BK Virus as a Cofactor in the Etiology of Prostate Cancer in Its Early Stages

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Prostate cancer has been projected to cause almost 10% of all male cancer deaths in the United States in 2007. The incidence of mutations in the tumor suppressor genes Rb1 and p53, especially in the early stages of the disease, is low compared to those for other cancers. This has led to the hypothesis that a human virus such as BK virus (BKV), which establishes a persistent subclinical infection in the urinary tract and encodes oncoproteins that interfere with these tumor suppressor pathways, is involved. Previously, we detected BKV DNA in the epithelial cells of benign and proliferative inflammatory atrophy ducts of cancerous prostate specimens. In the present report, we demonstrate that BKV is present at a much lower frequency in noncancerous prostates. Additionally, in normal prostates, T-antigen (TAg) expression is observed only in specimens harboring proliferative inflammatory atrophy and prostatic intraepithelial neoplasia. We further demonstrate that the p53 gene from atrophic cells expressing TAg is wild type, whereas tumor cells expressing detectable nuclear p53 contain a mix of wild-type and mutant p53 genes, suggesting that TAg may inactivate p53 in the atrophic cells. Our results point toward a role for BKV in early prostate cancer progression.

Prostate cancer is the leading cause of male cancer deaths in the United States. The latest statistics for 2007 estimate 218,890 new prostate cancer cases and 27,050 deaths (39). Prostate cancer is a slowly progressing disease; therefore, identification of its precursor lesions has become the primary focus of many studies (reviewed in references 14, 20, 30, and 54). An increased understanding of the molecular mechanisms associated with prostate cancer initiation and progression would be useful for developing strategies for its early detection and treatment.

Proliferative inflammatory atrophy (PIA), proposed as the potential precursor to adenocarcinoma due to its proliferative nature, is prevalent in the peripheral zone of the prostate gland, where prostatic intraepithelial neoplasia (PIN) and carcinoma also occur (20, 58, 66, 78, 89). It has been postulated that PIA may transition to carcinoma without an intermediate stage or may lead to carcinoma through PIN. Frequent histological transitions between PIA and PIN have been observed (19, 66). Several crucial prostate tumor suppressor genes, such as NKK3.1, CDKN1B (which codes for p27, a cyclin-dependent kinase inhibitor that regulates cell cycle progression), and PTEN (phosphatase and tensin homologue), are all expressed at very low levels in PIA, a pattern similar to their expression pattern in PIN and carcinoma (reviewed in reference 30).

The occurrence of mutations in p53 and Rb1 in the early stages of prostate cancer is relatively low (1, 7, 21, 56). This has suggested the possibility that a viral agent such as BK virus (BKV), which infects the urinary tract and encodes tumor antigens that inactivate these tumor suppressors, may play a role in the etiology of prostate cancer. This would be very similar to the way in which the E6 and E7 oncogene products of human papillomavirus (HPV) inactivate p53 and pRB, respectively, in cervical carcinomas (13, 22, 27, 37, 49, 72, 73). It has been suggested that exposure to infectious agents can cause injury to the normal prostate epithelium, leading to the development of PIA (reviewed in reference 20).

BKV, a member of the polyomavirus family, was first isolated from the urine of a renal transplant patient (28) and infects almost 90% of the human population by early childhood (34, 41, 76). It resides in a subclinical persistent state in the urinary tracts of healthy individuals and reactivates in immunosuppressed transplant patients, in whom it is associated with hemorrhagic cystitis and polyomavirus nephropathy (5, 35, 57, 68). BKV transforms rodent cells in culture (64), causes kidney tumors in transgenic mice (15), and immortalizes primary human cells alone (32, 65, 77, 82) or in the presence of other oncogenes such as c-ras (59) and adenovirus E1A (90). A possible role for BKV in human cancers is controversial because such a high percentage of the human population is exposed to the virus at a very early age, precluding the use of epidemiologic methods to test an association (16).

The genome of BKV is divided into early, late, and regulatory regions and codes for at least six proteins, two from the early region and four from the late region. The early proteins, large tumor antigen (TAg) and small tumor antigen (tAg), are the first to be expressed during infection. When TAg accumulates to high levels, it initiates viral DNA replication in the cell nucleus by recruiting the DNA polymerase α/primase complex to the viral origin of DNA replication, shuts off early gene transcription, and stimulates expression of the late genes, VP1, VP2, VP3, and agnoprotein (36). In a nonproductive infection, which usually occurs as a result of a cellular environment that is not conducive to viral replication, BKV induces oncogenesis.
through the expression of its two tumor antigens (reviewed in reference 86). TAg promotes cellular transformation by interfering with the tumor suppressor functions of p53 and pRB (reviewed in reference 3). TAg upregulates p53 levels in the cell by stabilizing the protein but functionally inactivates it by sequestering it in an inert form (44, 45, 48, 62, 91). Therefore, expression of TAg and subsequent inactivation of p53 mimic the same phenotypic effect as those caused by mutations in the p53 gene. Similarly, TAg functionally inactivates pRB by binding it and causing the release of E2F (reviewed in reference 4). TAg induces tumorigenesis and promotes anchorage-independent growth of transformed cells by the negative regulation of protein phosphatase 2A (2A, 63, 93).

In a previous report, utilizing in situ analysis, we demonstrated the presence of BKV DNA sequences in epithelial cells of benign and PIA ducts of cancerous prostate specimens (17). Additionally, BKV TAg expression was observed specifically in the atrophic epithelial cells but not in the normal epithelium. TAg was detected in the cytoplasm and colocalized with p53 in the atrophic cells, suggesting that neither protein could carry out its normal nuclear function. However, BKV was not detected in cells in the more advanced stages of cancer progression. In the present study, we have extended our analysis to noncancerous prostates. In normal prostates, BKV was present at a lower frequency than in cancerous prostates, and TAg expression was observed only in specimens containing PIA and PIN lesions. We also detected TAg expression in the same PIA ducts containing BKV DNA, confirming that the TAg is that of BKV. Utilizing laser capture microdissection (LCM) on cancerous prostates, we further show that the p53 gene from TAg-expressing PIA cells was wild type, whereas tumor cells expressing nuclear p53 contained a mix of wild-type and mutant p53 genes. Additionally, we demonstrate that the nuclear localization sequences (NLS) of cytoplasmically localized TAg and p53 were wild type, indicating that the sequestration of TAg and p53 in the cytoplasm was not due to mutations in the NLS of these genes. Together, these results support a causal role for BKV in PIA and the early development of prostate cancer.

MATERIALS AND METHODS

Human tissue specimens. Paraffin-embedded adenocarcinoma prostate resection specimens from radical prostatectomies and cystoprostatectomy specimens from bladder cancer patients with the diagnosis of muscle-invasive high-grade urothelial carcinoma, with no prostate cancer histology, were obtained from the Tissue Procurement Core at the University of Michigan Comprehensive Cancer Center. One section from each of the specimens was stained with hematoxylin and eosin and was evaluated for the presence of benign, atrophic, or tumor cells by the pathologist. Additionally, autopsy specimens were obtained commercially from the Tissue Procurement Core at the University of Michigan Comprehensive Cancer Center. One section from each of the specimens was stained with hematoxylin and eosin and was evaluated for the presence of benign, atrophic, or tumor cells by the pathologist. Additionally, autopsy specimens were obtained commercially as normal prostate tissue microarray from U.S. Biomax, Inc.

DNA extraction and PCR amplification. DNA extraction was performed using the method previously described by Das et al. (17) in a BKV-free area, and cross-contamination was avoided by frequent changing of gloves between samples. The sequences of the oligonucleotide primers used for these studies are listed in Table 1. The BKV early-region oligonucleotide probe for in situ DNA hybridization (ISH) [BKV in (1434-1473)], and a scrambled control probe with the same length and G + C content were characterized in our previous study (17).

Extracted DNA from entire thin tissue sections was amplified using Titanium Taq DNA polymerase (Clontech) in a ThermoHybaid thermocycler (P2c). All reactions were performed in a final volume of 100 μl containing 200 mM each primer, 200 μM deoxynucleoside triphosphates, 2 μl of the template, and 1 U polymerase in 0.5× buffer (20 mM Tricine-KOH [pH 8.0], 8 mM KCl, 1.75 mM MgCl2, and 1.87 μg/ml bovine serum albumin). For TAg NLS amplification, two rounds of 45 cycles each were used. The template for second-round amplification consisted of 30 μl of the product from the first-round amplification. The program consisted of initial denaturation for 5 min at 94°C followed by denaturation at 94°C for 30 s, annealing at 45°C for 30 s, and elongation at 72°C for 1 min, with a final elongation at 72°C for 7 min. For p53 gene amplification, 1× buffer was used, with two rounds of 45-cycle amplification. The program for exons 5 through 8 consisted of initial denaturation for 5 min at 94°C followed by denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 1 min, with a final elongation at 72°C for 7 min. The program for exon 9 was the same except that the annealing temperature was 51°C. The negative-control tube contained all the PCR components except the DNA template. p53 NLS amplification was done using PCR conditions identical to those for exons 7 through 8 and 9.

Sequence analysis. PCR products were separated by agarose gel electrophoresis, extracted (Qiagen gel extraction kit; Qiagen), and sequenced by the DNA Sequencing Core at the University of Michigan. Sequences were analyzed using Lasergene software from DNASTAR.

RPTE cells. Renal proximal tubular epithelial (RPTE) cells were grown and infected with BKV on two-well chamber slides (Fisher) as reported previously (46). Cells were fixed and stained 4 days after infection as previously published (17).
ISH and IHC. ISH and immunohistochemistry (IHC) were performed using our previously published protocol (17). For IHC with anti-VP1, 1% sodium dodecyl sulfate retrieval was performed for 8 min at room temperature. Monoclonal antibody anti-VP1 (P5G6 BKVVP1), a gift from Denise Galloway, was used at a 1:600 dilution.

Immunol-CM. Formalin-fixed, paraffin-embedded 4-μm-thick prostate tissue sections were deparaffinized and rehydrated using our previously published protocol. Immunohistochemistry was performed as previously described except that the antibody dilution for anti-TAg was 1:300. After IHC and counterstaining with hematoxylin, the sections were dehydrated in graded ethanol solutions (95% ethanol twice for 5 min each time; 100% ethanol three times for 5 min each time) and cleared in xylene three times for 5 min each time. The slides were air dried for 30 min in a fume hood and stored in a desiccator until LCM. Laser capture was performed using the PixCell II laser capture microscope from Arcturus Engineering. The TAg-positive PIA cells and nuclear p53-immunolabeled areas were visualized directly, after which the laser pulse was applied to activated thermoplastic film mounted on LCM caps to capture cells of interest. The following parameters were set on the PixCell II LCM system: laser spot size, 7.5 μm; power, 85 mW; current, 250 mV. DNA was extracted from approximately 2,500 captured cells by treatment with 40 μg/ml proteinase K buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0], 1% Tween 20, and 0.1 mg/ml proteinase K) for 48 h at 45°C. Proteinase K was first inactivated at 95°C for 10 min. The LCM DNA extracts were flash spun in a microcentrifuge and stored at −20°C. A 20-μl aliquot of the LCM DNA template was routinely used for first-round PCR amplification.

RESULTS

BKV is present at significantly higher frequencies in cancerous prostates. If BKV plays a role in prostate cancer progression, one would expect to find it at lower frequencies in normal prostates than in cancerous prostates. For these experiments, a cancerous prostate is defined as a prostate that has been surgically removed due to a diagnosis of prostate cancer and has been histologically confirmed to contain malignant cells. A normal prostate is defined as a prostate that has been surgically removed as part of a resection to remove a cancerous bladder (cystoprostatectomy) or during autopsy and has been histologically confirmed to contain only nonmalignant cells.

We initially analyzed the normal prostates using the same ISH technique that we previously used with cancerous prostates (17). BKV DNA was not detected in the majority of the normal specimens (Fig. 1A). However, in a small number of normal specimens, BKV DNA was observed in both normal and atrophic epithelial cells, as demonstrated by nuclear hybridization to the BKV-specific probe (Fig. 2E) but lacked TAg expression (Fig. 2F). We did not detect any BKV signal in the stromal cells. The ISH studies on normal prostates demonstrated the presence of BKV DNA sequences in 4/15 of the specimens, a frequency of detection significantly lower than that in cancerous prostates (Table 2).

Next, we used IHC with a specific anti-TAg monoclonal antibody to determine whether TAg was being expressed in the normal prostate tissue specimens (Fig. 1B and C). In the majority of the normal prostate tissue specimens, there was no detectable TAg (Fig. 1B). However, in a small number of normal specimens, we observed expression of TAg in the cytoplasm of atrophic epithelium (Fig. 1C). The staining pattern of the atrophic cells was identical to that observed with cancerous prostates. The IHC analysis of normal prostates demonstrated the expression of TAg in 4/29 of the specimens, an incidence significantly lower than that for cancerous prostates (Table 2). We also examined the status of the p53 protein in the normal prostate tissue specimens. In our previous report, we had observed the cytoplasmic localization of p53 in atrophic cells in the cancerous prostate specimens that also expressed detectable TAg (17). Representative IHC of anti-p53 staining on a serial section from the same normal prostate that was positive for TAg is shown in Fig. 1D. p53 expression was cytoplasmic, localized specifically to the atrophic cells that expressed TAg, and was detectable only in TAg-positive specimens (Table 3). The ISH and the IHC analysis for normal prostate tissue specimens suggest that BKV is not highly prevalent in the epithelia of normal prostates.

We wanted to confirm whether TAg was expressed in the same ducts that contained BKV DNA. To determine this, we aligned ISH and IHC slides from serial sections that showed the presence of viral DNA and TAg expression (Fig. 2). In a BKV-positive specimen, BKV DNA (Fig. 2B and G) and TAg expression (Fig. 2C and H, respectively) were observed in the same PIA duct. In contrast, a normal duct from the same BKV-positive specimen showed robust nuclear staining with the BKV probe (Fig. 2E) but lacked TAg expression (Fig. 2F).

Wild-type p53 gene in atrophic cells expressing TAg. We next wanted to test the hypothesis that TAg inactivates p53 in the early stages of prostate cancer, specifically in PIA. Our hypothesis regarding the status of the p53 gene in BKV-related prostate cancer is derived from the situation in another virally induced cancer, cervical carcinoma. Like TAg, the HPV E6 oncoprotein inactivates p53. HPV-induced cancers contain wild-type p53, while HPV-negative cancers have mutant p53 (13, 27, 37, 72). We predicted that in BKV TAg-expressing cells, a wild-type p53 gene would be observed, and in BKV TAg-negative tumor cells with detectable nuclear p53, a mutant p53 gene would be detected. To analyze this, we designed PCR primers to amplify exons 5 to 9 of the p53 gene. This region spans the sequence-specific DNA binding domain of p53; these exons are the sites for the most frequent mutations in the p53 gene in various cancers, including prostate cancer.

FIG. 1. ISH and IHC of normal prostates. (A) Section stained with a BKV-specific probe; (B) section immunostained with anti-TAg antibody; (C) PIA duct from a section that was positive for the presence of BKV DNA, showing immunostaining with anti-TAg antibody; (D) PIA duct from the same specimen as that in panel C, immunostained with anti-p53 antibody. Magnifications, ×400 (A, C, and D) and ×100 (B).
By utilizing LCM, BKV TAg-positive PIA cells and BKV TAg-negative tumor cells expressing nuclear p53 were isolated from cancerous prostate tissue sections immunostained with either anti-TAg or anti-p53, respectively. Amplification was performed both with DNA extracted from entire thin tissue sections and with DNA from the laser-captured cells, and the products were sequenced. The entire thin tissue sections contain a mixture of normal, atrophic, tumor, and stromal cells. In both the TAg-positive and TAg-negative captured specimens, there was slight stromal cell contamination (Fig. 3C and F). Chromatograms were carefully examined for the presence or absence of mutations in the p53 gene. A partial comparative chromatogram of exon 5 of the p53 gene from isolated TAg-positive cells and an entire thin tissue section of one specimen shows that the p53 sequence is wild type in the laser-captured atrophic cells expressing TAg, whereas it is a mixture of wild type and mutant in the entire section (Fig. 3). All the laser-captured TAg-positive PIA cells we analyzed contained wild-type p53, whereas cells from entire thin sections of some of these specimens contained a mixture of wild-type and mutant p53 (Table 4). In contrast, captured

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>BKV DNA result</th>
<th>TAg result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>No. negative</td>
</tr>
<tr>
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<tr>
<td>Cancerous</td>
<td>11</td>
<td>3</td>
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</table>

*P values for differences between normal and cancerous specimens, obtained by using the interactive calculation tool for Fisher’s exact probability test for 2-by-2 tables (K. J. Preacher and N. E. Briggs [www.quantpsy.org]), were 0.007 for BKV DNA and 0.008 for TAg.
tumor cells from a specimen that was BKV TAg negative but
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we had observed the presence of BKV DNA in normal and
stages. In our initial in situ analysis with cancerous prostates,
BKV plays a role in the etiology of prostate cancer in its early
NLS was also wild type (data not shown).
Additionally, exons 8 and 9
of the
three specimens (data not shown). Additionally, exons 8 and 9
with the antibody to VP1, indicating that viral replication was
occurring. To test this, IHC analysis was performed on a subset
of BKV TAg suggested that viral replication, which relies
on the host DNA synthetic machinery in the nucleus, cannot
be occurring. To test this, IHC analysis was performed on a subset
of the TAg-positive specimens using an antibody to the viral
late protein VP1, which is a BKV replication marker (Fig. 4).
We did not detect any positive signal in the tissue specimens
with the antibody to VP1, indicating that viral replication was
not occurring. As a positive control, BKV-infected human
RPTE cells were analyzed in parallel (Fig. 4C and D). Finally,
due to the cytoplasmic localization of TAg and p53, we determined
the integrity of the NLS of these two proteins. To accomplish
this, DNA was extracted from three TAg-positive tissue
specimens, and nested PCR was used to amplify and
sequence the TAg NLS. A wild-type NLS was observed in all
three specimens (data not shown). Additionally, exons 8 and 9
of the p53 gene, which contain a bipartite NLS, were
sequenced from three specimens. In these specimens, the p53
NLS was also wild type (data not shown).

**DISCUSSION**

The experiments in this study tested the hypothesis that
BKV plays a role in the etiology of prostate cancer in its early
stages. In our initial in situ analysis with cancerous prostates,
we had observed the presence of BKV DNA in normal and
PIA epithelium, with TAg expression specifically localized to
the atrophic but not the normal epithelium (17). BKV was not
detected and was assumed to be lost as the cancer advanced to
PIN or invasive carcinoma. We have extended our previous
studies to determine if BKV is also present in nondiseased
prostates, or whether its detection in cancerous tissue is simply
a reflection of its ubiquitous presence in the human popula-
tion. When we compared the presence of BKV in diseased and
nondiseased prostates, BKV was found at a higher frequency
in cancerous prostates. Viral DNA was detected in 79% of
cancerous prostates but only in 27% of nondiseased prostates,
and TAg expression was observed in 47% of cancerous pros-
tates but only in 14% of nondiseased prostates. Fisher’s exact
test both for ISH (P = 0.007) and IHC (P = 0.008) shows a
significant difference between the presence and expression of
BKV in normal and cancerous prostates. Interestingly, in both
types of specimens, TAg expression was detected only in PIA
cells. In nondiseased prostates, however, TAg expression in
PIA lesions was observed only in those specimens also con-
taining PIN lesions. Additionally, TAg expression is localized
to the cytoplasm in both types of specimen, but the NLS is wild
type. The comparative analysis between cancerous and nondis-
eased prostate supports our hypothesis that BKV is a cofactor
in PIA.

PIA has been observed in the vicinity of carcinoma lesions
and has been reported to sometimes merge with adenocarci-
noma (18; reviewed in reference 20). epithelial cells in PIA are
highly proliferative, exhibit phenotypic characteristics interme-
tiate between those of secretory and basal cells, and have been
proposed to be precursors of prostatic neoplastic transforma-
tion (89). Merging of focal areas of PIA with PIN has also been
reported (18). Interestingly, the occurrence of mutations in p53
or Rb1 in PIA is low, supporting a viral cause for the transition
of benign epithelium to PIA lesions through the inactivation of
these tumor suppressors by viral oncoproteins.

There are two additional reports on the presence of BKV
DNA in prostate carcinomas (43, 94). Zambrano et al. dem-
strated the presence of BKV DNA in 3/12 prostate speci-
mens by using PCR (94), and Lau et al. detected BKV DNA
in tumor cells in 2/30 prostate specimens using ISH (43). Our
previous study, however, was the first to demonstrate the pres-
ence of both viral DNA and oncoprotein expression in PIA
lesions of neoplastic prostate (17). In the current report, we
specifically examined the expression of TAg in the same PIA
ducts that contain BKV DNA, further supporting our previous
conclusion that the TAg is indeed that of BKV. Additionally, in
this study, we show that although the normal ducts may have
BKV DNA, there is no apparent expression of TAg in those
ducts. This suggests the intriguing possibility that BKV infects
the normal epithelium and resides in a latent state and that
activation of TAg expression in PIA may promote the transi-
tion from a benign to an atrophic state, ultimately leading to
prostate cancer. The fact that TAg is detected only in the PIA
lesions of nondiseased prostates that also have signs of PIN
also supports a possible link between BKV and cancerous
lesions. It is tempting to speculate here that these nondiseased
prostates that have PIN are already on their way to cancer, and
TAg expression may act as a cofactor that promotes this tran-
sition. Interestingly, prostate cancer is a slowly progressing
disease, focal areas of atrophy are a common occurrence in the

**TABLE 3. Summary of normal-prostate data**

<table>
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<tr>
<th>Specimen</th>
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</table>

* N, normal; A, atrophy (prostatic atrophy and PIA); B, benign prostatic hyperplasia; P, PIN; T, a very small area of incidental tumor; Pos, positive; Neg, negative; ND, not determined. ISH was performed with an early-region DNA probe for BKV.
aging prostate (20, 26, 29, 47, 69), and BKV is a relatively poor transforming agent (6, 33).

Detection of p53 by IHC usually requires that the p53 be stabilized in some manner (23, 24). Two known means of stabilization are binding by TAg (reviewed in reference 62) and mutation (60, 80). Our analysis of the p53 gene from laser-captured TAg-positive PIA cells and TAg-negative tumor cells with nuclear p53 is therefore relevant to the question of whether the virus is involved in oncogenesis. The p53 sequence is wild type in laser-captured PIA cells expressing TAg. This is similar to the status of p53 genes in cervical carcinomas expressing the HPV E6 oncoprotein (reviewed in reference 84) and suggests that the wild-type p53 is being inactivated by sequestration in the cytoplasm. However, when the p53 sequences from entire thin sections from cancerous prostates were analyzed, a mixture of wild-type and mutant p53 was sometimes, but not always, observed. Since p53 mutations are not frequent in prostate cancer, it is not surprising that we did not detect more mutations (21). We predicted that the mutant p53 sequence that we observed in TAg-positive thin sections was derived from tumor cells. LCM of p53-positive tumor cells supported this, because we detected a mix of wild-type and mutant p53 in those specimens. In spite of slight stromal cell contamination in the LCM samples, the mutant form of p53 was readily detectable in the specimen expressing nuclear p53. An earlier study using LCM demonstrated a very low occurrence of p53 gene mutations in PIA lesions (88). Since the p53 gene in TAg-positive cells in cancerous prostates is wild type, we did not perform this analysis on normal specimens. p53 expression in both types of specimens was detectable only in PIA cells and was cytoplasmic despite the protein having a wild-type NLS. For p53 to function as a tumor suppressor, its translocation and retention in the nucleus is required (40, 52, 71). Nuclear p53 overexpression was not observed in any of the nondiseased prostates.

It is interesting that TAg, which is normally a nuclear protein, is localized in the cytoplasm of the epithelial cells in these PIA lesions. The tumorigenic potential of a simian virus 40 (SV40) TAg containing a mutation in the NLS, causing it to remain in the cytoplasm, has been reported previously (10, 42, 61). This cytoplasmic SV40 TAg has the ability to induce tumors in transgenic mice at a rate equivalent to that of nuclear
However, in our studies, the NLS of BKV TAg was wild type. In addition, BKV replication, as measured by VP1 expression, was not observed in the TAg-positive specimens, consistent with the observation of TAg expression in the cytoplasm. Nonproductive infections leading to oncogenesis are known to occur with the gammaherpesvirus Epstein-Barr virus, as well as with oncogenic retroviruses, which generally require a helper virus for replication.

Additionally, cytoplasmic p53 expression in transformed cells in the presence of a cytoplasmically localized SV40 TAg has been reported previously (42). Cytoplasmic localization of p53 has also been observed in various human tumors (8, 9, 51, 53, 75, 79). This suggests that in certain cancers, functional p53 inactivation occurs by the sequestration of the protein in the cytoplasm, leading to acceleration of tumor progression by the accumulation of chromosomal mutations during cell proliferation.

The fact that the NLS of TAg and p53 were wild type in BKV-positive prostates suggests that there is a cellular factor(s) in the cytoplasm that is sequestering both proteins. Wild-type p53 has been shown to be retained in the cytoplasm as a result of interactions with proteins such as Hdm-2 and heat shock proteins (81). Phosphorylation of serine residues adjacent to the NLS of SV40 TAg by casein kinase II facilitates the nuclear translocation of TAg, and this process of nuclear import is inhibited by the p34cdc2-mediated phosphorylation of a nearby threonine residue (38, 70, 74). Complexes of p34cdc2 and p53 have been observed in TAg-transformed cells (50), and TAg has been shown to stimulate the expression of the cdc2 gene (12, 55). Interestingly, serine 315 of p53 is adjacent to one of its NLS and is also phosphorylated by p34cdc2 (2), and exclusion of p53 from the nucleus due to the phosphorylation of serine 315 was recently demonstrated (67). It is tempting to speculate that in the prostate specimens that express TAg, p34cdc2 phosphorylates TAg and/or p53, which impairs the ability of both proteins to translocate to the nucleus.

Based on our findings, we present the following model (Fig. 5). BKV infects normal epithelial cells and induces a change of the normal cells to PIA through the expression of TAg; alternatively, the transition to PIA induces TAg expression. This results in the induction of proliferation and sequestration of p53 in the cytoplasm. As the cells proliferate, they accumulate mutations at a higher-than-normal rate due to the absence of p53 activity. Eventually, a cell accumulates enough mutations to completely lose growth control and clonally expands into a tumor. The loss of BKV in the tumor cells could be due to selection against TAg by the immune system, dilution of viral episomes due to lack of replication, or proapoptotic effects mediated by TAg that are not compatible with the other growth control mutations in the tumor cells (83, 85, 87), resulting in selection against TAg expression. Loss of TAg expression has been reported in studies of TRAMP (transgenic adenocarcinoma of mouse prostate) mice, which develop tu-

<table>
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<th>Specimen</th>
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a Nuc, nuclear; Cyto, cytoplasmic.
b wt, wild type; mt, mutant; ND, not determined.
c LCM was performed on TAg (specimens C1 to C5) and on Tag (specimens C6 to C8). Asterisks indicate specimens for which the exon 8 sequence could be amplified but good-quality sequence could not be obtained.

FIG. 4. IHC for VP1. (A and B) TAg-positive cancerous prostate tissue sections immunostained with an immunoglobulin G2a isotype control or an anti-VP1 monoclonal antibody, respectively. (C and D) Mock- or BKV-infected kidney epithelial cells in culture, respectively, immunostained with an anti-VP1 antibody. Magnifications, ×100 (A and B) and ×400 (C and D).
FIG. 5. Model of induction of prostate cancer by BKV. See Discussion for details.

so, the unique viral properties of BKV can be explored for the possibility of prophylactic or therapeutic vaccination or for treatment by designing drugs that target TAg.

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