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Advances in Tumor Virology

A Potential Linkage Between the JC and BK Polyomaviruses and Brain and Urinary Tract Tumors: A Review of the Literature

Silvia Carluccio[1], Lucia Signorini[1], Francesca Elia[2], Sonia Villani[1], Serena Delbue[1,3] and Pasquale Ferrante[1,2]

[1]Department of Biomedical, Surgical and Dental Sciences, University of Milano, Milano, Italy. [2]Fondazione Ettore Sansavini, Health Science Foundation, Lugo, Italy. [3]Istituto Clinico Città Studi, Milano, Italy.

ABSTRACT: JC virus (JCV) and BK virus (BKV) belong to the *Polyomaviridae* family and were the first human polyomaviruses (PyVs) discovered. Both JCV and BKV remain latent in the kidneys and can reactivate in immunocompromised hosts, causing progressive multifocal leukoencephalopathy (PML) and nephropathy, respectively. PyVs induce cell transformation *in vitro* and in animal models, where they infect non-permissive cells. The molecular mechanisms of tumor formation are based on the large tumor antigen (T Ag) protein, which is able to bind and inactivate pRb and p53, inducing uncontrolled cell cycle progression. Many studies on clinical samples also indicate a positive association between JCV and BKV and human solid tumors; however no direct involvement of PyVs in the development of human cancers has been demonstrated. In this review we focused on the potential association between JCV and BKV with brain and urinary tract tumors, respectively.

KEYWORDS: polyomaviruses, large tumor antigen, cell transformation

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CORRESPONDENCE: serena.delbue@unimi.it

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Introduction

The name "polyomavirus" (PyVs) is derived from a Greek word meaning "many tumors"; the term describes the ability of the first isolated murine PyVs to induce multiple tumors when inoculated into newborn mice.[1] However, to date, the direct involvement of PyVs in the development of human cancers has not been demonstrated.[2]

JC virus (JCV) and BK virus (BKV) belong to the *Polyomaviridae* family and were the first described human PyVs.

JCV was isolated in 1971 from the brain of a patient with progressive multifocal leukoencephalopathy (PML).[3] In the same year, BKV was recovered from the urine of a renal transplant patient.[4] The designations "JCV" and "BKV" were derived from the initials of the patients from whom the viruses were first isolated.

PyVs are nonenveloped viruses with an icosahedral capsid, containing a circular, double-stranded DNA genome approximately 5.2 kb in length. The 40–45-nm capsid is composed of three structural viral proteins (VP1, 2 and 3) organized into 72 pentamers. Each pentamer includes five VP1 molecules (the major capsid protein) associated with the VP2 and VP3 minor structural proteins.[5]

The genomic structure is divided into three regions: the early and the late coding units, which are transcribed in opposite directions starting from a common non-coding control region (NCCR). The NCCR is also comprised of the origin of DNA replication (ori), the TATA BOX, several cellular transcription factor binding sites, promoters and the enhancer for transcription of the viral genome. The early region encodes the alternatively spliced proteins large tumor

antigen (T Ag) and small tumor antigen (t Ag), which are multifunctional regulatory proteins essential for viral replication. The T antigens are also capable of promoting cellular transformation *in vitro* and potentially *in vivo*. The late region encodes the three viral structural proteins by alternative splicing of a common mRNA and a small accessory agnoprotein.[6]

A common feature of PyVs is that the occurrence of viral-associated diseases is a rare event. Despite this, antibodies to the viruses can be detected in a large percentage of healthy subjects, indicating that the infection is commonly widespread. The asymptomatic primary infection occurs in childhood and is usually subclinical; JCV infects approximately 50%–80% of healthy subjects, while the percentage of BKV seroprevalence is approximately 80%-90%.[7]

Both JCV and BKV infect and remain latent primarily in the kidney tissue and can reactivate in cases of host immunosuppression. In cases of severe immunodeficiency, JCV can lytically infect oligodendrocytes, causing a fatal demyelinating disease of the central nervous system (CNS) known as PML. BKV reactivation is associated with hemorrhagic cystitis and PyV-associated nephropathy (PVAN) following renal transplantation.[8]

Molecular mechanisms of viral tumorigenesis. PyVs are able to induce cell transformation when they infect non-permissive cells where viral replication is not supported; this is in contrast to permissive cells, where the virus can replicate.[9]

Because of this, JCV and BKV are considered good candidates for playing a role in the pathogenesis of human tumors.[10]

The main viral factor involved in cellular transformation *in vitro* and *in vivo* is the T Ag. The T Ag is capable of neural cell transformation *in vitro*[11] and induces tumor formation in small rodents, including hamsters and rats,[12–16] transgenic mouse models[17] and non-human primates,[18] although the virus is not permissive in hosts other than humans.

BKV is able to transform embryonic fibroblasts and cells cultured from the kidneys and brains of hamsters, mice, rats, rabbits and monkeys,[19–21] leading to the development of several types of tumors. BKV-infected or -transfected human cells generally do not display a completely transformed phenotype, characterized by immortalization, anchorage independence, and tumorigenicity, although they exhibit morphological alterations and an increased lifespan.[22,23]

Three models that challenge the "driver" role of PyVs in cancers were reviewed by Dalianis and coworkers.[24] The first model is the "hit-and-run" mechanism, where PyV infection is associated with the early stages of tumorigenesis, resulting in chromosomal instability. This model posits the involvement of PyVs in tumorigenesis even if the PyVs can no longer be detected in the later stages of disease. In the second mechanism, called the "passenger" model, the PyVs find favorable conditions for replication in cells that have already undergone transformation but that play no role in the acquisition of the typical oncogenic features of the cells. The last model is the "by-stander" model, in which PyVs are able to infect neighboring cells and can be detected in anatomic regions connected to the tumor but that are unrelated to the malignancy. However, the long elapse period between initial viral infection and evidence of tumor development complicates the argument that PyVs contribute to cancer.

The detailed molecular mechanisms by which PyV products may support tumorigenesis have been extensively studied and reviewed.[21,25–28]

T Ag is a nuclear phosphoprotein of 688–695 amino acids that interacts with DNA at the viral origin of replication to initiate PyV replication. T Ag is able to bind and inactivate cellular proteins, such as the retinoblastoma family of tumor suppressors (Rb, p130, and p107)[26,27,29–31] and oncosuppressor p53,[25,27,31] that prevent cell cycle passage in S phase. T Ag binding to pRb enables the activation of the transcription factor E2F, promoting cell cycle progression,[32] while the interaction with p53 seems to compromise its protective role against both DNA damage and oncogenic transformation.

Additionally, other cellular proteins, such as insulin receptor substrate 1 (IRS-1),[33] β-catenin,[34,35] the neurofibromatosis type 2 gene product[36] and the anti-apoptotic protein survivin,[37] are implicated in binding to JCV T Ag. These bonds promote the impairment of the homology-directed DNA repair mechanism,[38] the enhancement of c-myc and cyclin D1 gene expression,[34,35] the lack of positive regulation of p53[36] and the significant decrement of the apoptotic process. BKV T Ag activates the DNA methyltransferase 1 gene[39] that is associated with tumorigenesis through tumor suppressor gene hypermethylation (Fig. 1).

PyVs t Ag, a viral protein of 172 amino acids, interacts with protein phosphatase 2A (PP2A), a serine/threonine specific phosphatase implicated in the regulation of the mitogen-activated protein kinase (MAPK) pathway.[40] This interaction most likely leads to uncontrolled cell growth, as demonstrated for SV40 t Ag.[41,42]

Although attention has been focused on the transforming proteins T Ag and t Ag during the last two decades, it has become increasingly evident that JCV and BKV agnoprotein, a small highly basic viral protein of 71 amino acids, is involved in the dysregulation of cell cycle progression and cell accumulation at the G2/M phase.[43] Agnoprotein has also been implicated in the impairment of DNA damage-induced cell cycle arrest.[44]

Many studies have reported data regarding the association between JCV and CNS tumors. This evidence was first reported in 1961, when Richardson,[45] who first described PML, diagnosed an oligodendroglioma in a patient with concomitant chronic lymphocytic leukemia and PML. After the identification of JCV as the etiologic agent of PML, investigations were conducted to determine whether brain

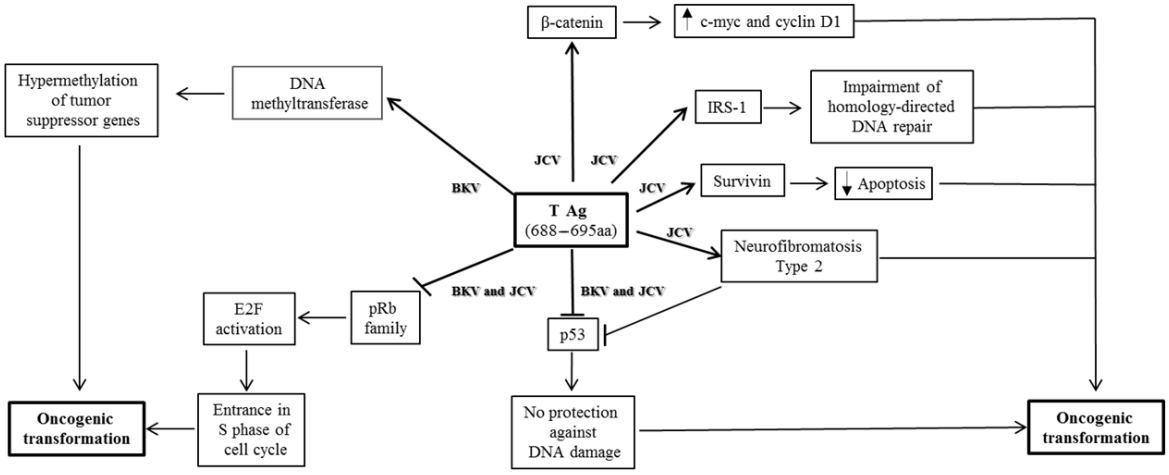

Figure 1. The interaction of JCV and BKV T Ag with cellular proteins. JCV T Ag interacts with β-catenin, IRS-1, survivin and neurofibromatosis type 2 proteins. BKV T Ag binds DNA methyltransferase. T Ags from both viruses inhibit the oncosuppressor proteins pRb and p53. These interactions may drive PyV-infected cells to oncogenic transformation.

tumors could be related to JCV in patients without PML. JCV sequences have been detected in several tissues, including kidneys, the main latency site of the virus, brain, liver, lung, spleen, lymph nodes, and colorectal epithelium.[46,47] In contrast, BKV is known to persist in the kidney and urinary tract.[48,49] Consequently, it is not surprising that BKV DNA has been identified mainly in urothelial carcinomas of the renal pelvis, prostate, and bladder as well as renal cell carcinomas.[50,51]

Contrasting data on a possible role for BKV in other human cancers, such as brain, lung, liver, pancreas, lymphomas, adrenal tumors, colorectal tumors, rhabdomyosarcoma and Kaposi's Sarcoma,[20,28,52–54] have accumulated slowly in recent years.

In this review we describe the potential association between JCV and BKV with brain and urinary tract tumors, respectively.

JCV and CNS Tumors

Medulloblastoma. Medulloblastoma is a highly malignant tumor that represents greater than 25% of childhood intracranial neoplasms. The investigation of the role of JCV in human medulloblastoma pathogenesis began with evidence that JCV was able to cause medulloblastoma in animal models.[55] Kyrnska and colleagues analyzed 23 tumor samples using PCR and found JCV T Ag sequences in 47.8% of them. Immunohistochemical (IHC) analysis of the same tumor samples showed T Ag protein expression in the nuclei of the tumor cells.[56]

Accordingly, two additional studies showed the presence of both T Ag gene and protein expression in approximately 25% of analyzed medulloblastoma samples.[57,58] However, Vasishta and colleagues failed to demonstrate an association between JCV and medulloblastoma.[59]

Astrocytoma and oligoastrocytoma. Since 1983, the presence of JCV genome sequences in multiple malignant astrocytoma samples has been described.[60] Subsequently, Rencic and colleagues reported the case of an immunocompetent subject with a diagnosis of oligoastrocytoma characterized by the presence of DNA sequences corresponding to the JCV NCCR and T Ag. In this study, the NCCR sequence was defined as the oncogenic strain Mad4.[61] IHC assays showed that T Ag protein was expressed in the oligodendroglial region but not in the astrocytic area. Following this observation, it was hypothesized that the T Ag protein may be secreted by the oligodendrocytes and may contribute to the uncontrolled growth of the neighboring astrocytic cells. Similar results were obtained by Caldarelli-Stefano and co-workers, who analyzed tumor tissues from 10 patients with astrocytoma; T Ag sequences were found in 4/10 samples (40%) by means of nested PCR. The JCV NCCR sequence, characterized as Mad4, was amplified in 1/10 (10%) tissue samples, and T Ag protein expression was detected in 1/10 (10%) tissue samples.[58] Del Valle and colleagues searched for JCV sequences in several subtypes of astrocytoma. The results obtained by performing multiple experiments revealed that 10/13 astrocytoma (76.9%), 4/5 pilocytic astrocytoma (80%) and 5/8 oligoastrocytoma (62.5%) tissues contained JCV early gene sequences, but IHC analyses contained some discrepancies. It was suggested that mutations in the viral genome may abrogate T Ag expression in tumor cells of astrocytoma patients.[62]

Xanthoastrocytoma. Boldorini and colleagues found JCV DNA sequences in the brain of an immunocompetent 9-year-old child with a pleomorphic xantoastrocytoma. Nested PCR on DNA extracted from tumor tissue revealed the presence of genomic sequences corresponding to the JCV T Ag, NCCR, and VP1 regions. Sequence analysis showed that the NCCR was rearranged, yielding the Mad4 strain that was previously reported as being oncogenic in animals.[63]

Glioblastoma multiforme. Glioblastoma multiforme (GBM) is the most aggressive malignant primary brain

tumor in humans, involving glial cells and accounting for 52% of all functional tissue brain tumor cases and 20% of all intracranial tumors. Two case studies showed a potential association between JCV and this type of tumor. A peculiar case of a patient affected with both multiple sclerosis (MS) and GBM and infected with JCV was reported. Both the GBM tissue and the demyelinating area of the brain showed the presence of JCV genome sequences belonging to two different strains: Mad4 and W1.[64] Pina-Oviedo and colleagues conducted their investigations on brain tumor samples from a 54-year-old immunocompetent subject and found that JCV T Ag protein was expressed in the cell nuclei, whereas the agnoprotein was expressed in the cell cytoplasm. Interestingly, the T Ag positive cells were microdissected, resulting in the isolation of the complete viral genome that was molecularly characterized as Mad1, the strain usually associated with the poorest prognosis of infection[65] A larger study reported the presence of JCV early gene sequences and protein expression in 12/21 (57.1%) GBM tissues.[64] A similar percentage of JCV positivity (52.4%) was reported by Delbue and colleagues.[66] Contrasting results were observed in two other surveys, conducted on 80 and 225 GBM tissues, that failed to amplify the viral genome.[67,68] The differences in the employed molecular techniques may explain the divergence of the obtained results.

Oligodendroglioma. Tumors derived from oligodendrocytes include oligodendrogliomas and anaplastic oligodendrogliomas. Caldarelli-Stefano et al were able to amplify JCV T Ag sequences in 1/5 (20%) oligodendroglioma tissues.[58] A higher percentage (57.1%) of JCV-positive tumor samples was obtained by Del Valle. In this work, T Ag-positive nuclei were found in both oligodendrogliomas and anaplastic oligodendrogliomas by IHC.[69]

In a subsequent study, a total of 20 formalin-fixed and paraffin embedded surgical and postmortem tumor samples were collected, including 18 classical oligodendrogliomas and 2 anaplastic oligodendrogliomas. Gene amplification of JCV DNA sequences in the tumor samples was performed by PCR using specific pairs of primers targeting four different regions of the viral genome, including the NCCR, the NH2-terminal region of T Ag, the agnoprotein gene, and the region corresponding to the VP1 encoding sequence. The T Ag gene was detected in 13/18 (72.2%) oligodendrogliomas and in the two anaplastic oligodendrogliomas, while VP1 sequences were amplified in 66.6% and 100% of samples, respectively. The viral NCCR sequence, characterized as either Mad4 or an archetype, was amplified in 7/18 (38.8%) oligodendrogliomas. IHC analysis showed the presence of the viral protein T Ag in the nuclei of 8/18 oligodendrogliomas (44.4%) and in both cases of the anaplastic oligodendroglioma, while agnoprotein was found in 10/18 oligodendrogliomas (55.5%) and in 1/2 (50%) anaplastic oligodendrogliomas. No evidence of capsid protein VP1 expression was reported. Consequently, it was hypothesized that there was infection but no virion production of the tumor cells.[69]

Ependymomas. The ependymoma is a brain tumor derived from ependymal cells.[70] Caldarelli-Stefano and colleagues analyzed 5 ependymoma tissues, finding one of them positive for the presence of the JCV genome Mad4 strain.[58] One year later, 83.3% of ependymomas from a different cohort of patients showed the presence of JCV early DNA sequences and protein expression and the absence of late protein expression.[62]

CNS lymphoma. The association of JCV with B-cell lymphomas of the CNS has been investigated because B lymphocytes are known to be a site of JCV latency and reactivation. Del Valle and coworkers[71] examined 27 tumor specimens by means of PCR and IHC, showing the presence of agnoprotein and T Ag sequences in 22/27 samples and of the VP1 encoding region in 8/27 specimens. JCV T Ag and agnoprotein expression was detected by IHC in 6 tumor samples (22.2%) and localized to the cell nuclei and cytoplasm, respectively. In contrast, the JCV VP1 protein was not detected, indicating the lack of complete viral replication. The nuclear presence of the T Ag protein was associated with cell oncosuppressor p53 (Table 1).[62,71]

Table 1. Studies with clinical samples indicating a positive association between JCV and CNS tumors.

TUMOR	T Ag	VP1	AGNO	NCCR	METHODS	REFERENCES
Medulloblastoma	+	/	/	/	PCR, IHC, Southern blot	56–58
Astrocytoma	+	/	/	+ (Mad4)	PCR, IHC, Southern blot	58,62
Oligoastrocytoma	+	/	/	+ (Mad4)	PCR, IHC, Southern blot	61,62
Xanthoastrocytoma	+	+	/	+ (Mad4)	PCR	63
Glioblastoma multiforme	+	+	+	+ (Mad1–4)	PCR, IHC, Southern blot	64–66
Oligodendroglioma	+	+	+	+	PCR, IHC, Southern blot	58,62,64
Ependymomas	+	/	/	+ (Mad4)	PCR, IHC, Southern blot	58,62
CNS lymphoma	+	+	+	/	PCR, IHC, Southern blot	71

Abbreviations: PCR, polymerase chain reaction; IHC, immunohistochemistry; ISH, *in situ* hybridization; +, positive; /, not determined.

BKV and Human Tumors

Carcinoma of the urinary tract. BKV infection has been repeatedly suggested to be associated with cancers of the genitourinary tract. However, due to conflicting results regarding the detection of specific sequences and proteins in human cancers, the oncogenic role of this virus remains controversial.[72] Profound immunological impairment, like that experienced by transplant organ recipients, may be necessary for BKV reactivation. Virus replication has been associated with a number of diseases, such as hemorrhagic cystitis, ureteric stenosis and PVAN, but the potential association of BKV infection with malignancy, particularly of urothelial and renal tubular origin, is still being debated.[73]

Less is known about the molecular mechanisms of BKV's contribution to hemorrhagic cystitis in the bladder. It is not clear whether there is a reservoir of BKV in the bladder that reactivates upon immunosuppression or whether BKV moves to the bladder from the kidney at the time of reactivation, although there is some evidence of BKV in normal bladder tissue.[52] In the studies by Fioriti et al and Monini et al, BKV sequences were found to be present at a high frequency in bladder carcinomas.[78,79] Weinreb et al reported a significant association between urine cytology suggestive of PyV infection and subsequent diagnosis of bladder carcinoma.[75] Alexiev et al reported two cases of kidney transplant patients who developed PVAN and unspecified BKV infection followed by the development of carcinoma of the urinary bladder several years post transplantation. They proposed a model to explain the relationship between BKV reactivation in immunosuppressed patients and oncogenesis. In particular, they hypothesized that an "uncoupling" of T Ag expression from the late genes may lead to oncogenic transformation, while an efficient release of progeny virions from the nucleus resulting in cell lysis may be inconsistent with oncogenesis.[76] In another study, they reported that decoy and malignant cells strongly stained positive for T Ag and that the urothelial carcinoma showed diffuse expression of T Ag, p53 and p16 in both the primary tumor and the metastatic carcinoma. Otherwise, no T Ag staining was reported in adjacent uninvolved metastatic sites (lymph node tissue). This result demonstrated unequivocally that the BKV viral genome was incorporated into the genetic material of tumor cells, suggesting a causative role for BKV in the development of urothelial carcinoma. [77]

In contrast, Roberts et al, found no evidence of T Ag expression in urothelial carcinoma tissues obtained from 20 immunocompetent patients.[78] This may account for the fact that immunosuppression, such as that experienced by transplant recipients, appears to be a major risk factor for the development of BKV-associated urinary tract carcinomas. Taking advantage of the immune system weakness, BKV-associated urothelial carcinomas usually develop as high grade and aggressive subtypes.

Herawi et al detected the presence of the T Ag protein in both benign and cancerous bladder tissues, but only urothelial carcinoma cells showed strong and diffuse nuclear T Ag signals.[79]

A tissue-based study showed the detection of BKV DNA by PCR in 5.5% of urothelial carcinomas, all of which were negative for BKV T Ag by IHC.[78] Rollison et al concluded that BKV did not play a major role in the pathogenesis of bladder carcinoma, as only 5.5% of bladder samples were positive for the presence of the BKV genome and none of them showed T Ag expression.[80]

In a case report, a single patient who developed PVAN and a subsequent carcinoma of the bladder that was diffusely positive for BKV T Ag expression was described.[81] The involvement of BKV in kidney cancer is not well established. There are several case studies reporting either the presence or the absence of BKV DNA sequences or protein expression in renal carcinomas developed after renal transplant.[82–84] The presence of BKV sequences in kidney cancer samples also seems to vary among different cohorts of patients.[50,51,74–84] Kausman et al reported the case of a 10-year-old child with a carcinoma of the donor renal pelvis following BKV allograft nephropathy. Removal of the primary tumor and cessation of immunosuppression led to regression of the secondary tumors and a return to health. However, *in situ* hybridization (ISH) for BKV was negative within the tumor.[83] In contrast, Narayanan et al reported a case of a poorly differentiated, high grade renal carcinoma occurring in a solid organ adult transplant recipient following BKV nephropathy causing renal allograft loss.[82]

Prostate cancer. Many groups have examined the hypothesis that BKV may play a role in the etiology of prostate cancer. Dweepanita et al proposed an interesting model: BKV may infect normal epithelial cells and induce a transition of the normal cells to proliferative inflammatory atrophic (PIA) cells due to the expression of BKV T Ag. Alternatively, it was hypothesized that transition to PIA may enhance T Ag expression, resulting in the sequestration of p53 in the cytoplasm and uncontrolled cell proliferation. 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 subsequent loss of BKV from the tumor cells could be due to selection against the T Ag by the immune system, dilution of viral episomes due to lack of replication, or to the proapoptotic effects mediated by T Ag.[85–88] In support of this theory, Monini et al reported the detection of BKV sequences by means of PCR and Southern blotting in more than 50% of both normal and tumor tissues obtained from the urinary tract and prostate.[50] Viral DNA load was found to be significantly higher in neoplastic tissues compared to non-neoplastic tissues, suggesting that there may be selection for BKV-containing tumor cells.[50] Moreover, BKV DNA in these tumor samples seemed to exist in a rearranged episomal and/or integrated form, and the simple restriction pattern of these sequences was suggestive of early acquirement of viral DNA during cancer progression and subsequent clonal expansion. Delbue et al found that the prevalence of BKV DNA was significantly higher in tissue specimens from prostate

Table 2. Studies with clinical samples indicating a positive association between BKV and Urinary tract tumors.

ANATOMIC SITE	SAMPLE	T Ag	VP1	AGNO	NCCR	METHODS	REFERENCES
Urothelial tract	Non neoplastic tissue	–/+	/	/	/	PCR, IHC, Southern blot	50,74–79,81
	Bladder carcinoma	+	+	/	+		
	Urothelial carcinoma	+	/	/	/		
Kidney	Non neoplastic tissue	–	/	/	/	PCR, IHC, ISH, Southern blot	50,79,82–84
	Renal carcinoma	+	/	/	+		
Prostate	BPH	+	+	/	/	PCR, IHC, ISH, Southern blot	50,85,89–92,101–103
	PIA	+	+	/	/		
	PCA	+	+	/	+		
	PIN	+	+	/	/		

Abbreviations: PCR, polymerase chain reaction; IHC, immunohistochemistry; ISH, *in situ* hybridization; +, positive; /, not determined. BPH, benign prostate hyperplasia; PIA, proliferative inflammatory atrophy; PCA, prostate cancer; PIN, prostatic intraepithelial neoplasia.

cancer patients compared to benign prostate hyperplasia (BPH) patients, suggesting an etiological role for BKV in the early stages of prostate cancer progression.[89] Das et al reported that BKV DNA sequences were specifically found in the epithelium of benign ducts and PIA lesions in more than 70% of the neoplastic prostate tissues examined by both PCR and ISH.[85] The frequency of BKV DNA was much lower (<30%) in normal prostates, ruling out the possibility that the detection of BKV DNA in cancerous tissue was due to its ubiquitous presence in humans. Furthermore, IHC revealed that the percentage of T Ag positive samples was also significantly higher in cancerous prostates than in normal prostates. A report by Russo et al suggested the role of BKV in the pathogenesis of prostate cancer.[90] BKV DNA was detected by PCR in 85% of the prostate cancer specimens, but in none of the BPH control group specimens. Moreover, IHC analysis revealed that both T Ag and p53 proteins were present in the cytoplasm in 77% of the cancer samples, whereas p53 was found in the nucleus of the T Ag negative tumor cells. These results are consistent with the findings by Das et al.[85,90,91]

Lau et al examined 30 cancerous prostate tissues. While they did not observe T Ag expression by IHC in any of their samples, ISH revealed the presence of BKV DNA in four non-neoplastic, two neoplastic and one PIN tissues.[92] There are also negative reports regarding the potential association between BKV and prostate cancer in the literature. Using nested PCR, Sfanos et al analyzed a total of 338 samples from 200 patients and found only one sample positive for BKV;[93] Akgul and colleagues detected the BKV genome in one out of 85 PCA analyzed tissue samples;[94] Bergh and colleagues did not amplify the BKV genome in either 171 prostate cancer tissues nor in 181 control tissues.[95] Negative results were also obtained by Martinez Fierro and Groom and colleagues in two different cohorts of 55 and 100 patients, respectively.[96,97]

Recently, papers aimed at exploring the BKV-prostate cancer link were subjected to an updated systematic review and analysis to interpret the contrasting results. The authors concluded that there is evidence for a significant link between BKV expression and prostate cancer development, particularly between BKV infection and cancer risk (Table 2).[98]

Conclusions

In this article, we have reviewed data concerning the possible link between JCV and BKV with CNS and urinary tract human tumors, respectively. Although a role for JCV and BKV in malignant transformation was proposed more than 40 years ago, and although *in vitro* studies supported the oncogenic properties of the T Ag protein, there is insufficient evidence of a casual association between these human PyVs and solid cancer development. Indeed, some biological features of these viruses make the establishment of a direct association with tumors difficult: JCV and BKV are ubiquitous in the human population, their primary infection is often asymptomatic, the length of infection is not determinable, and they do not productively infect animal models.[17]

To settle this issue, JCV and BKV should fulfill criteria that have been fixed for establishing a causal relationship between a virus and a tumor.[99] These criteria include the detection of the viral genome and/or proteins in cancerous tissues, proven molecular mechanisms for inducing tumorigenesis and consistency of association. Regarding the first criterion, numerous reports on the presence of the viral genome and protein expression in *ex vivo* studies showed discordant results that make their interpretation extremely difficult. Additionally, current epidemiological data and the presence of JCV and BKV sequences in normal tissues surrounding neoplastic tissues does not support a causative role in several cancers. While the second criterion may be fulfilled by the two polyomaviruses, evidence is still lacking for the third criterion.

Despite the "inadequate evidence of carcinogenicity in humans", the WHO International Agency for Cancer Research Monograph Working Group decided to classify JCV and BKV as "possibly carcinogenic to humans", belonging to group 2B, on the basis of the "sufficient evidence in

experimental animals".[100] Therefore, only further solid, clear-cut epidemiologic, histopathologic and DNA evidence will ultimately settle this urgent issue and will help to answer the still unsolved question: "Do JCV and BKV cause tumors in the human population?" When a complete understanding is reached, a vaccination approach for the prevention of polyomavirus infection may be proposed.

Author Contributions

Conceived and designed the experiments: SD, PF. Analyzed the data: SD, SC, FE. Wrote the first draft of the manuscript: SC, LS, SV. Contributed to the writing of the manuscript: SD, FE. Agree with manuscript results and conclusions: SD, PF. Jointly developed the structure and arguments for the paper: FE, SV. Made critical revision and approved final version: SD, PF. All authors reviewed and approved of the final manuscript.

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