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Brain tumors and polyomaviruses

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Polyomaviruses, including JC virus (JCV), BK virus (BKV), and simian virus 40 (SV40) have attracted much attention in the past decade due to their repeated isolation from various human tumors, including those originating from the central nervous system (CNS). JCV and BKV are considered to be ubiquitous human pathogens that become reactivated under impaired physiological conditions such as immunosuppression. Productive replication of JCV and BKV induces diseases such as progressive multifocal leukoencephalopathy in the brain and hemorrhagic or nonhemorrhagic cystitis and nephritis in the kidney. JCV DNA sequences have been isolated from a number of human CNS tumors, including medulloblastoma, ependymoma, and a broad range of glial-origin neoplasms. SV40, once believed to be a monkey virus, has now been isolated from a variety of human cancer cells, including mesothelioma, ependymoma, and non-Hodgkin's lymphoma. In this mini-review, the authors focused their attention on the possible involvement of polyomaviruses, such as JCV, BKV, and SV40, with human brain tumors. *Journal of NeuroVirology* (2003) **9,** 173–182.

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Introduction

Brain tumors

New cases of brain tumors in humans are diagnosed annually in approximately 1 in 9000 people. In the adult population, they account for 9% to 10% of all primary tumors. In children, the annual incidence of tumors of the central nervous system (CNS) is about 3.7 cases per 100,000, making it the second most common neoplastic disease in the pediatric population.

Classification of brain tumors is based on our current understanding of the cellular development of the nervous system. Neuronal precursors give rise to immature neoplasms known as primitive neuroectodermal tumors (PNETs), whereas glial precursors give rise to a wide range of tumors from various specialized cellular subsets, including ependymocytes, astrocytes, and oligodendrocytes. The neural crest gives rise to the cells that form the peripheral nervous system and the meningeal coverings of both central and peripheral structures. In the peripheral nervous system, neural crest–derived tumors include schwannomas and neurofibromas, whereas the arachnoidal cells of the meninges give rise to meningiomas.

The major factors in disease severity, due to their location, are that brain tumors produce both focal and generalized disabilities. Many brain tumors, including gliomas, tend to widely infiltrate the CNS, making surgical cure virtually noneffective. In most cases, treatments are only palliative and long-term survival with most tumors is limited to a few years (Greenberg *et al*, 1999).

Our present knowledge of the causes of these tumors is limited. From an environmental perspective, ionizing radiation is an established risk factor, particularly at high doses. Nonionizing radiation may also be a risk factor as well, as attested to by an increased risk for brain tumor development in individuals who are routinely subjected to high electrical current flow. Chemical agents that have been implicated in brain tumorigenesis include *N*-nitroso compounds, tobacco, alcohol, and vinyl chloride. Occupational risk appears to be elevated for electricians,

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petrochemical workers, rubber tire fabricators, aircraft pilots, farmers, and health professionals. The degree to which these factors account for the total number of human brain tumors is not known but appears to be small (Davis and Preston-Martin, 1998).

An increase in brain tumors within families and the association of brain tumors with known genetic syndromes suggests a hereditary role for the development of brain tumors. Heritable syndromes with a predisposition for nervous system tumors include Turcot's syndrome, which is associated with astrocytomas, glioblastomas, and medulloblastomas; Gorlin's syndrome associated with medulloblastomas; neurofibromatosis type 1 associated with astrocytomas and optic nerve gliomas; and neurofibromatosis type II associated with multiple schwannomas, meningiomas, and neurofibromas. The relative risk of brain tumors in first and second degree relatives of affected patients may be as high as 8.9% (Bondy *et al*, 1994). Inherited predisposition may also account for 5% of all pediatric brain tumors (Bondy *et al*, 1991). Cytogenetic and molecular studies have identified numerous markers for brain tumors and implicated the involvement of specific pathways such as the p53/MDM2 pathway. Mutations in p53, the basis of the Li-Fraumeni syndrome, are also found in one-third of astrocytomas. The gene for MDM2, which interacts with p53, is amplified in 10% of astrocytomas. Similarly, unraveling Turcot's syndrome has placed emphasis on the wingless pathway leading to a mutated APC (adenomatous polyposis coli) gene, Gorlin's syndrome on the hedgehog/patched signaling pathway, and neurofibromatoses I and II on the gene products neurofibromin and merlin, respectively (Biegel, 1999; Nozaki *et al*, 1999).

Several studies have reported an increased risk of childhood brain tumors in individuals whose mothers were infected with various viruses during pregnancy (Adelstein and Donovan, 1972; Linet *et al*, 1996). One particular area of inquiry into the possibility of an infectious etiology of brain tumors comes from studies on polyomaviruses due to their established ability to transform cells *in vitro* and to induce tumors in experimental animals (Butel, 1997; Khalili, 2001).

Polyomaviruses

Human polyomaviruses, which include the well-studied simian virus 40 (SV40), JC virus (JCV), and BK virus (BKV), are icosahedral nonenveloped DNA viruses with capsid diameters of approximately45 nm. The genome consists of covalently bound, double-stranded, circular supercoiled DNA with an average length of 5 kb. The polyomaviral genomes have common structural features consisting of a noncoding regulatory region, an early region encoding the regulatory protein, T antigen, and its various isoforms, and a late region that encodes for the structural capsid proteins (Frisque and White, 1992).

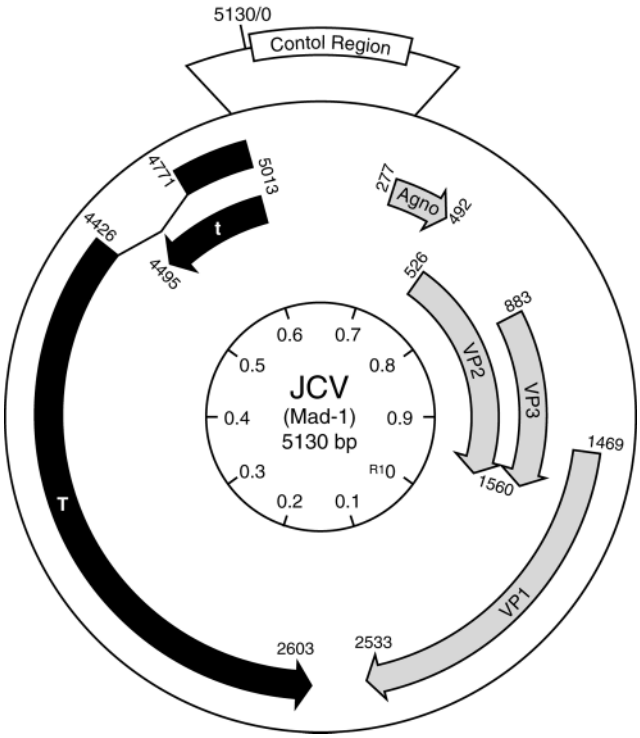

Figure 1 Organization of the polyoma genome. The genome of JCV is schematized as an exemplar. The circular genomes of all polyoma viruses are similar, with three general regions identified based on function. These comprise the regulatory (noncoding) region, the early coding region and the late coding region.

The regulatory region separates the coding regions and contains sequences that are necessary for the initiation of viral gene transcription and viral DNA replication. Transcription of the early and late genes proceeds in opposite directions around the viral DNA. Figure 1 illustrates the structural organization of the human neurotropic polyomavirus JCV genome.

In humans, JCV causes the subacute fatal CNS demyelinating disease progressive multifocal leukoencephalopathy (PML) due to productive infection of oligodendrocytes (for details, see the article by Seth *et al* [2003] in this issue). Although this rare syndrome was originally described in patients with systemic immunosuppression due to lymphoproliferative and myeloproliferative disorders and/or chemotherapy, it has subsequently been recognized as a concomitant of human immunodeficiency virus (HIV)-1 infection, accounting for a distinct rise in the incidence of PML in the acquired immunodeficiency syndrome (AIDS) population (Berger *et al,* 1998). BKV most commonly causes a self-limited hemorrhagic cystitis during pregnancy and has also been associated with urinary tract, respiratory tract, and meningeal inflammatory diseases in children, HIV-1–infected individuals, and transplant recipients (Arthur *et al*, 1986). SV40, although originally classified as a monkey polyomavirus, has recently been shown to infect humans as well. Serological

surveillance of worldwide populations has led investigators to conclude that primary human polyomavirus infections are extremely common, occur during youth, and are often subclinical. Following acute infection, viral persistence over prolonged intervals has been demonstrated in brain, lung, kidney, bone, and blood by a variety of techniques, including Southern blotting and polymerase chain reaction (PCR) (Arthur *et al*, 1989).

These findings have suggested that many symptomatic polyoma infections represent reactivation in immunocompromised carriers and that the conditions under which these occur alter the ability of the organism to abrogate viral reproduction. Support for this notion stems from sequence analysis of JCV and BKV isolates. The regulatory regions of archetype strains cloned from healthy, asymptomatic individuals lack the large tandem repeats found in many of the isolates from symptomatic patients. This suggests an adaptation of the virus, including spontaneous alterations of the regulatory region following an initial infection with an archetype strain. This adaptation may allow for expression in tissue types such as brain or the urogenital tract under immunosuppressive conditions, which result in manifest pathologies such as PML and cystitis (Frisque and White, 1992).

Polyomaviruses and human brain tumors

The initial evidence that human brain tumors might be associated with polyoma virus infection came from retrospective analyses of tumor incidence versus SV40 polio vaccine contamination (Farwell *et al*, 1984, Heinonen *et al*, 1973). Although this hypothesis remains controversial, the rationale for a possible link emerged as a result of two concurrent research projects. During the initial manufacture of the Salk polio vaccine from 1954 to 1960, the vaccine was grown on monolayers of rhesus monkey kidney cells, which were then treated with graded concentrations of formalin to inactivate virus infectivity while preserving antigenicity. Following the isolation of the SV40 virus in 1960, there was fairly rapid recognition of SV40 contamination of these lots because SV40 is found frequently in rhesus monkeys but is more resistant to formalin inactivation than polio. Concurrent with these studies were observations that SV40 could cause tumors when inoculated into animal species that were not the natural host. The range of tumors in these initial experiments included ependymomas produced by intracranial injections (Rabson *et al*, 1962) and sarcomas from intramuscular inoculations (Eddy *et al*, 1961). Although the administration of SV40-contaminated polio vaccine was eliminated by 1961, it is not known how many of the 90 million people who were given the Salk vaccine were exposed to SV40. Estimates of between 10 and 30 million people have been made (Shah and Nathanson, 1976).

Along with the epidemiologic evidence, case reporting and pathologic analyses initially strengthened the association of polyomaviruses with human CNS tumors as summarized in Table 1. The first evidence for polyoma involvement in choroid plexus tumors came from the electron microscopic observation of viral particles most consistent with polyoma virus in surgically excised papilloma from a 33-year-old woman (Bastian, 1971). Gliomas and CNS lymphomas coexisting in the brains of patients with PML provided a link between JCV and these tumors (Castaigne *et al*, 1974; GiaRusso and Koeppen, 1978). In particular, the histopathology of PML is suggestive of astrocytic neoplasias in that the "bizarre astrocytes" typically observed in PML lesions are thought to resemble the transformed cells of glioblastoma. Although JCV infection of oligodendrocytes that express both T antigen and capsid protein is lytic, astrocytes may be non- or semipermissive for JCV. In this setting, expression of T antigen in the absence of lytic infection may alter the cell cycle, resulting in tumors of astrocytic origin.

SV40 detection in human brain tumors

Using anti-SV40 T-antigenantibody, the polyomavirus early protein, T antigen, was first observed in human brain tumors by immunohistochemical analysis (Tabuchi *et al*, 1978). Further investigation using Southern blotting revealed the presence of episomal SV40 DNA in human CNS tumors (Krieg *et al*, 1981). This has subsequently been confirmed in studies employing PCR amplification of archival specimens (Huang *et al*, 1999; Weggen *et al*, 2000). Similar sequences have also been detected in a more mature neuronal tumor, the ganglioneuroma (Lednicky *et al*, 1995). Historically, the molecular evidence for SV40 in human glial tumors has been investigated most extensively in ependymal tumors. Both Southern blotting and PCR amplification have been used to analyze these tumors and demonstrate a large number with sequences specific for SV40 and JCV (Bergsagel *et al*, 1992; Huang *et al*, 1999; Lednicky *et al*, 1995; Martini *et al*, 1996). An initial analysis of amplified SV40 sequences revealed an archetype arrangement of the enhancer region (Lednicky *et al*, 1995). This finding raised the possibility that the archetype sequence might confer some degree of tissue specificity and increase the likelihood of tumor formation. A more intensive analysis performed on a number of these cases from which full-length polyoma sequences could be amplified suggests that SV40 has a relatively broad host and tissue range and that the archetype sequence can be found in both tumorous and nontumorous sources (Stewart *et al*, 1998). These studies and others demonstrate SV40 sequences in other glial tumors, including choroid plexus tumors, low grade astrocytomas, malignant astrocytomas, and oligodendrogliomas (Bergsagel *et al*, 1992; Huang *et al*, 1999; Kouhata *et al*, 2001; Kreig *et al*, 1981; Krieg and Scherer 1984; Lednicky *et al*, 1995, Malkin *et al*, 2001; Martini *et al*, 1996; Meinke *et al*, 1979). Furthermore, SV40 sequences have been detected in meningiomas, which are

Table 1 Association of human brain tumors with polyomaviruses

Polyomavirus	*Tumor histology*	*References*	*Methodologies employed*
SV40	Astrocytoma	Huang *et al*, 1999	PCR
		Kreig *et al*, 1981	Southern blot
		Kreig and Scherer 1984	Sequencing
		Martini *et al*, 1996	PCR
	Anaplastic astrocytoma	Huang *et al*, 1999	PCR
	Gemistocytic astrocytoma	Huang *et al*, 1999	PCR
	Glioblastoma	Kouhata *et al*, 2001	PCR
		Martini *et al*, 1996	PCR
		Meinke *et al*, 1979	Southern blot
	Giant cell glioblastoma	Huang *et al*, 1999	PCR
	Gliosarcoma	Huang *et al*, 1999	PCR
	Oligodendroglioma	Huang *et al*, 1999	PCR
		Kreig *et al*, 1981	Southern blot
	Ependymoma	Bergsagel *et al*, 1992	PCR
		Huang *et al*, 1999	PCR
		Lednicky *et al*, 1995	PCR and sequencing
		Martini *et al*, 1996	PCR
		Weggen *et al*, 2000	PCR
	Malignant ependymoma	Tabuchi *et al*, 1978	IHC
	Subependymoma	Weggen *et al*, 2000	PCR
	Choroid plexus papilloma	Bergsagel *et al*, 1992	PCR
		Lednicky *et al*, 1995	PCR and sequencing
		Malkin *et al*, 2001	PCR and IHC
		Martini *et al*, 1996	PCR
		Tabuchi *et al*, 1978	IHC
	Choroid plexus carcinoma	Lednicky *et al*, 1995	PCR and sequencing
		Malkin *et al*, 2001	PCR and IHC
	Meningioma	Kreig *et al*, 1981	Southern blot
		Kreig and Scherer, 1984	Sequencing
		Martini *et al*, 1996	PCR
		Weggen *et al*, 2000	PCR
	Medulloblastoma	Kreig *et al*, 1981	Southern blot
		Weggen *et al*, 2000	PCR
	Ganglioneuroma	Lednicky *et al*, 1995	PCR and sequencing
JCV	Astrocytoma	Caldarelli-Stefano *et al*, 2000	PCR and IHC
		Del Valle *et al*, 2001	PCR and IHC
	Anaplastic astrocytoma	Del Valle *et al*, 2001	PCR and IHC
	Glioblastoma	De Valle *et al*, 2000	PCR and IHC
		Del Valle *et al*, 2001	PCR and IHC
		Del Valle *et al*, 2002	PCR and IHC
	Gliosarcoma	Del Valle *et al*, 2001	PCR and IHC
	Pilocytic astrocytoma	Del Valle *et al*, 2001	PCR and IHC
	Pleomorphic xanthoastrocytoma	Boldorini *et al*, 1998	PCR
	Oligodendroglioma	Caldarelli-Stefano *et al*, 2000	PCR and IHC
		Del Valle *et al*, 2001	PCR and IHC
	Anaplastic oligodendroglioma	Del Valle *et al*, 2001	PCR and IHC
	Oligoastrocytoma	Del Valle *et al*, 2001	PCR and IHC
		Rencic *et al*, 1996	PCR, IHC, and Western blot
	Ependymoma	Caldarelli-Stefano *et al*, 2000	PCR and IHC
		Del Valle *et al*, 2001	PCR and IHC
	Subependymoma	Del Valle *et al*, 2001	PCR and IHC
	Meningioma	Weggen *et al*, 2000	PCR
	Medulloblastoma	Del Valle *et al*, 2002	PCR and IHC
		Khalili *et al*, 1999	PCR and IHC
		Krynska *et al*, 1999	PCR and IHC
	Gliomatosis cerebri	Del Valle *et al*, 2001	PCR and IHC
BKV	Astrocytoma	Corallini *et al*, 1987b	Southern blot and sequencing
	Glioblastoma	Corallini *et al*, 1987b	Southern blot and sequencing
		Dörries *et al*, 1987	Southern blot
	Oligodendroglioma	Corallini *et al*, 1987b	Southern blot and sequencing
		Dörries *et al*, 1987	Southern blot
	Ependymoma	Corallini *et al*, 1987b	Southern blot and sequencing
	Meningioma	Corallini *et al*, 1987b	Southern blot and sequencing
		Dörries *et al*, 1987	Southern blot
	Schwannoma	Corallini *et al*, 1987b	Southern blot and sequencing
		Dörries *et al*, 1987	Southern Blot

Each human polyomavirus has been associated with human tumors, particularly those originating in the CNS. The positive published findings for human brain tumors are enumerated in this table by virus, tumor type, and the detection methods that have been employed successfully.

believed to originate from cells of neural crest origin (Kreig *et al*, 1981; Krieg and Scherer, 1984; Martini *et al*, 1996; Weggen *et al*, 2000).

BKV detection in human brain tumors
In two separate studies (Corallini *et al*, 1987b; Dörries *et al*, 1987), BKV sequences have been detected in ependymomas, astrocytomas, both benign and malignant, oligodendrogliomas, meningiomas, and schwannomas. Additional research has detected BKV in more than 85% of CNS cell lines (De Mattei *et al*, 1995).

JCV detection in human brain tumors
Investigations of JCV in human brain tumors have employed molecular amplification of the viral DNA and examination of viral gene expression. In a set of 23 primitive neuroectodermal origin tumors, i.e., medulloblastomas, 20 demonstrated N-terminal T-antigen sequences, 13 contained C-terminal T-antigen sequences, and 20 had sequences from the VP1 region (Khalili *et al*, 1999; Krynska *et al*, 1999a). In a subset of 16 of these tumors, further PCR analysis demonstrated sequences that code for the viral accessory protein, Agnoprotein. Immunohistochemistry has demonstrated T antigen in the nuclei of 25% of these tumors. Surprisingly, the Agnoprotein is detectable in the perinuclear cytoplasmic compartment in 55% of cases, frequently in the absence of T antigen. The VP late proteins were not detected in any of these cases (Del Valle *et al*, 2002). As with SV40, JCV sequences have been detected in a variety of both low- and high-grade glial tumors, including those of ependymal, astrocytic, and oligodendroglial origin (Boldorini *et al*, 1998; Caldarelli-Steffano *et al*, 2000; Del Valle *et al*, 2000, 2001; Rencic *et al*, 1996). In a survey of 85 glial tumors conducted in our laboratory, JCV early sequences were detected in 69% of samples by PCR and 33% of the tumors showed immunohistochemical evidence of T-antigen expression (Del Valle *et al*, 2001). As in the case of SV40, JCV sequences were detected in meningiomas as well (Weggen *et al*, 2000).

Tumors induced in experimental animal models
Since the initial descriptions of SV40-induced tumors (Eddy *et al*, 1961; Rabson *et al*, 1962), a body of work has accumulated in a variety of experimental systems, expanding the role of polyomavirus in CNS tumors, as summarized in Table 2. Inoculation studies with SV40 produced lymphoma, leukemia, and sarcomas with intravenous injections of virus (Diamandopoulos, 1972). Mesotheliomas have resulted from intrapleural, intraperitoneal, and intracardiac injections of virus (Rizzo *et al*, 2001). BKV is only weakly oncogenic when inoculated into rodents subcutaneously but induces tumors in 70% to 80% of hamsters when injected intracerebrally or intravenously. Although the majority of tumors are ependymomas, pancreatic islet tumors, and osteosarcomas, occasional neuroblastomas, pineal tumors and fibrosarcomas are produced as well. Mice and rats develop a slightly different spectrum of tumors characterized by fibrosarcoma, liposarcoma, osteosarcoma, nephroblastoma, glioma, and choroid plexus papilloma (Corallini *et al*, 1987a; Dougherty, 1976; Shah *et al*, 1975; Uchida *et al*, 1979; Watanabe *et al*, 1982). JCV has been similarly implicated in experimental tumorigenesis of the nervous system. Inoculation of JCV intracerebrally into newborn Golden Syrian hamsters produces CNS tumors in more than 85% of the animals. The most common tumors found are primitive neuroectodermal tumors (PNETs), including medulloblastomas and pineocytomas (ZuRhein, 1983). Astrocytomas, glioblastomas, and peripheral neuroblastomas are

Table 2 Experimental brain tumors produced by human polyomavirus inoculation

Polyomavirus	*Species*	*Tumor histology*	*References*
SV40	Hamster	Ependymoma	Eddy *et al*, 1962
	Rat	Ependymoma	Rabson *et al*, 1962
	Mouse	Choroid plexus papilloma	Brinster *et al*, 1984; Van Dyke *et al*, 1987
JCV	Monkey	Astrocytoma	London *et al*, 1978
		Glioblastoma multiforme	London *et al*, 1978
	Hamster	Astrocytoma	ZuRhein, 1983
		Ependymoma	ZuRhein, 1983
		Medulloblastoma	ZuRhein, 1983
		Pineocytoma	ZuRhein, 1983
		Primitive neuroectodermal	ZuRhein, 1983
	Rat	Primitive neuroectodermal	Ohsumi *et al*, 1985
	Mouse	Adrenal neuroblastoma	Small *et al*, 1986; Franks *et al*, 1996
		Medulloblastoma	Krynska *et al*, 1999b
		Malignant peripheral nerve sheath	Gordon *et al*, unpublished observations
		Pituitary adenoma	Gordon *et al*, 2000
BKV	Hamster	Ependymoma	Corallini *et al*, 1987a; Dougherty 1976; Uchida *et al*, 1979; Watanabe *et al*, 1982
	Mouse	Ependymoma	Corallini *et al*, 1987a

The human polyomaviruses SV40, JCV, and BK have each produced a spectrum of brain tumors that are tabulated here.

also commonly seen in the hamster model. Of interest, JCV is the only human virus known to induce solid tumors in nonhuman primates, as owl and squirrel monkeys injected intracerebrally with JCV develop astrocytomas (London *et al*, 1978).

In these experimental animals, T antigen can be detected in the absence of viral capsid protein expression (Frisque and White, 1992). Although SV40 has been isolated from transformed cells (Soriano *et al*, 1974), there has been only one report to date of infectious JCV recovery from an experimental animal (Major *et al*, 1987). In this instance, a JCV-induced glioblastoma detected in an owl monkey was cocultured with permissive human fetal glial cells, resulting in virion production. The inference drawn from these studies is that many tissue types may be infected with JCV, and their intracellular environments may permit early but not late gene expression, therefore not allowing the production of complete virus. T-antigen expression in the absence of late gene products may result in cell cycle dysregulation without cellular lysis leading to the expansion of these infected cells and their resulting transformation.

Further evidence for the direct association of T antigen with tumors of the nervous system emerges from studies of transgenic mice containing the polyoma early but not late genes. These animals constitutively produce early proteins under the control of the natural viral promoter/enhancer region in the absence of the influence of any late genes or gene products. Due to restrictions in lytic infection believed to occur upon infection of human polyomaviruses in rodent models, the use of early gene constructs lacking the late gene sequences may recapitulate the situation seen in abortive infections in human. SV40 transgenic mice have developed choroid plexus papillomas (Brinster *et al*, 1984; Messing *et al*, 1988; Van Dyke *et al*, 1987), whereas JCV transgenic mouse lines have developed medulloblastomas, peripheral neuroectodermal origin tumors including adrenal neuroblastomas and primitive mesenteric tumors, pituitary adenomas, and malignant peripheral nerve sheath tumors (Franks *et al*, 1996; Gordon *et al*, 2000; Gordon *et al*, unpublished observations, Krynska *et al*, 1999b; Small *et al*, 1986).

Mechanisms of polyomavirus-induced CNS tumorigenesis

The evidence from both human and experimental systems suggests that the oncogenecity of polyomavirus T antigens is due at least in part to their ability to bind and functionally inactivate cellular tumor suppressor proteins, including p53, and the retinoblastoma gene product pRb (Dyson *et al*, 1990). These two phosphoproteins normally affect cell growth and differentiation via control of the cell cycle. Deregulation of this function can result in uncontrolled cellular proliferation, setting the stage for tumorigenesis. It is worth noting that p53 was initially characterized by its ability to bind to SV40 T antigen (Levine, 1997) and has been shown to be stabilized by cells transformed by SV40. The association of T antigen with p53 may block the induction of p21/WAF-1, which normally contributes to the G1 stabilization of the cell cycle. Low levels of p21/WAF-1 can release the inhibition on the activity of cyclin:cdk complexes, which leads to phosphorylation of pRb. Whereas in G1-arrested cells, hypophosphorylated pRb binds to the transcriptional regulator E2F-1, hyperphosphorylated pRb cannot complex with E2F-1. The liberation of E2F-1 may also be accomplished by the direct interaction of T antigen with pRb. An increase in the cellular level of E2F-1 may lead to induction of its own gene expression and expression of other S phase–specific promoters such as proliferating cell nuclear antigen (PCNA), promoting entry of cells into S phase.

Although the scheme outlined above has helped us elucidate a large number of pathways involved in polyoma tumorigenesis, other lines of evidence should be considered as well. For instance, SV40 T antigen has been shown to be capable of regulating p53-mediated transcription in the absence of a direct interaction with p53 (Rushton *et al*, 1997). Interestingly, SV40 T antigen interacts with the hyperphosphorylated forms of the pRb family members p130 and p107, rather than with the hypophosphorylated form as in the case of pRb. In addition, T antigen may also change the phosphorylation state of p107 and p130 (Knudsen and Wang, 1998). Furthermore, BKV T antigen has been shown to induce E2F-1 in the absence of T antigen binding to pRb (Harris *et al*, 1998).

The role of two additional signaling mechanism, the Wnt/wingless and insulin-like growth factor I receptor (IGF-IR) pathways, in relation to T antigen–mediated cellular transformation has recently been explored. The Wnt signaling pathway plays a crucial role in a number of developmental processes, including neurogenesis, through a family of short-range signaling molecules (Cadigan and Nusse, 1997). Signaling in this pathway revolves around a number of key molecules, including adenomatous polypopsis coli (APC) and β-catenin. In the absence of Wnt signaling, β-catenin is targeted for rapid degradation by the ubiquitin-proteasome pathway, whereas in the presence of Wnt signaling, β-catenin is hypophosphorylated and stabilized (Staib *et al*, 1996). β-Catenin then accumulates in cells and translocates to the nucleus where it binds to the T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factor. Formation of the β-catenin:LEF complex results in interaction with specific DNA sequences (5′-CCTTTGAACT-3′) positioned in the promoters of Wnt-responsive genes, including cell cycle regulators such as myc and cyclin D, as well as transcription of genes involved in cell growth, including c-myc and cyclin D (Behrens *et al*, 1996). In this respect, β-catenin plays a

central role in regulating this pathway. Somatic mutations in APC, which typically lead to a truncated protein with no regulatory activity, can cause the accumulation of free β-catenin, and mutations in β-catenin itself have also been detected in greater than 4% of medulloblastomas (Huang *et al*, 2000). These mutations may increase the half-life of β-catenin, allowing free β-catenin to translocate to the nucleus. The accumulation of β-catenin has also been associated with mutational inactivation of p53 (Wetmore *et al*, 2001). Of note, in a murine medulloblastoma cell line derived from JCV T antigen–transgenic mice, T-antigen expression was associated with higher levels of cellular β-catenin (Gan *et al*, 2001). These observations suggests that the interaction between T antigen and β-catenin may play an important role in regulating cellular growth *in vitro* and *in vivo*.

The IGF-IR system has been implicated in the growth of normal and transformed cells, acting through its intermediate, insulin receptor substrate-1 (IRS-1). Both human and JCV T antigen–transgenic medulloblastomas grossly over express IRS-1. In addition, when human and transgenic medulloblastoma cell lines are exposed to IGF-I, phosphorylation of IGF-IR and IRS-1 occurs with concomitant cell proliferation. Both of these responses, as well as anchorage-independent growth of T antigen–transformed medulloblastoma cells, are strongly inhibited by a dominant-negative mutant of the IGF-IR (Lassak *et al*, 2002; Wang *et al*, 2001). T antigen may well contribute to these observed effects in that translocation of IRS-1 to the nucleus has been observed in T antigen–positive human medulloblastoma biopsies and in JCV T antigen–transgenic mouse tumors. These results suggest that the IGF-IR system itself and in concert with JCV T antigen may play a role in these tumors.

In addition to cell cycle dysregulation, polyomaviruses may contribute to genetic instability. In support of this notion, SV40 T antigen has been associated with chromosomal instability of human fibroblast cell lines *in vitro* and JCV infection with chromosomal abnormalities *in vivo* (Neel *et al*, 1996; Ray *et al*, 1990). In addition, abnormal DNA repair has been observed in cells possessing the SV40 origin of replication and a portion of the early genes in the absence of T antigen (Hunter and Gurney, 1994). SV40 small T antigen itself can dysregulate the cell cycle of normal human fibroblasts (Gaillard *et al*, 2001). In more recent studies, an increase in telomerase activity and induction of Notch-1 upon infection of cells with SV40 suggest the potential involvement of additional regulatory pathways in response to polyomavirus infection (Foddis *et al*, 2002).

Of interest is the observation that expression of T antigen in human biopsies and tissues from a number of transgenic models is not detected in all tumor cells. Indeed, it is not clear that long-term maintenance of T-antigen expression is required beyond the initiation stage for continued tumor growth and progression to malignancy. In SV40 transgenic mice, T antigen can be detected in tissues prior to the appearance of tumors, whereas only a proportion of preneoplastic foci that express T antigen become full-fledged tumors (Van Dyke *et al*, 1987). In transgenic mice expressing SV40 T antigen under the control of an inducible promoter, silencing T-antigen expression after the creation of hyperplastic foci failed to stop the progress of tumor development, and in at least two different experimental systems involving SV40 and JCV, spontaneous loss of T-antigen has been associated with concomitant mutations in p53 (Ewald *et al*, 1996; Krynska *et al*, 2000; Salewski *et al*, 1999). These observations suggest that T antigen may operate via a "hit-and-run" mechanism whereby T-antigen expression in dysplastic cells or preneoplastic lesions may result in chromosomal instability or gene inactivation at an earlier stage of transformation, rendering T-antigen expression unnecessary for tumor maintenance.

Perspectives

The evidence accumulated over the last 40 years clearly associates polyomavirus infection with brain tumors. The T antigen of polyomaviruses with its transforming ability has been a powerful tool in unraveling several molecular pathways involved in the control of cell proliferation, including the cell cycle, Wnt and IGF signaling pathways, as well as many others. Although laboratory and animal studies have provided crucial information on polyomavirus-mediated tumorigenesis in the brain, the remaining challenge rests on the demonstrations of whether polyomaviruses may function as the etiologic agent of brain tumors in the human population or acts as a cofactor. In any event, due to the ability of viral proteins to deregulate many important biological events that can lead to cancer development, one must take seriously the observed association of this group of viruses with human cancers and put efforts in blocking its direct and/or indirect contributions to cancer development in the brain.

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