DNAs, thus ensuring good quality DNA and absence of PCR inhibitors. Of eight DNA viruses tested, only EBV and JCV were found in the prostate tissue. This observation is in accord with previous studies (Grinstein et al, 2002; Zambrano et al, 2002).

5.8S gene

| Microorganism | Position | Sequence | Amplimer size (nt) |
|---------------|----------|----------|-------------------|
| JC virus      | 2656–2677 | 5′ TGC AGT TTT CCT GTG TGT C T3′ | 259 |
|               | 2914–2893 | 5′ TTT AGG CCA GTT GCT GAC TTG G3′ | |
|               | 2722–2743 | 5′ CAG TGC TTG ATC CAT GTG CAG A3′ | 167 |
|               | 2888–2867 | 5′ TGC CAT TCA TGA GAG GAT TGT G3′ | |
| BK virus      | 1452–1472 | 5′ GAA AAA ACT ATT GCC CCA GGA G3′ | 192 |
|               | 1643–1625 | 5′ AGT TTT GCC ACT TGC AGC G3′ | |
|               | 1487–1508 | 5′ AAC TGC TCC TCA ATG GAT GTT G3′ | 114 |
|               | 1600–1579 | 5′ CCC CTG GAC ACT CTC CTT TTC T3′ | |
| C. albicans   | 5.85 gene | 5′ GCC TGT TTG AGC GTC GTT TC3′ | 82 |

*Sequence positions refer to the JC virus isolate SK-6. *Sequence positions refer to the BK virus Dunlop strain sequence.

Table 1 Oligonucleotide primer sequence for polyoma viruses JC virus and BK virus and C. albicans

To the best of our knowledge, this is the first study investigating the presence of eight different DNA viruses and C. albicans in a large series of men with BPH. Owing to the case–control design of the present study, it had a potential to evaluate if viral/fungal infection could precede and, possibly, contribute to prostate inflammation and prostate cancer development. Only archival samples positive for the β-globin gene were subsequently tested for microbial DNAs, thus ensuring good quality DNA and absence of PCR inhibitors. Of eight DNA viruses tested, only EBV and JCV were found in the prostate tissue. This observation is in accord with previous studies (Grinstein et al, 2002; Zambrano et al, 2002).

These viruses are unlikely to contribute to prostate cancer development in the patients studied owing to the similar occurrence in the case and control groups. Data on the presence of HPV in benign and malignant prostate tissues are contradictory. Some groups have reported high rates of detection (Noda et al, 1998; Serth et al, 1999; Zambrano et al, 2002), whereas others have not found HPV (Effert et al, 1992; Strickler et al, 1998). All samples tested in this study were negative for HPV, thus making it an unlikely contributing factor for subsequent cancer development in the 352 patients studied. Our study did not find any association between the presence of EBV and JCV and histological inflammation in the prostate. These viruses are therefore unlikely as triggering factors of chronic prostate inflammation. In conclusion, our study has shown that the prostate can harbour mixed microbial communities. Epstein–Barr virus, JCV and C. albicans do not appear to contribute to chronic prostate inflammation and subsequent prostate cancer development.

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