Polyomavirus and Human Cancer

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The polyoma viruses which affect humans (JC, BK, and SV40) have been implicated in several human diseases and are undergoing increased scrutiny as possible cofactors in human cancer. These viruses contain DNA which encodes for structural proteins as well as T antigens. These T antigens are tumorigenic in animal models and can cause human cell transformation and immortalization. Viral DNA and proteins from BK, JC, and SV40 have been detected in various brain, pleural, bone, gastrointestinal and endocrine tumors. Despite increasing evidence that these viruses are associated with human cancer, their exact role remains unclear. BKV and JCV have only recently been implicated as possible cofactors in tumorigenesis; SV40 has been the more studied of the three in terms of cancer, ever since millions of people were exposed inadvertently via contaminated polio vaccines. The authors give a brief overview of the viruses and review the data which link each with human cancer.

Introduction

The human polyoma viruses (JCV and BKV), along with their simian cousin (SV40), are ubiquitous viruses that are primarily associated with progressive multifocal leukoencephalopathy (PML) and hemorrhagic cystitis, respectively, under specific conditions in immunocompromised individuals. Currently, polyoma viruses are now undergoing increasing scrutiny as possible causes for several human cancers. In animal studies, polyoma viruses have been found to be viral agents for oncogenesis. It is known that polyoma viruses produce a wide range of pathological lesions in experimental animals, including a variety of neoplastic tumors. Evidence has been mounting recently that JCV, BKV, and SV40 are potential oncogenic viruses in humans as well.

Structure and Function

The polyoma viruses have an icosahedral structure of 45 nm diameter. Its DNA is arranged in a double-stranded, circular chromosome of 5 kb. The three viruses share approximately 69% genomic identity,[1] and they are distinguishable both at the DNA and protein levels. The capsid consists of three structural proteins: VP1, VP2, and VP3. The viral DNA encodes for two early proteins that regulate gene expression: large T and small t (Table 1), which are derived from alternatively spliced mRNA.

The receptor for entry of the virus into the cell is not clearly defined at this time. However, VP1 binds to sialic acid, which is an important component of many cell receptors.[2] Current theory is that the virus is able to use a class of sialoglycoproteins as primary receptors for entry. These proteins are abundantly expressed on cell surfaces. Studies of mouse polyoma virus have shown that the virus is able to replicate in at least 40 different cell types.[3] It is likely that the entire particle moves into the nucleus via a nuclear pore. The early proteins expressed (mainly the large T antigen) accumulate and recruit host DNA polymerase to the viral DNA origin. The virus then uses host enzymes to replicate the viral DNA and produce the structural proteins. New viral particles are then formed and leave the cell via a yet to be determined mechanism.

Replication of polyoma viruses is regulated from an enhancer region, which has binding sites for multiple cellular transcription factors, some of which are tissue specific and thought to determine tropism of the virus. For example, Purα is a transcription factor that interacts with the enhancer region for JCV; Purα DNA binding is found only in brain tissues.[4] Polyoma viruses are generally de-

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Table 1 | **Components Encoded by Polyomavirus DNA**

	Function
Structural (late) proteins	
VP1	Major structural protein thought to be a mechanism of entry into the cell. The gene is highly conserved.
VP2	
VP3	
Functional (late) proteins	
Agnoprotein	May modify large T antigen function in JCV
Functional (early) proteins	
Large T antigen	Share ~70% amino acid identity between three viruses
Small T antigen	Studied mainly in simian virus 40 (SV40)
17kT"	Found experimentally in SV40; relevance unknown
T'_{135}, T'_{136}, T'_{165}	Defined in JCV as products of further processing of viral transcripts; may complement or modulate large T antigen function
T' in BKV	Described in BKV, but relevance not fully defined

Table 2 | **Proposed Mechanisms of Tumor Induction by T Antigens**

Antigen	Function	Outcome
Large T	Binds pRb family proteins via LXCXE	Increase in cell proliferation
Large T	Binds hsc70 molecular chaperone protein affecting degradation of pRb family proteins via J domain	Increase in cell proliferation
Large T	Binds p53 tumor suppressor proteins	Blockade of apoptosis
Large T	Damages chromosomes in unknown manner	Increase in mutations
Small T	Binds PP2a affecting the cell cycle	Increase in cell proliferation under certain conditions

scribed as archetypal or rearranged, depending on the structure of the transcriptional control region. Archetypal virus contains a single copy of the promoter and enhancer, whereas rearranged strains have significant variation in this region.[5,6] The differences between the strains (i.e., duplications of the promoter or enhancer) may help to explain the variability of viral replication and why some strains are virulent but others are not. Nonarchetypal virus will often have duplication of the enhancer region, which promotes viral growth. However, both archetypal and rearranged strains are found in normal and diseased human tissues;[7,8] there is no obvious association between DNA structure and disease. Host factors likely play a role in this regard. It is possible that inefficient replication of archetypal virus may not elicit a strong immune response, thus creating a low-level persistent infection.

The oncogenic potential of polyoma viruses is mediated through their T antigens, mainly the large T antigen (LT) (Table 2). LT has three main regions that are central to its ability to transform cells. Two of these regions alter the retinoblastoma susceptibility (pRb)–related tumor suppressor proteins found in normal cells. pRb normally binds to transcription factor E2F. The pRb family–E2F complexes repress the transcription of genes involved in S phase progression and DNA synthesis, and free E2F activates these genes. When pRb is phosphorylated, free E2F is released and activates genes, which lead to the progression of the cell cycle and growth.[9-11] BKV T antigen stimulates the premature release of free E2F from pRb complexes by binding to the hypophosphorylated form of pRb.[12] Near the N terminal of LT is the J domain, which by an independent mechanism mediates degradation of pRb family protein complexes. Another domain is also located in the N terminal region and binds the pRb complexes.[12-14] A third domain of LT binds to p53,[15] whose normal function is to detect DNA damage in the cell and either pause the cell cycle or activate an apoptotic pro-

gram. There has also been a suggestion that LT can damage the human chromosome in another as yet unspecified way.[16]

There are some differences between the polyoma viruses and the function of the large T antigen. SV40 LT reaches higher concentrations compared to BKV—and thus the binding of the pRb sites is more prominent in SV40—whereas BKV affects this system mainly via the J domain. JCV LT has not been studied to such a great extent, but it is believed to function in a similar manner.

Small T antigen (ST) has been studied in SV40 oncogenesis. ST shares a common N terminus with LT but has a unique C-terminal end. This protein has been shown to bind and inhibit cellular protein phosphatase 2A,[17] which regulates signal transduction pathways in the cell. ST plays a complementary role in cell transformation, and cells infected with SV40 strains that produce ST are more easily transformed and produce a wider range of tumors compared to cells infected with ST-deficient mutants.[18,19] It has been suggested that ST may help transform cells during times of stress, such as nutrient starvation, which would normally suppress proliferation.[20] ST has not been studied extensively in JCV or BKV, but there is some sequence homology with SV40 that may infer a similar mechanism of action.

Alternative splicing of T antigen transcripts gives rise to other smaller proteins that share identity with LT. These proteins have been studied in JCV and are designated T'. These T' proteins share the amino terminus with LT; the J domain and the LXCXE domain are preserved and have been shown to contribute to JCV replication.[21] T' proteins may have other roles besides complementing cell transformation—it has been suggested that these proteins may function in JCV similarly to ST in SV40. These smaller protein products have also been found in BKV-infected cells[15] but as of yet have not been further characterized.

Clinical Characteristics and Association with Human Cancer

BK Virus

BKV was first isolated from the urine of a renal transplant recipient in 1971.[22] The seroprevalence of this entity has been measured in early childhood at a range from 60% to 100%.[23,24] The mode of transmission is not fully understood, but several mechanisms have been proposed. These include respiratory, body fluids (semen, urine, and blood) and transplacental.[25-27] BKV can remain latent in a variety of tissues—the most commonly reported are kidney and brain. There is also some evidence of dormant BKV in leukocytes, lung, liver, bone, and reproductive tissue.[25,26,28,29] This virus may cause primary or reactivation disease in many sites, including the brain, liver, eye, lung, and kidney. Pathological lesions are generally seen in immunocompromised individuals, with most of attention paid to transplant patients.[30-33] Urinary tract pathology is the most common sequelae of active BKV infection, including hemorrhagic cystitis,[31] ureteric stenosis,[32] and interstitial nephritis.[33] These entities have been reported primarily in the transplant literature and may be worsened with higher degrees of viral replication. A study of bone marrow transplant patients showed that hemorrhagic cystitis was associated with increased BKV viruria.[34] Rarely, cases of meningoencephalitis, pneumonitis, and retinitis have also been attributed to BKV. There is no specific treatment aside from an attempt to improve the host immune response in affected patients. There have been several different strains of BK virus isolated, which may represent geographic differences as well as differences in oncogenic potential.

The ability to detect viral DNA in tumor specimens has become more precise over the past two decades, which may explain discrepancies between reports of BKV in tumors from previous studies. BKV DNA has been isolated from many different types of human tumors, both integrated into the genome and episomally. Sites include bone, pancreatic islet cells, kidney, urinary tract, and a wide variety of brain tissues.[28,35-41] Some of the larger studies show data as follows (Fig. 3): BKV has been detected episomally in 4 of 9 tumors of pancreatic islet cells and 19 of 74 brain tumors via Southern blot.[38] Further work with polymerase chain reaction (PCR) on a large group of tumor cells as well as normal tissue demonstrated BKV early DNA encoding LT in 58 of 68 of brain tumors, 21 of 27 osteosarcomas, and 5 of 13 Ewing's tumors. The same

Table 3 | **Evidence of BKV in Human Tumors and Tumor Cell Lines**

Site	Evidence	Method	Reference
Central Nervous System			
Neuroblastoma	18/18 + DNA	PCR	36
	16/18 + LT	Immunohistochemistry	36
	4/5 + DNA	PCR	28
	2/6 + DNA	Southern blot	35
Astrocytoma	7/7 + DNA	PCR	28
	2/11 + DNA	Southern blot	38
Papilloma	6/6 + DNA	PCR	28
Ependymoma	10/11 + DNA	PCR	28
	1/3 + DNA	Southern blot	38
	2/3 + DNA	Southern blot	35
Glioblastoma	19/22 + DNA	PCR	28
	4/4 + LT mRNA	RT-PCR	28
	9/18 + DNA	Southern blot	38
	1/10 + DNA	Southern blot	35
	1/5 + DNA	Southern blot	41
Meningioma	5/8 + DNA	PCR	28
	1/1 + LT mRNA	RT-PCR	28
	2/20 + DNA	Southern blot	38
	2/2 + DNA	Southern blot	35
	5/6 + DNA	Southern blot	41
Oligodendroglioma	5/7 + DNA	PCR	28
	1/1 + DNA	Southern blot	38
	1/1 + DNA	Southern blot	41
	2/2 + DNA	PCR	28
Spongioblastoma	1/3 + DNA	Southern blot	38
Bone			
Giant cell			
Ewing's tumor	5/5 + DNA	PCR	28
	5/13 + DNA	PCR	28
	5/5 + LT mRNA	RT-PCR	28
Osteosarcoma	1/5 + DNA	Southern blot	35
	21/27 + DNA	PCR	28
	9/14 + LT mRNA	RT-PCR	28
Kidney/urinary tract			
Carcinomas	31/52 + DNA	PCR	37
	1/1 + DNA	Southern blot	39
Insulinoma	1/1 + DNA	Southern blot	40
	4/9 + DNA	Southern blot	38

sequences were found in 13 of 13 of normal brain tissue, 2 of 5 normal bone tissue, and 25 of 35 peripheral blood cells.[28] RT-PCR showed that most of these samples also expressed LT mRNA as well. A study of 18 cases of neuroblastoma showed that all 18 contained BKV DNA, and 16 of 18 expressed LT—compared to none in the controls (adrenal medulla). These samples were further studied with immunoprecipitation to demonstrate LT binding with p53.[26] In Italy, a strain of BKV, designated URO1, was identified in the examination of several types of urinary tract tissues.[37] The prevalence of viral DNA was similar between neoplastic (31/52) and nonneoplastic (21/37) samples. However, BKV DNA sequences were present in sufficient amounts to be detected by Southern blot in a portion of uri-

Table 4 | **Evidence of JCV in Human Tumors and Tumor Cell Lines**

Site	Evidence	Method	Reference
Central Nervous System			
Medulloblastoma	22/23 + DNA	PCR	55
	4/16 + LT	Immunohistochemistry	55
	17/20 + DNA	PCR	56
	9/20 + LT	Immunohistochemistry	56
	11/20 + agnoprotein	Immunohistochemistry	56
	26/35 + DNA	PCR	58
Astrocytoma	14/40 + LT	Immunohistochemistry	58
	5/6 + DNA	PCR	58
Ependymoma/subependymoma	5/6 + LT	Immunohistochemistry	58
	13/22 + DNA	PCR	58
Glioblastoma/gliosarcoma	6/27 + LT	Immunohistochemistry	58
	4/7 + DNA	PCR	58
Oligodendroglioma	2/10 + LT	Immunohistochemistry	58
	1/131 + DNA	PCR	43
Meningioma	12/46 + DNA (normal/cancer matched pairs)	PCR	48
Colon	48/54 + DNA (normal/cancer matched pairs)	PCR with topoisomerase	48

nary tract tumors but not in that of normals. The DNA material appeared to be integrated into the host cell genome.

The causal link between BK virus and cancer has not been established. Some studies have looked for BKV DNA in tumors and have not found it.[42,43]

JC Virus

The first report of JCV isolation was in 1971 from a patient who was suffering from progressive multifocal leukoencephalopathy.[44] The virus infects more than 70% of humans during childhood and adolescence.[45] Like BKV, the mode of transmission is not fully understood, although JCV is easily retrievable from human sewage. Using DNA analysis of different strains of JCV, it has been suggested that the intake of water or food may be a primary portal of entry and that parent-to-child transmission is a common occurrence.[46,47] JCV commonly resides in the kidney and may also be found in lymphoid tissue and gastrointestinal tissue.[48,49] Primary infection is generally asymptomatic. Reactivation of disease in immunosuppressed hosts is thought to be the cause of progressive multifocal leukoencephalopathy, a fatal demyelinating condition. Most of the evidence for the oncogenic potential of JCV has been in experimental models: brain tumors in owl monkeys and Golden Syrian hamsters.[50,51] Several years ago, case reports began appearing of JCV DNA detected in brain tumors.[52-54]

Recently, there have been more reports linking the virus with cases of human medulloblastoma as well as other brain tumors (Fig. 4). In 1999, 23 cases of pediatric medulloblastomas were evaluated for evidence of JCV;[55] 20 of 23 were positive for DNA encoding the N-terminal region of LT, 13 of 23 contained C-terminal sequences, 20 of 23 had VP1 sequences, and 11 of 23 had all three sequences present. Further analysis showed that 4 of 16 available samples had detectable T antigen by immunohistochemistry. SV40 DNA was also detected in 5 of 23 of these samples, but BKV DNA was not found. Other experiments have looked for late JCV gene products (agnoprotein) in 20 medulloblastoma specimens.[56] The function of agnoprotein is not clear at this time, but there is some evidence that it may have a complementary role in replication of the viral genome.[57] DNA sequences for LT were found in 13 of 20 specimens and sequences for agnoprotein in 11 of 16 specimens. Immuno-

histochemistry revealed LT in 9 of 20 and agnoprotein in 11 of 20 specimens. When different varieties of brain tumors were analyzed (including oligodendroglioma, astrocytoma, glioblastoma, gliosarcoma, ependymoma, and subependymoma—a total of 85 specimens), all types of brain tumors showed evidence of early JCV DNA sequences, ranging from 57% to 83%.[58] T antigen was also found in all varieties of tumors except anaplastic astrocytoma, to a lesser extent. None of the samples showed immunohistochemical evidence of VP1. JC virus has also been associated with nonneurologic disease. Studies of colorectal cancer and matched normals found that 12 of 46 samples contained JCV DNA.[48] When another 25 pairs were analyzed after treatment with topoisomerase, 48 of 54 samples tested positive for JCV by PCR. Viral DNA sequences were also detected in a human colorectal cancer cell line and in colon cancer xenografts. Data from this study also suggest that JCV DNA is present at 10 times higher numbers in cancerous tissue compared to the paired normal tissue.

SV40 Virus

Although similar to JCV and BKV, SV40 is not a human polyomavirus. In its natural hosts, SV40 is thought to be transmitted via urine, respiratory, oral, and subcutaneous routes.[59] It is of interest in human disease because it has been estimated that millions of people in the United States were exposed to SV40 via contaminated virus vaccines (mainly polio) from 1955 to 1963, and SV40 is thought to be transmitted between humans. These vaccines were prepared from infected rhesus monkey kidney cells, some of which had been infected with SV40. Over the next 20 years, evidence had not shown any increase in disease or cancer in this population,[60] and the concern waned. Recently, however, there has been more evidence that may link SV40 with human tumors; some have questioned whether the initial studies were correct in stating that there was no increase in incidence of human cancer.[61] Serologic surveys found evidence of SV40 antibodies in a significant number of individuals too young to have received contaminated vaccines.[62] It follows that there is an alternative route of infection—likely human to human as there are no known intermediate reservoirs or vectors. It is possible that SV40 has been a low-level human pathogen all along or that there is some cross-reactivity with other polyomavirus antibodies. However, there is in vitro evidence that SV40 can replicate in human cells, and SV40 DNA sequences have been detected in normal human tissue.[63,64]

Unlike its cousins, SV40 DNA has been reported in numerous human tumors, mainly mesotheliomas and brain and bone tumors. A large focus of study has been the association of SV40 and malignant mesothelioma. Several investigators have found evidence of the virus in this cancer; prevalence ranges from 40% to 50% in some areas.[65-68] Viral components have been detected with microdissection of tumor tissue[67] and with in situ hybridization.[66] There has also been some evidence that the presence of SV40 may have a negative impact on prognosis in mesothelioma.[69] There is geographic variability in these findings; SV40 DNA was not found in mesothelioma samples from Finland or Turkey.[70,71] Contaminated polio vaccines were not distributed in these countries. Despite these data, the relationship between SV40 infection and mesothelioma is not defined, and some investigators believe it is premature to link the two.[72] A multicenter study formed specifically to address these issues did find evidence of SV40 in 83% of samples.[68]

Studies have shown SV40 LT DNA in approximately 30% of bone tumors, including osteosarcoma, giant cell tumors, and various others.[73-75] One report found that as high as 46% of osteosarcomas had viral sequences.[75] Various central nervous system tumors have also shown evidence of viral gene sequences, although at lower levels and with much variability.[43,55,76-79] One of these studies demonstrated LT binding with both pRb and p53.[76]

New studies have examined the correlation of this virus with non-Hodgkin's lymphoma (NHL). Two separate studies found that 43% of NHL patients had SV40 (compared to none in cancer and noncancer controls).[80,81] The sequences were found most commonly in B cell and follicular variants of the disease. EBV was also detected at a significantly lower level, and the association occurred in both HIV-positive and HIV-negative patients.

Conclusion

In this article, we have reviewed the data that link the polyoma viruses with human cancer. There are numerous difficulties in establishing a causal link between polyoma viruses and human cancer. The viruses are ubiquitous, and primary infection is mild; it is often impossible to determine length of infection. The incubation period after initial exposure is very long, and cancers produced are very rare. There may be many other environmental and host factors that play a role in the production of tumor. Studies to this point have demonstrated the presence of these viruses in human tumors, but the causal link has not been proven. Nevertheless, data are accumulating that point to an association of these viruses (especially SV40) and a role in cancer.

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