No Evidence of Human Polyomavirus 9, WU and KI DNA in Kidney and Urinary Bladder Tumour Tissue Samples

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Polyomavirus · KI polyomavirus · WU polyomavirus · Human polyomavirus 9 · Urinary tract · Tumour

Abstract
Background/Aims: The oncogenic potential of human polyomaviruses (HPyVs) has been proposed, but so far only Merkel cell carcinoma polyomavirus seems to be associated with a human tumour. The role of BK polyomavirus (BKPyV) in human tumourigensis remains controversial. BKPyV establishes persistent infection in the urinary tract, and renal and bladder neoplasms have been studied extensively, but conflicting prevalence data are reported. KI, WU and HPyV9 were detected in urine samples suggesting that these viruses may also infect the urinary tract, but their presence in urinary tract tumours has not been studied. The aim of this work was to examine the prevalence of KIPyV, WUPyV, HPyV9 and BKPyV by PCR in renal and bladder neoplasms. Methods: A total of 190 formalin-fixed paraffin-embedded renal neoplasms, bladder cancer and kidney biopsy samples were analysed for the presence of BKPyV, KIPyV, WUPyV and HPyV9 DNA by real-time and nested PCR. Results: Amplifiable DNA was extracted from all the samples, but none of the studied viruses were detected in benign renal neoplasia (0/23), malignant renal tumours (0/89) or bladder cancer (0/76). Conclusion: Our study did not find any evidence that BKPyV, KIPyV, WUPyV or HPyV9 are associated with bladder and renal tumours.

Introduction

As all known human pathogenic viruses of the Polyomaviridae family encode early regulatory tumour antigens, the potential oncogenic role of these viruses has been suggested [1–4] since the discovery of the first human polyomavirus (HPyV), BK polyomavirus (BKPyV) [5]. This suggestion was strengthened by in vitro and animal experiments with BKPyV that proved its transforming ability as reviewed previously [1, 3, 6]. After the primary infection, BKPyV establishes a lifelong persistent infection in the urinary tract and renal cells [2], hence there exist large number of studies of urinary tract neoplasia and cancers [7–15]. DNA, RNA or even proteins of the BK virus have been detected in different human tumours (brain, neuroblastoma, osteosarcoma, bladder,
kidney, adrenal, prostate, genital, colon), but the role of BKPyV in human cancers is still controversial [1, 4, 6, 16–19].

Although 11 new human pathogenic polyomaviruses (KI, WU, Merkel cell polyomavirus, HPyV6, HPyV7, trichodysplasia spinulosa-associated polyomavirus, HPyV9, HPyV10, STLPyV, HPyV12 and New Jersey polyomavirus) have been discovered since 2007 [20–32], so far only Merkel cell polyomavirus has been associated with a rare, cutaneous tumour. This was named Merkel cell carcinoma as an aetiological agent [33].

KI and WU polyomaviruses (KIPyV, WUPyV) were first described in 2007 from respiratory samples [20, 21]. Subsequent seroepidemiological studies revealed that both viruses are widespread, the primary infections of which may occur during the early years of life, and adulthood seropositivities are 55–100% [34–36]. Most of the important questions about the pathogenesis of KIPyV and WUPyV have not been clarified. Their respiratory transmission is suggested based on prevalence data from numerous studies, as has been summarized in review articles [19, 37, 38]. Both KI and WU viruses were detected in urine samples, which suggests that these viruses may also infect the urinary tract [39–41].

HPyV9 was first detected in plasma and urine samples from renal transplant patients [25], following which two teams also found the viral DNA in urine samples [42, 43]. The pathogenesis of the transmission of HPyV9 is not known, but it seems to be a frequent infectious agent since seropositivity in adults reaches 47% and increases with age [44].

Some publications about the prevalence of KIPyV, WUPyV and HPyV9 in different tumours are available, but these viruses have not been hitherto examined in bladder and renal cancers [45–57]. In the present study the prevalence of KIPyV, WUPyV, HPyV9 and BKPyV were examined by PCR in tissue samples from the urinary tract. In order to reveal a possible association of these viruses with urinary tract tumours, samples from renal and bladder neoplasia were examined.

### Materials and Methods

#### Patients and Samples

The Regional and Institutional Ethics Committee of the University of Debrecen approved the study (IX-R-052/00016-29/2012). One hundred and ninety formalin-fixed paraffin-embedded tissue samples were analysed. The samples were submitted for routine diagnosis to the Department of Pathology, University of Debrecen, between 2010 and 2014. Data relating to the number of kidney biopsies (from renal transplant patients), and benign and malignant renal and urinary bladder tumour samples of 180 patients are summarized in table 1. Ten-micrometre-thick tissue sections were deparaffinised with xylene, then nucleic acid was isolated using the High Pure FFPET DNA Isolation Kit (Roche, Basel, Switzerland) according to the manufacturer’s instructions. The quality and quantity of DNA eluted in 50 μl was checked with a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Waltham, Mass., USA) and stored at –20°C until use.

#### Detection of WUPyV, KIPyV, HPyV9 and BKPyV DNA

To confirm the effectiveness of nucleic acid extraction, the presence of amplifiable DNA, β-globin PCR was used with the primers PCO3 (5’ACACAACCTGTGTCCACTACG3’) and PCO4 (5’CAAATCATCCACGTCCAC3’). Five microliters of DNA

### Table 1. Patient and tissue sample data

<table>
<thead>
<tr>
<th>Tissue samples</th>
<th>Patients/samples, n</th>
<th>Female, n</th>
<th>Male, n</th>
<th>Min.–max. age of patients (median), years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal neoplasia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal adenoma</td>
<td>5/5</td>
<td>3</td>
<td>2</td>
<td>6–80.7 (64.3)</td>
</tr>
<tr>
<td>Renal angiomyolipoma</td>
<td>10/10</td>
<td>9</td>
<td>1</td>
<td>36.3–64.3 (58.7)</td>
</tr>
<tr>
<td>Renal oncocytoma</td>
<td>8/8</td>
<td>3</td>
<td>5</td>
<td>40.5–74.3 (62.2)</td>
</tr>
<tr>
<td>Leiomyosarcoma renis</td>
<td>1/1</td>
<td>1</td>
<td>–</td>
<td>57.1</td>
</tr>
<tr>
<td>Carcinoma renocellularis renis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary type</td>
<td>11/11</td>
<td>4</td>
<td>7</td>
<td>33.7–69.4 (58.7)</td>
</tr>
<tr>
<td>Chromophobe type</td>
<td>5/5</td>
<td>3</td>
<td>2</td>
<td>33.3–83.6 (59.5)</td>
</tr>
<tr>
<td>Clear cell type</td>
<td>69/71</td>
<td>21</td>
<td>48</td>
<td>31–81.2 (61.7)</td>
</tr>
<tr>
<td><strong>Bladder carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uroepithelial carcinoma</td>
<td>68/76</td>
<td>23</td>
<td>45</td>
<td>45.3–87.6 (68.6)</td>
</tr>
<tr>
<td>Renal biopsy from renal transplant patients</td>
<td>3/3</td>
<td>0</td>
<td>3</td>
<td>31.9–52.8 (37.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>180/190</td>
<td>66</td>
<td>114</td>
<td>6–87.6 (63.5)</td>
</tr>
</tbody>
</table>
One hundred and eleven tissue samples from different renal neoplasms (adenomas, angiomyolipomas, oncocytomas, leiomysarcoma and renal renocellular carcinomas), 3 kidney biopsy samples and 76 bladder carcinoma tissue samples were studied for the presence of HPyV9, BK virus DNA was checked by real-time PCR according to the protocol detailed previously [61]. A nested PCR was also carried out with previously published primers [62]. Plasmid containing the complete genome of BKPyV Dunlop strain (kindly provided by Serena Delbue from the University of Milan, Italy) was used as a positive control. The first round of BKPyV nested PCR was performed in a final volume of 20 μl containing 5 μl of template DNA, 0.4 U of Phusion Green hot start high-fidelity polymerase (Thermo Scientific), 4 nmol dNTPs and 10 –10 pmol of each primer (PM1+ sense and PM2– antisense). For the second round, 2.5 μl of PCR product from the first round was amplified in a 25-μl final volume using 0.5 U Phusion Green hot start high-fidelity polymerase, 5 nmol of dNTPs and 10 –10 pmol of each primer (BKV+ sense and PM2– antisense). The cycling conditions were: 98 °C for 30 s, 30 cycles; 98 °C for 10 s, 60 °C for 30 s (1st round)/55 °C for 30 s (2nd round), 72 °C for 30 s and a final extension at 72 °C for 5 min.

Results and Discussion

One hundred and eleven tissue samples from different renal neoplasms (adenomas, angiomyolipomas, oncocytomas, leiomysarcoma and renal renocellular carcinomas), 3 kidney biopsy samples and 76 bladder carcinoma tissue samples were studied for the presence of HPyV9,
BKPyV is able to induce malignant transformation in animals and in vitro cell cultures, but studies with human cancers are controversial. The presence or absence of BKPyV DNA and/or proteins has been reported, hence the role of this virus in human tumourigenesis is not clarified, as reviewed previously [1, 3, 19].

In our study, BKPyV DNA was not detected in benign and malignant renal neoplasia (0/111) and also not in bladder cancer (0/76). Nucleic acid extraction was successful, resulting in a good quantity of amplifiable DNA, and previously used PCR detection methods with good sensitivity levels were used (table 2). In addition, 1 out of the 3 examined renal biopsy samples was BKPyV DNA positive. Antigen expression was not examined.

Based on our data and previous studies, there is to date no evidence that WUPyV, KIPyV or HPyV9 have any role in oncogenesis; however, this cannot be definitively excluded. Although all three viruses have previously been detected in urine samples, there is no evidence that these viruses infect the urinary tract. Despite the growing volume of prevalence data, little or hardly anything is known about the pathogenesis of KIPyV, WUPyV and HPyV9. The cited studies and our new results provide essential data regarding the prevalence of novel HPyVs, but more samples and studies are needed. Identifying the susceptible cells, sites of infection and possible latencies, as well as the isolation of the virions and further in vitro studies are necessary for further progression.

Acknowledgements

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Disclosure Statement

References


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56 Ramqvist T, Nordfors C, Dalianis T, Ragnars-Olding B: DNA from human polyomaviruses, TSPyV, MWPyV, HPyV 6 and 7 was not detected in primary mucosal melanomas. Anticancer Res 2013;34:639–643.


