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The case for BK polyomavirus as a cause of bladder cancer

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In 2014, the International Agency for Research on Cancer judged Merkel cell polyomavirus (MCPyV) to be a probable human carcinogen. BK polyomavirus (BKPyV, a distant cousin of MCPyV) was ruled a possible carcinogen. In this review, we argue that it has recently become reasonable to view both of these viruses as known human carcinogens. In particular, several complementary lines of evidence support a causal role for BKPyV in the development of bladder carcinomas affecting organ transplant patients. The expansion of inexpensive deep sequencing has opened new approaches to investigating the important question of whether BKPyV causes urinary tract cancers in the general population.

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Introduction

In the late 1950s, Sarah Stewart and Bernice Eddy isolated a virus capable of causing multiple types of tumors in experimentally infected newborn mice [1]. Eddy went on to discover a different member of the 'poly-oma' virus family in rhesus monkey kidney cell cultures that were used to grow inactivated poliovirus vaccine stocks. The monkey-derived polyomavirus, known as SV40, was shown to cause brain tumors when inoculated into newborn hamsters and the discovery was rightly viewed with significant alarm [2]. Fortunately, large-scale epidemiological studies have conclusively shown that people exposed to SV40-contaminated poliovirus vaccines did not suffer a detectably increased risk of developing cancer [3].

Polyomaviruses appear to co-speciate with host animal lineages [4]. The absence of SV40 genomes in modern metagenomics surveys is consistent with the idea that the monkey-derived virus is not well adapted to replication in human hosts. In contrast, a human-specific SV40 homolog, BKPyV, ubiquitously infects human populations worldwide. The primary focus of this brief review is the question of whether BKPyV causes cancer in humans.

The non-enveloped polyomavirus virion encapsidates a ~5 kb circular double-stranded DNA (dsDNA) genome. The viral genome persists and replicates as an episome and integration into the host cell chromosome is not a normal feature of the viral life cycle. The genome is divided into three regions: the early region, which encodes the large and small tumor antigens (LT and sT, respectively); the late region, which encodes the virion structural proteins (VP1, VP2), and the control region, which encompasses the origin of replication and transcription regulatory elements. Eleven known human polyomavirus (HPyV) species generally establish infection at a young age, resulting in a lifelong subclinical infection in various organs. The epithelial surfaces of the bladder and kidney are the primary site of productive replication for BKPyV and its close relative JCPyV.

Polyomaviruses do not encode a DNA polymerase and are therefore wholly dependent on cellular DNA replication and DNA damage repair machinery for propagation. LT and sT are essential for unwinding the viral origin of replication and for reprogramming the cell cycle to promote DNA synthesis [5–8]. In animal model systems, expression of HPyV LT and sT, also referred to as the viral oncogenes, causes oncogenic transformation and host genome instability [9–14].

In 2008, Merkel cell polyomavirus (MCPyV) was discovered in cases of Merkel cell carcinoma (MCC), a rare but highly lethal form of skin cancer. It is now clear that in most MCC cases the MCPyV genome is no longer maintained as an episome, rather it is clonally integrated into the tumor genomic DNA. Integration is an accidental dead end for the virus but can result in persistent expression of the viral oncogenes, which is a critical factor in the ongoing development and survival of the tumor [15–18].

There is a large and conflicting body of older literature concerning the question of whether SV40 or BKPyV oncogenes can be found in human cancers (reviewed in Refs. [3,19]). A critical obstacle has been the problem of false-positive PCR and immunohistochemical detection [20]. The ubiquity of BKPyV infection and the fact that segments of the SV40 genome are in widespread use as

molecular biological tools has made it particularly difficult to rule out laboratory and environmental contamination. The unprecedented resolution provided by the advent of massively parallel sequencing has partly overcome this problem. In particular, the apparent absence full-length SV40 sequences in metagenomic deep sequencing surveys is consistent with the idea that that the virus rarely or never infects humans.

Comprehensive tumor deep sequencing studies have recently begun to settle the question of how frequently BKPyV might cause cancer through an MCPyV/MCC-like mechanism. In a comprehensive deep sequencing survey of various cancer types, BKPyV was only conclusively observed integrated into the genome of one out of 413 advanced muscle-invasive bladder tumors [21–23]. The observation conclusively shows that persistent integration of BKPyV into tumor genomes is a rare event in the general population.

Epidemiological evidence for virus-induced bladder cancer

Epidemiological studies on AIDS patients and solid organ transplant recipients inform us that immunosuppressed populations are at an increased risk for developing certain forms of cancer [24–27]. For example, the observation that AIDS patients are at increased risk of developing Kaposi's sarcoma led Moore and Chang to their discovery of the eighth human herpesvirus, KSHV [28]. Feng *et al.*'s successful search for viruses in MCC tumors was likewise guided by the observation that MCC incidence is higher among immunosuppressed individuals [18,29]. Similarly, the incidence of cervical cancer, which is caused by a group of high-risk human papillomavirus (HPV) types, is also elevated among immunosuppressed individuals [25].

Several common cancer types that have not yet been associated with viral infections show significantly increased incidence in immunosuppressed populations. Most notably, organ transplant patients show a roughly fourfold increase in risk of bladder and kidney carcinomas relative to the general population [24,25]. More recent studies investigating the relationship between kidney transplantation and the development of cancer have revealed that patients who develop BKPyV viremia or polyomavirus-associated nephropathy after transplantation have a further 4-fold to 11-fold increased risk of bladder cancer compared to transplant recipients without BKPyV disease [30–32]. These observations specifically implicate uncontrolled BKPyV replication in the development of bladder cancer, at least among immunosuppressed transplant recipients. Curiously, HIV+ individuals do not share this increased risk, which may reflect a difference in the biology of their immunosuppression or a yet to be discovered characteristic of BKPyV pathology in transplant recipients. 'Hitchhiking' of BKPyV in the engrafted organ could be a factor.

The idea that BKPyV plays a causal role in cancers of the renourinary tract has recently been confirmed by deep sequencing surveys identifying BKPyV DNA integrated into the cellular genomes of two post-transplant kidney carcinomas and three urothelial carcinomas affecting transplant patients [33–35]. The presence of BKPyV in cancers of the urinary tract is supported by a variety of other case studies (reviewed in Ref. [36]) [37–42]. Collecting duct carcinomas, also known as Bellini duct carcinomas, have emerged as a renourinary tumor type that is particularly likely to harbor BKPyV and exhibit active expression of LT [35,43–45]. As in tumor-associated MCPyV, tumor-associated BKPyV sequences generally appear to carry mutations that inactivate or remove VP1 and the helicase domain of LT. Additionally, these viruses frequently have disease-associated rearrangements in the viral control region that are known to enhance the expression of the T-antigens [38]. Together, this literature indicates that BKPyV can, at least in rare cases, cause cancers of the human urinary epithelia via a direct oncogenic mechanism, in which the virus clonally persists within tumor cells (Figure 1, 'Persistent Direct' column).

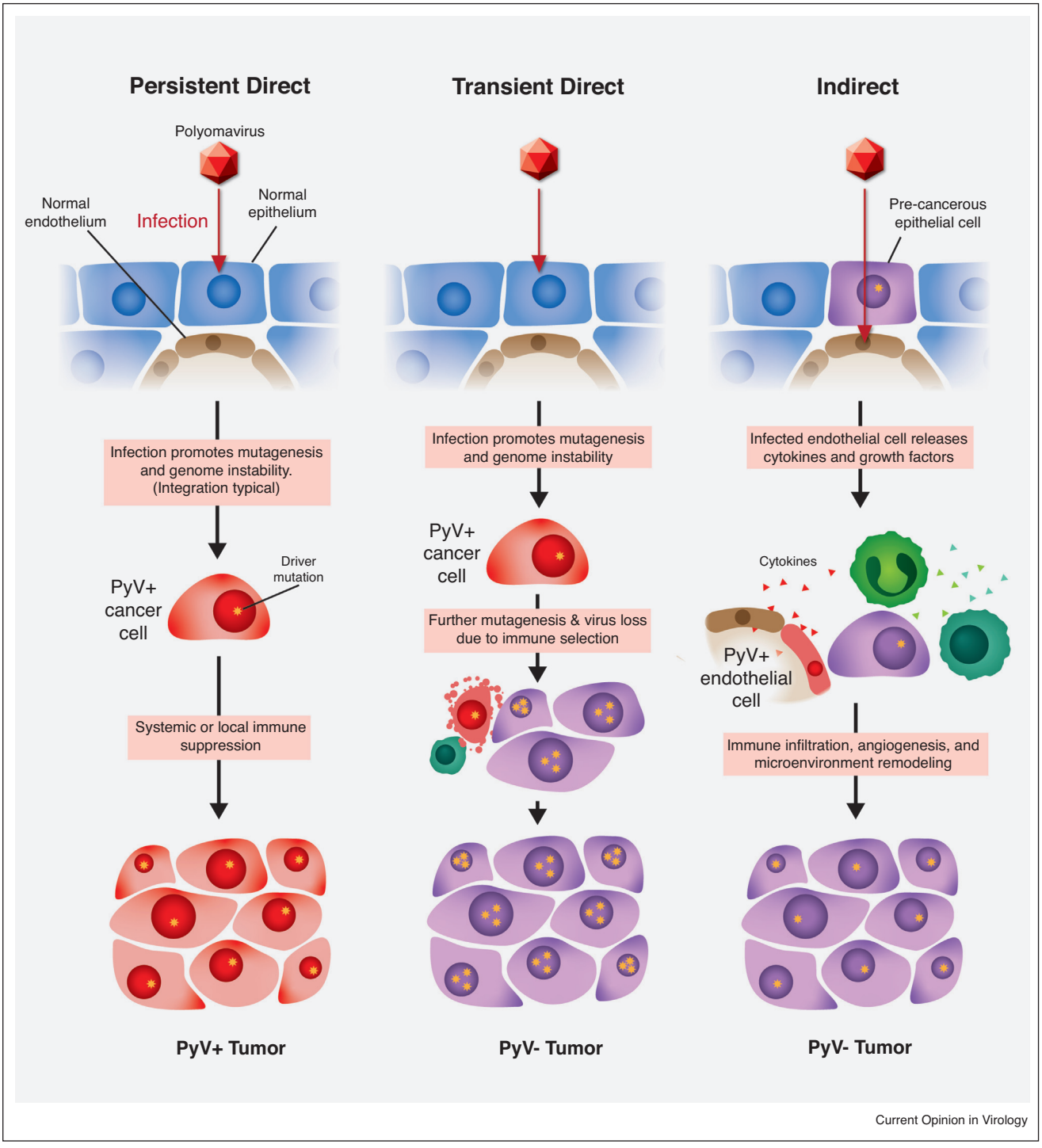
Hit-and-run carcinogenesis

Genotoxic carcinogens, such as tobacco smoke, can cause lasting damage that permanently increases the lifetime risk of developing cancer, even long after the carcinogen has been withdrawn [46]. Similarly, examples of 'covert pathogenesis' have been suggested for bacterial infections of the urinary tract where the true etiological agent is absent by the time disease symptoms manifest [47]. It has been unclear whether viruses cause cancer in humans through this type of mechanism.

Polyomaviruses share many physical and genetic properties with papillomaviruses. A subset of 'high risk' HPVs belonging to the genus *Alphapapillomavirus* are responsible for nearly all cases of cervical, vulvar, and penile cancers as well as an increasing majority of head and neck cancers [48]. Although HPV-induced carcinomas typically harbor chromosomally integrated HPV sequences, a minority of invasive tumors carry circular (non-integrated) HPV episomes [49]. HPV-induced tumors are generally dependent on the ongoing expression of the E6 and E7 oncoproteins, which, like polyomavirus LT proteins, inactivate p53 and pRb tumor suppressor proteins [50]. A small minority of HPV-induced cervical cancers may be at least transiently independent of viral oncogene expression, possibly due to compensatory expression of cellular oncogenes and mutation of tumor suppressor genes [51].

Although the direct persistent oncogenic mechanism generally observed for high-risk HPVs is similar to that of MCPyV in MCC, work in a variety of animal model systems has demonstrated that other papillomavirus types can cause carcinomas through 'hit-and-run' mechanisms

Figure 1



Models of polyomavirus (PyV) contribution to carcinogenesis. Persistent direct mechanism: PyV infects an epithelial cell and, through the expression of viral oncogenes and promotion of cellular genome instability, transforms the cell. In the absence of immune-mediated tumor cell death the transformed cell grows into a PyV-positive tumor. Transient direct mechanism (also known as 'hit-and-run' or 'covert' pathogenesis): virus-mediated cellular genome instability promotes the accumulation of driver mutations (gold stars), eventually enabling a population of tumor cells to lose the expression of viral oncogenes. Cells expressing viral oncogenes are killed through immune surveillance leading to a PyV-negative tumor. Indirect mechanism: PyV infects an endothelial cell adjacent to a pre-malignant epithelial cell. The infected endothelial cell expresses cytokines that recruit tumor-promoting immune cells. The pre-malignant epithelial cell undergoes transformation and grows into a PyV-negative tumor.

in which viral DNA is lost from the nascent tumor [52–54]. Epidemiologic and mechanistic studies suggest a scenario in which human *Betapapillomaviruses* are an important risk factor for the early development of non-melanoma skin cancer, but viral gene expression is not required for survival of the advanced tumor [54–56]. In this scenario, viral oncogene expression promotes cell survival in the face of otherwise lethal levels of UV-mediated mutagenic damage.

A long-standing problem with the hit-and-run hypothesis has been its failure to generate readily testable predictions. The availability of high-throughput deep sequencing methods has made it increasingly possible to test two predictions of the hit-and-run hypothesis. First, it would be expected that early cancer precursor lesions would be more likely to harbor BKPyV because the pre-malignant cells have not yet acquired enough mutagenic damage to become independent of viral oncogene expression. Another prediction is that immunocompromised individuals would be less likely to exert the immunological pressure against viral proteins (thus selecting for loss of the viral DNA) and would therefore be more likely to develop BKPyV-positive bladder carcinomas. The literature cited above suggests that this latter prediction has already begun proving true.

Genotoxic effects of viral oncogenes

Beyond the inactivation of cellular tumor suppressor proteins, papillomavirus E6 and E7 oncoproteins also drive tumor evolution in part through the upregulation of the APOBEC3 family of cytosine deaminases [57–61]. APOBEC3 enzymes normally serve as antiviral defenses, and the dramatic depletion of the APOBEC3-preferred deamination target motifs in *Alphapapillomavirus* genomes is evidence of a long-term evolutionary conflict [62]. Mutations in the viral genome consistent with APOBEC3 deamination have been observed in cervical cancer and in precursor cervical intraepithelial neoplasias [59,63].

In healthy cells, excessive unrepaired APOBEC3 damage typically leads to p53-mediated apoptosis [64]. This supports the hypothesis that the high level of APOBEC3 damage observed in cervical carcinomas is not merely triggered by E6/E7-mediated induction of APOBEC3 expression but also depends on the ability of E6 to inactivate p53 [60,61,65–70].

Much like the papillomavirus oncoproteins, the LT proteins of several polyomaviruses have the capacity to induce APOBEC3B expression in culture models of epithelial and endothelial cell infections [71–73]. Furthermore, increased expression of APOBEC3B has been observed in nephropathic lesions co-expressing BKPyV LT [74]. Like high-risk HPV types, BKPyV genomes show a strand-biased global depletion of APOBEC3-target motifs, suggesting a long evolutionary arms race between APOBEC3 enzymes and BKPyV [73]. These analyses also highlight a curious enrichment of preferred APOBEC3 target sites (TC motifs) overlapping the VP1 major capsid protein gene. It has recently been shown that BKPyV VP1 sequences observed in patients suffering from BKPyV nephropathy carry subclonal point mutations at these sites that confer resistance to antibody-mediated neutralization [74]. Much of the variation in this region is due to C-to-G transversions at TCA trinucleotides, which then produce an equally APOBEC3-mutable TCA motif on the opposite strand [74]. This raises the possibility of reversible mutational toggling through repeated rounds of APOBEC3 damage and error-prone repair. These mutations may serve as hallmarks of disease-associated BKPyV strains.

Much like cervical and HPV-positive head and neck cancers, bladder cancers show some of the highest APOBEC3-associated mutation loads of any cancer [65]. The fact that BKPyV LT expression simultaneously induces APOBEC3B expression and prevents the p53-mediated cell death that APOBEC3 damage would generally trigger suggests a possible scenario in which the virus promotes accumulation of mutations in driver genes, eventually rendering viral gene expression dispensable and allowing loss of the virus in the face of immunological pressure. Under this hypothesis, APOBEC3 damage could be thought of as a fossil record of the past presence of the virus (Figure 1, 'Transient Direct' column). The triggers for APOBEC3 overexpression in tumors remain unclear and APOBEC3-mediated mutagenesis in xenograft model systems is puzzlingly episodic [75].

Indirect effects

Although the primary site of productive BKPyV replication is the urothelium, infection of a wide variety of other cell types, including fibroblasts, salivary epithelium, endothelium, lymphocytes, neurons, and glial cells has been documented [76–81]. In particular, infection of endothelial cells has been observed for many different species of human and non-human polyomaviruses. In immunocompromised patients, the appearance of polyomavirus-infected endothelial cells has correlated with vasculopathy and even cardiac arrest [82–85]. A recent study of BKPyV-infected primary endothelial cells revealed activation of the interferon β pathway [72]. Infected endothelial cells released the cytokine CXCL10, which has been previously identified in the urine of kidney transplant recipients as a potential biomarker of subclinical BKPyV infection [35]. Although CXCL10 is a pro-inflammatory cytokine associated with the recruitment of tumor-infiltrating immune cells and in some cases improved outcome, other cases have shown that CXCL10 and its receptor CXCR3 can promote tumor growth and metastasis [86–88]. In some cases, tumor-associated macrophages may be recruited which can

remodel of the extracellular matrix, mobilizing growth factors and promoting migration and invasion by pre-malignant and malignant cells [89,90]. BKPyV-infected endothelial cells could also have effects on angiogenesis through the release of VEGF and enhanced survival of infected cells [91]. Such early 'bystander' effects could hypothetically promote tumorigenesis in a manner that would not be reflected by the presence of viral sequences in the malignant tumor (Figure 1, 'Indirect' column).

Other cancers

There have been numerous reports of polyomaviruses, especially BKPyV, in cell lines and cancers from various origins (previously reviewed in Refs. [9,92]). For example, LT expression has been detected in inflammatory precursor lesions that are thought to give rise to prostate cancer [93,94]. JC polyomavirus (a close relative of BKPyV) has been inconclusively implicated in colorectal and cerebrospinal tumors and human polyomavirus seven has been reported in thymic neoplasias [95,96]. A major confounding factor in some of these studies has been a reliance on PCR, which is susceptible to false-positive artifacts, especially in nested reactions. Without deep sequencing of the virus present in these samples and validation of distinct viral genetic features (e.g. the appearance of APOBEC3 damage or rearranged control region), it is difficult to rule out environmental contamination. The advent of inexpensive high-throughput sequencing methods should allow for a comprehensive re-evaluation of the presence of polyomaviruses in cancer and putative cancer precursor lesions.

Concluding remarks

BKPyV can play a direct persistent causal role in bladder carcinoma and other renourinary cancers, particularly among organ transplant patients. Polyomaviruses could theoretically also act in a much wider capacity as transient direct carcinogens by promoting cell survival while also enhancing the accumulation of driver mutations in the genome of the nascent tumor, eventually facilitating escape from dependence on viral oncogene expression. In theory, polyomavirus infection of non-tumor cells, such as endothelial cells and fibroblasts, could establish a niche that promotes the survival of nascent tumors. It will be important to apply modern high-resolution approaches, such as deep sequencing, to address the open question of whether BKPyV causes a larger fraction of renourinary carcinomas or other cancers through these occult mechanisms.

A vaccine against BKPyV is currently in commercial development [97]. An approach that could conclusively test the hypothesis that uncontrolled BKPyV replication causes cancer through as-yet-undiscovered mechanisms would be to administer kidney transplant recipients a BKPyV vaccine (for the purpose of preventing BKPyV nephropathy) and then several years later retrospectively compare the incidence of cancer among vaccines to historical baselines. This natural experiment would be akin to results showing a dramatic reduction in HPV-induced cervical pre-cancers in HPV vaccine recipients and reductions in liver cancer incidence in populations vaccinated against hepatitis B virus [98].

Conflicts of interest statement

Nothing declared.

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