BK Virus as a Potential Co-factor for HPV in the Development of Cervical Neoplasia

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Abstract. Cervical cancer is the third most common type of cancer in women worldwide. A persistent infection with high risk (HR) human papillomavirus (HPV) is necessary for cervical cancer to occur. However, the great majority of women that are infected with HR-HPV will not develop cervical cancer, indicating that HR-HPV alone is not adequate to drive the development of cervical cancer, suggesting the involvement of cofactors. The BK polyomavirus (BKV) establishes latency near cervical tissue in the urogenital tract and is frequently detected in the urine, especially in immunosuppressed patients, and hence may coexist with HR-HPV. Current experimental evidence indicates that both HR-HPV and BKV are capable of altering cell-cycle control and inhibit apoptosis. Therefore, they may act additively or synergistically to promote malignant transformation. We hypothesize that BKV is a co-factor for HR-HPV in cervical cancer. In this study, we examined 249 cervical swabs that were submitted for routine HR-HPV screening test in the Molecular Diagnostics Laboratory at the University of Texas Medical Branch (UTMB). Our results showed that 107 samples contained HR-HPV at an overall rate of 43% (107/249); BKV was present in 4 (3.7%) of the 107 HR-HPV positive specimens and in 12 (8.5%) of the 142 HR-HPV negative samples with an overall positive rate of 6.4% (16/249). Although there was no statistical significance between HR-HPV and BKV co-infection (P=0.19, Fisher’s exact test), our results support the hypothesis that BKV can co-exist with HR-HPV in cervical specimens.

Key words: BKV, HR-HPV, cervical cancer, co-factor

Introduction

Cervical cancer is the third most common type of cancer in women worldwide. Each year, about 12,000 women are diagnosed with cervical cancer in the U.S. (http://www.cdc.gov/hpv/). Cervical cancer is neoplasia of cells on the surface of the cervix, the portion of the uterus at its junction with the vagina. Almost all cervical cancers are associated with human papillomavirus (HPV) [1, 2]. Genital HPV infections are very common. Most of these infections clear spontaneously, but in 5-10% of women, these infections are persistent and pose a risk of development of dysplasia that can progress to invasive cervical cancer [2, 3]. The Papanicolaou (Pap) test can detect abnormal cells on the cervix so that the disease can be removed before development of invasive cancer. HPV testing of cervical specimens adds sensitivity and positive predictive value to the Pap test [2, 3].

HPV are members of the papillomavirus family of viruses that establish productive infections in the keratinocytes of skin or mucous membranes. HPV are non-enveloped, double-stranded circular DNA viruses consisting of a genome of 6800 to 8000 base pairs (bp) [2]. Of the more than 100 types of HPV, types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 are classified as high-risk (HR) because they are associated with the malignant transformation of cervical epithelial cells. Types 16 and 18 are generally acknowledged to cause...
approximately 70% of cervical cancer cases (http://www.cancer.org/). The HPV genome encodes six early (E1, E2, E3, E4, E6, and E7) and two late (L1 and L2) proteins. After the host cell is infected, E1 and E2 are expressed first. High levels of E2 repress expression of the E6 and E7 proteins. When HPV integrates into the host genome, E2 function is disrupted, preventing repression of E6 and E7. Importantly, E6 and E7 are the HPV proteins associated with cancer by inactivating tumor suppressors p53 and RB, respectively [2, 3]. It is believed that persistent HR-HPV infection is required for the malignant initiation of cervical cells and progression to cervical cancer, which fits the theory of oncogene addiction [4, 5]. Although HPV infection with high-risk types has been shown to be a necessary factor in the development of cervical cancer, the great majority of genital HR-HPV infections do not result in cervical cancer. Therefore, one or more co-factors are required for HR-HPV to drive malignant transformation.

BK virus (BKV) belongs to the polyomavirus family and is characterized by a naked, circular, double-stranded DNA genome. BKV DNA is approximately 5,300 bp and is functionally divided into three regions: early, late, and transcriptional control. The early region encodes the large T and small t antigens (T-ag, t-ag), and the late region encodes the three structural proteins VP1, VP2, and VP3 along with agnoprotein [6]. There are six BKV subtypes based on DNA sequence variations, including type I (Ia and Ic), II, III, IV, V, and VI. Type I (e.g., the BKV prototype Dunlop) is by far the most prevalent subtype in all patient groups [7]. BKV ubiquitously infects the human population. Following a typically subclinical primary infection, BKV establishes life-long latency in the urogenital tract. BKV is known to reactivate and can cause severe disease in immunosuppressed patients, particularly renal and bone marrow transplant recipients. BKV T-ag can interact with tumor suppressor proteins pRb and p53, leading to a variety of transforming effects, and is a potential oncogene [8, 9]. BKV t-ag can also play an important role in transformation by inhibiting protein phosphatase 2A (PP2A), resulting in stimulation of the MAP kinase pathway and subsequent cellular proliferation [8, 9].

Since BKV lies dormant near the reproductive organs and can be shed in urine, we hypothesize that the virus may infect cervical tissue and co-exist with HPV. It is conceivable that BKV T-ag and t-ag may functionally interact with HPV E6/E7 proteins, additively and/or synergistically, to promote cellular transformation and tumor initiation of cervical cancer. To test our hypothesis, we examined 249 HR-HPV infected and uninfected cervical samples for the presence of BKV.

**Materials and Methods**

**Specimens.** The study included a total of 249 cervical swabs obtained and transported using the Digene Specimen Collection Kit (Digene, Inc, Gaithersburg, MD) that were submitted for routine HR-HPV screening test in the Molecular Diagnostics Laboratory at the University of Texas Medical Branch (UTMB) between April and December of 2009. The study was approved by the UTMB Institutional Review Board (IRB).

**Detection of High-Risk HPV.** HR-HPV was detected using Hybrid Capture 2 High-Risk HPV DNA Test according to the manufacturer’s instructions (Digene Inc, Gaithersburg, MD). This nucleic acid hybridization and signal amplification assay detects 13 HR-HPVs (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Basically, HPV DNA in the specimen was denatured and hybridized with the HPV RNA probe, and the DNA-RNA hybrid was captured using monoclonal antibodies on microtiter plates. A second monoclonal antibody conjugated to alkaline phosphatase was added, and alkaline phosphatase cleaved a substrate to produce a chemiluminescent signal. The intensity of light emitted indicated the presence or absence of target DNA in the specimen (Digene Inc, Gaithersburg, MD). The limit of detection of the assay for HR-HPV was 4000 copies/sample.

**BK Virus Real-Time PCR Assay.** After HR-HPV testing, the remaining specimens in the denaturation solution (Digene Inc, Gaithersburg, MD) were tested for BKV DNA. DNA was precipitated using isopropanol, dissolved in 50 μl of DNase-free water, then stored at -20°C before use. DNA concentration was measured using a spectrophotometer. To ensure the presence of adequate DNA in the
specimens, only samples with DNA concentrations greater than 0.1 ng/µl were included in the study. BK virus DNA was detected by real-time PCR using minor groove binding (MGB) Alert BK Virus Probe and Primers (Nanogen, San Diego, CA), with PCR enzyme mix LightCycler FastStart DNA Master Hyb Probe (Roche, Indianapolis, IN) on a Smart Cycler II real-time PCR machine (Cepheid, Sunnyvale, CA), as described [10]. The dynamic range of the BKV assay was 390-3.9 x 10^8 copies/ml. The lower limit of the quantification value of 390 copies/ml served as the limit of detection for this study.

Statistical analysis. Statistical analysis was performed using SAS 9.2 software (SAS Institute Inc. Cary, NC). Fisher’s exact test was used to examine the independence hypothesis between HR-HPV and BKV positivity in cervical specimens and p<0.05 was considered significant.

Results and Discussion

A total of 249 cervical swabs were tested for the presence of HR-HPV and BKV DNA. One hundred and seven samples were positive and 142 negative for HR-HPV, and the overall positive rate was 43% (107/249). BKV was detected in 4 (3.7%) of the 107 HR-HPV positive samples, and in 12 (8.5%) of the 142 HR-HPV negative specimens (Table 1). There was no statistically significant association between HR-HPV and BKV positivity (P=0.19, Fisher’s exact test). Patients positive for BKV had an average age of 45 years; the youngest patient was 26 while the oldest was 70. Out of the 16 BKV positive samples, nine were Caucasian, four were African American, and three were Hispanic. No patient developed cervical cancer during the follow-up period (from several months to several years, Table 2). Abnormal cytology was observed in a total of six HR-HPV containing cases (with or without BKV co-infection), but not in the 12 BKV only cases. In addition, the highest-grade lesion (CIN3) cases were in HR-HPV only positive rather than HR-HPV-BKV co-infected cases (Table 2).

The results of our study did not reveal a statistically significant correlation between HR-HPV and BKV co-infection in cervical samples, or a role of BKV in the initiation of cervical cancer. Indeed, there were no patients with cervical cancer in this study. Our study, however, did not rule out the possibility of a link between the two viruses and subsequent disease in the cervix. First of all, the abnormal cytology rate was higher in the HR-HPV-BKV co-infection cases (1/4, 25%) than in the HR-HPV only group (5/107, 5%). However, increased sample sizes are necessary to confirm the rate difference. A recent publication by Cormer et al revealed a high prevalence of HR-HPV and BKV co-infection in precancerous cervical lesions [11]. They detected BK sequences in 37% (34/93) of samples with high-grade squamous intraepithelial lesions (HSIL, generally equivalent to CIN 2-3) that were all positive for HR-HPV; therefore, all the BKV positive samples were co-infected with HR-HPV. They also showed that while HR-HPV was positive in 50% of the 80 cases of low-grade squamous intraepithelial lesion (LSIL, generally equivalent to CIN 1), none was positive for BKV. Furthermore, BKV was also absent in all 100 normal cervical specimens, of which 8% contained HR-HPV [11]. The incremental increases of HR-HPV from 8%, 50%, to 100% in normal, LSIL, and HSIL samples suggests a role for HR-HPV in the initiation of malignant transformation of cervical cells, as well as in the support of “oncogene addiction” [4, 5] of LSIL and HSIL cells for HR-HPV in tumor progression. The results also elucidate a potential role of BKV in the tumor progression step of cervical cancer development. While both Cormar et al (2011) and our study support the notion that BKV and HR-HPV can co-infect cervical specimens, the disparities in the positive rates and the order of HR-HPV and BKV infection may reflect variation in the study populations or differences in the technical performance of the assays, as has been demonstrated in the analysis of SV40 in

<table>
<thead>
<tr>
<th>BK +ve</th>
<th>BK -ve</th>
<th>Total</th>
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<tbody>
<tr>
<td>HR-HPV +ve</td>
<td>4</td>
<td>103</td>
</tr>
<tr>
<td>HR-HPV -ve</td>
<td>12</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>233</td>
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Table 1: Detection of high-risk HPV and BK viral DNA in cervical specimens.
Is BKV a co-factor for HPV in cervical cancer?

mesothelioma and BKV in kidney transplant recipients [7, 12].

Secondly, most HR-HPV infections are temporary and have no long-term significance. However, when the infection persists, in 5-10% of infected women, there is a high risk of developing precancerous lesions of the cervix, which can progress to invasive cervical cancer. This process usually takes 15–20 years. Due to the short follow-up time of our study (Table 2), the long-term effect of HR-HPV-BKV co-infection was not evaluated. Direct testing of cervical cancer specimens for HR-HPV and BKV may reveal the link between the co-infection and cervical cancer. However, unlike HR-HPV, the absence of BKV cannot rule out its role in cervical cancer due to, for example, the hit-and-run theory of polyoma virus in cancer development [13, 14]. Further studies are necessary to determine whether HR-HPV and BKV infections persist over a longer follow-up period. For example, if patients are tested annually and continue with infection with the same genotypes/subtypes of HR-HPV and BKV, it is likely that the infections are persistent, which may correlate with an increased risk of cervical cancer.

Finally, our data showed that BKV DNA can be present in the absence of HR-HPV infection, raising the possibility that BKV infection may occur prior to HR-HPV infection. This notion is supported by a recent study showing the presence of BKV DNA in 6 sperm fluids that were negative for HPV [15]. However, this finding requires further investigation for clinical significance. It is believed that in most people, the immune system eliminates HPV infection [16]. BKV positivity may be a marker for immune suppression and an indication of the body’s inability to clear HR-HPV infection. Our study supports the notion that HR-HPV and BKV can co-infect cervical specimens. Further studies are warranted to understand the role, if any, of BKV and HR-HPV co-infection in the tumorigenesis of cervical carcinoma. For example, HR-HPV tests are used to screen patients for cervical cancer, and a positive test result often leads to unnecessary further testing and treatment in women who likely would not develop cancer as a result of HR-HPV. If co-infection with BKV is necessary for HR-HPV to drive the development of cervical cancer, co-testing for BKV and HR-HPV should increase the specificity of cervical cancer screening.

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References


