

Tumor induction by simian and human polyomaviruses

Review Article

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Abbreviations: BK virus, (BKV); central nervous system, (CNS); CREB-binding protein, (CBP); human immunodeficiency virus, (HIV); insulin-like growth factor receptor, (IGF-IR); JC virus, (JCV); Jun N-terminal kinase, (JNK); Kaposi's sarcoma, (KS); myelin basic protein, (MBP); nuclear localization signal, (NLS); polymerase γ -primase, (Pol γ); progressive multifocal encephalopathy, (PML); proteolipid protein, (PLP); simian virus 40, (SV40)

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Summary

Human [(JC virus, JCV) and BK virus, BKV] and simian virus 40 (SV40) polyomaviruses induce numerous of tumors in experimental animals. In addition, the detection of viral genomes belonging to this group of viruses in a variety of human tumors raises the possibility of the association of the viral oncogenic proteins, large T and small t antigens, in the induction of such tumors. It has been already demonstrated that large T antigen primarily targets two major tumor suppressor proteins, p53 and retinoblastoma gene product, Rb, but there appears to be much more to uncover with respect to the molecular targets of these two oncogenic proteins at the cellular level. It has been suggested that in the absence of productive replication, the expression of the early genomes of these viruses leads to the production of tumor antigens, deregulation of cellular growth mechanisms due to the inactivation of tumor suppressors by tumor antigens, and possibly the selection of transformed phenotype. Studying the molecular targets of tumor antigens of polyomaviruses may help us to trace the molecular pathways induced by these viruses and perhaps such findings might in turn enable us to treat tumor-related cases in an effective way.

I. Introduction

The genome structure and gene products of polyomaviruses have been under intense investigation in recent years for several reasons. First, their small, circular genomes serve as miniature model systems to study many aspects of DNA structure for more complex eukaryotic genomes. Second, their oncogenic proteins can transform cells under certain conditions in both tissue culture and experimental animals in a manner resembling malignancies seen in humans. Particularly, recent findings regarding the detection of the genomes of both human (JCV and BKV) and simian virus 40 (SV40) polyomaviruses in a variety of human tumors suggest that this group of viruses may play a role in the induction of certain human tumors, although controversy still remains as to whether these viruses indeed induce such tumors. Such observations have led to investigators to further

study the mechanisms of tumor induction by these viruses. In this short review, we focused our attention to recent developments with respect to polyomavirus-induced tumors in experimental animals and the detection of viral genomes in a variety of human malignancies.

II. JC Virus (JCV)

JCV is a small human DNA virus with a double-stranded, covalently linked circular genome, 5130 base pair in size. It is classified in the Papovaviridae family within the polyomavirus genus (Frisque, Bream, and Cannella, 1984). JCV genome is composed of bidirectional regulatory elements and coding regions (**Figure 1**). The regulatory region contains the origin of DNA replication and promoter/enhancer elements for viral early and late genes. The coding regions can be divided

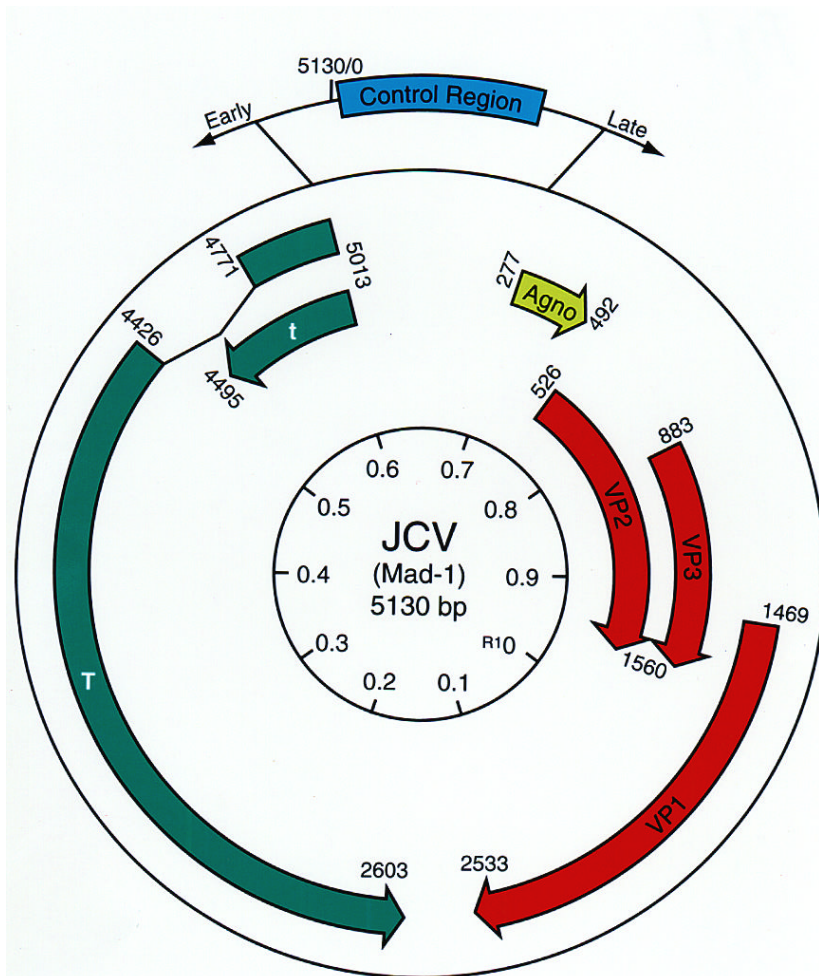


Figure 1. Genomic organization of JCV. JCV genome is composed of regulatory and coding regions. The regulatory region contains the origin of DNA replication and promoter/enhancer elements. The coding regions are divided into an early and late region. The early region encodes regulatory proteins, small and large T antigen. The late coding region encodes viral structural proteins (VP-1, VP-2 and VP-3) and a short regulatory peptide, agnoprotein.

into early and late regions. The early coding region primarily encodes two regulatory proteins, small and large T antigen although recent findings indicate that this region also encodes three additional small peptides called T's (Bollag et al, 2000). The late coding region encodes structural capsid proteins (VP-1, VP-2 and VP-3) and a small regulatory agnoprotein. Structural and antigenic studies demonstrated that JCV is related to other polyomaviruses such as human BK virus, and a primate virus, simian virus 40 in the genus. Serological data indicate that, unlike SV40, JCV and BKV share the property of hemagglutination of human type O erythrocytes. It should also be noted here that there is lack of convincing sera conversion data for wide infectivity of SV40 in human population as seen for JCV and BKV. Seroepidomological data shows that overwhelming majority of the world's population is infected by JCV (Frisque, 1992; Major et al, 1992; Berger and Concha, 1995) and the virus establishes a persistent infection in the kidneys (latent infection) after a subclinical primary infection. Recent reports indicate that peripheral blood B lymphocytes, hematopoietic progenitor cells, and tonsillar

stromal cells could also harbor JCV. These sites, therefore, can be considered additional potential sites for JCV infection and latency (Atwood et al, 1992; Monaco et al, 1996, 1998a,b, 2001; Frisque, 1998).

JCV was first isolated from brain tissue of a PML patient by Padgett et al, in 1971. The brain tissue was used as a source of inoculum to infect primary cultures derived from human fetal brain and the virus was successfully isolated from long-term cultures mainly consisting of glial cells (Padgett, 1971). This was the first direct evidence suggesting that a neurotropic viral agent was associated with the occurrence of PML. Shortly after its isolation, the oncogenic potential of the virus was tested both in tissue culture and experimental animals. Particularly, recent findings regarding the detection of JCV genome in a variety of human tumors indicate that JCV may be associated with the induction of human tumors.

JCV is a neurotropic virus that lytically infects oligodendrocytes in the central nerves system and causes a neurodegenerative disease of the white matter in the human brain, progressive multifocal encephalopathy (PML). The disease develops mostly in patients with

underlying immunosuppressive conditions, including Hodgkin's lymphoma, lymphoproliferative diseases, and AIDS (Major, 1992; Berger and Concha, 1995; Berger and Major, 1999). In a small number of cases, however, PML was also found to affect individuals with no underlying disease (Major, 1992; Berger and Concha, 1995). While PML was previously considered a rare complication of middle-aged and elderly patients with lymphoproliferative diseases, due to the AIDS epidemic in recent years, it is now a commonly encountered disease of the CNS in patients of different age groups. This suggests that human immunodeficiency virus (HIV) infection may directly or indirectly participate in this process. Recent estimates indicate that the incidence of PML in HIV-seropositive patients reached up to 5%, compared to that 0.8% before the AIDS epidemic (Aksamit et al, 1990; Aksamit, 1995; Berger and Concha, 1995; Berger et al, 2001).

A. Tumor induction by JCV in experimental animals

Following its isolation, JCV has not only been shown to cause a variety of tumors in experimental animals (Walker et al, 1973; Varakis et al, 1978; London et al, 1978, 1983; Krynska et al, 1999) but also shown to have the ability to induce neoplastic cell transformation in tissue culture. Since JCV induced tumors arise in tissues of neural origin (Walker et al, 1973; Varakis et al, 1978), tissue-specific expression of JCV regulatory region is thought to play a major role in this process. Inoculation of JCV into several experimental animal models, including hamsters, nonhuman primates, and transgenic mice, resulted in variety of tumors depending on the animal type, age and site of inoculation. For instance, more than 80% of newborn Syrian hamsters when inoculated intracerebrally and subcutaneously with the Mad-1 strain of JCV developed glioblastomas, neuroblastomas and medullablastomas (Walker et al, 1973; Varakis et al, 1978). Even the presence of an entire biologically active JCV genome was demonstrated when cells from these tumors were co-cultivated with permissive glial cells (Walker et al, 1973). JCV was also inoculated intraocularly into newborn hamsters and resulted in abdominal neuroblastomas developing in several locations of the body (Walker et al, 1973).

Unlike the other members of the polyomavirus family (BKV and SV40), JCV is the only polyomavirus shown to induce tumors in nonhuman primates, such as monkeys. When owl squirrel monkeys were inoculated with live JCV subcutaneously, intraperitoneally, and intracerebrally (London et al, 1978, 1983), the animals developed tumors at different time intervals. One owl monkey developed a malignant cerebral tumor similar to an astrocytoma seen in humans after 16 months of inoculation. Another one developed a malignant neuroblastoma 25 months after inoculation. Further analysis of the tumors for the expression of JCV large tumor antigen which is the main viral regulatory protein involved in tumor induction revealed both the presence of large T antigen and complex formation with tumor suppressor protein p53 (Dyson, 1990).

Mechanistically, the tumorigenic potential of JCV T antigen appears to be, at least in part, mediated by its interaction with tumor suppressor genes including p53 and retinoblastoma gene products, pRb and p130. Upon binding, T antigen appears to interfere with the cell cycle progression properties of these proteins. Coimmunoprecipitation assays using cellular extracts from JCV-transformed glial cells show T antigen complex formation with pRb, p53 and p107 (Monier, 1986). A report by Rencic et al, (1996) also suggests a role for T antigen in the induction of oligoastrocytomas in an immunocompetent patient. JC virus large T antigen has also been shown to interact with cellular and viral proteins including YB-1, Pur, JCV agnoprotein, and insulin receptor substrate 1 (IRS-1) (Gallia, 1998; Safak et al, 1999, 2002; Lassak et al, 2002). IRS-1 is the major signaling molecule for the type I insulin-like growth factor receptor (IGF-IR) (Baserga, 1999). In addition, recent reports also indicate a possible communication between JCV T antigen and the Wnt signaling pathway in induction of tumor formation because T antigen expressing cells express higher levels of β -catenin and its partner LEF-1 (Gan et al, 2001).

Our group also described the formation of different tumors in tissues that derived from neural origin in transgenic mice models (Franks et al, 1996; Krynska et al, 1999; Gordon et al, 2000). JCV early coding region, driven by its own promoter, was utilized to create these transgenic animal models. Histological and histochemical analysis of the tumor masses demonstrated the expression of JCV large T antigen in tumors versus control tissues. In contrast to previous observations by Small et al (Small et al, 1986a,b), transgenic animals created with the early region of JCV archetype strain (Krynska et al, 1999) did not show any sign of hypomyelination in the central nervous system which was a feature observed in transgenic mouse models. On the contrary, cerebellar tumors that resemble human medullablastomas appeared in the transgenic animals (Krynska et al, 1999). In another line of transgenic mouse, half of the animals developed large, solid masses within the base of the skull by one year of age. Histological evaluation of the tumors by location and by histochemical studies demonstrated that these tumors arose from the pituitary gland (Gordon et al, 2000). **Figure 2** exemplifies a variety of tumors induced by JCV in an experimental animal model system.

In addition to the evaluation of tumorigenic activity of JCV in mice, transgenic mice were also used to study the process of the acute demyelination occurring in PML-affected brain tissue. Some of the offsprings of a transgenic mouse created with the regulatory and coding sequences of JCV T-Ag (Small et al, 1986a; Small et al, 1986b) exhibited mild to severe tremor phenotypes with hypo and dysmyelination occurring in the central nervous system (CNS). In addition, dysmyelination was further characterized in transgenic animals by Trapp et al, (Trapp et al, 1988) by examining the expression of the JCV and myelin-specific genes. Initial examination of brain tissue from transgenic mice revealed relatively low expression levels of proteolipid protein (PLP), myelin basic protein

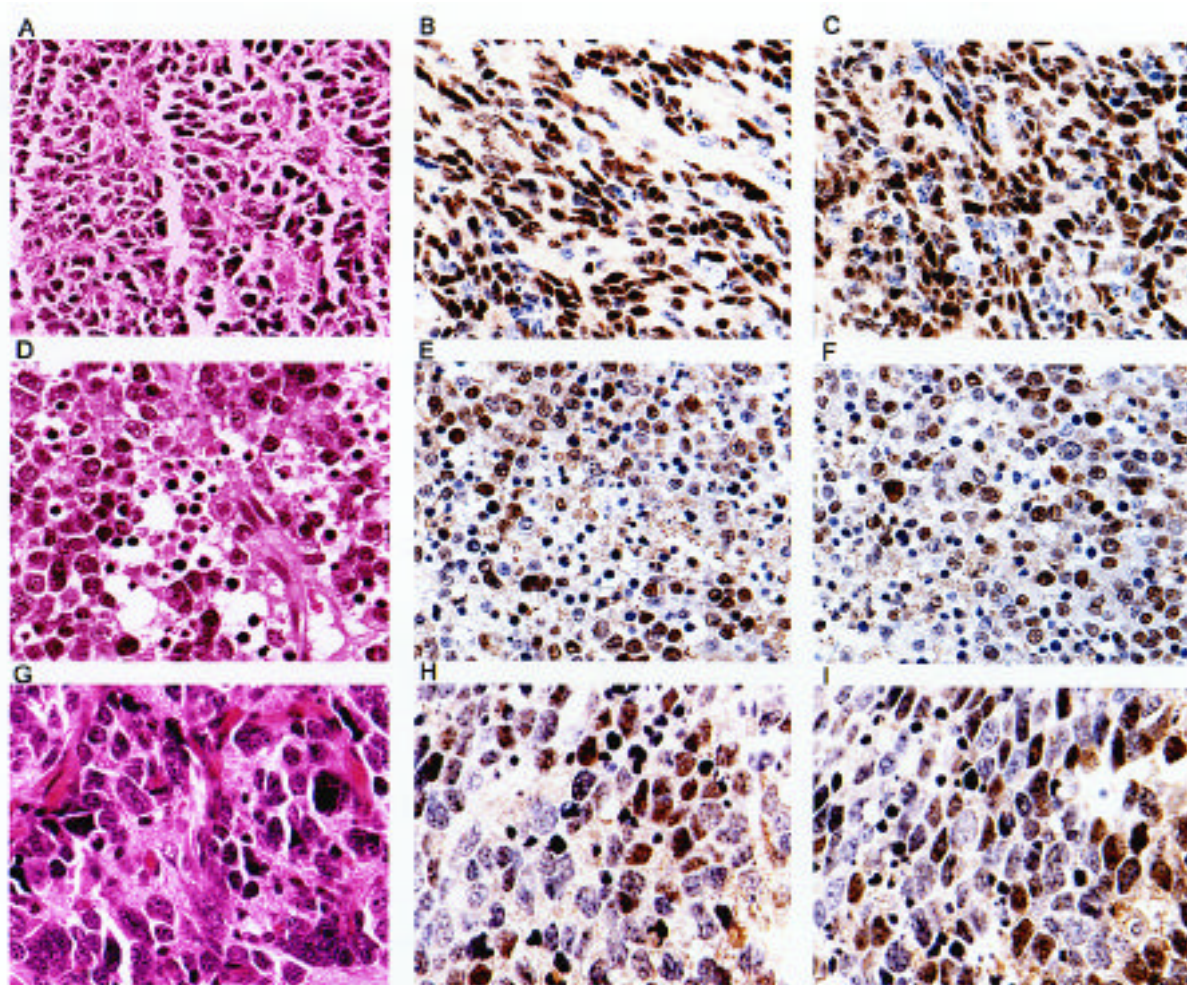


Figure 2. JCV transgenic animal models. Transgenic mice containing the full sequence of the JCV genome (archetype), develop primitive neuroectodermal tumors in the brain, characterized by numerous packed cells with an elongated nuclei and scant cytoplasm (**Panel A**, Hematoxylin & Eosin). Immunohistochemistry against the early gene product T-antigen, demonstrates the nuclear localization of the protein (**Panel B**), and in the same group of cells there is intense immunoreactivity for p53 (**Panel C**). Transgenic animals containing only the early sequence of JCV, develop a variety of neural-origin tumors, including adrenal neuroblastomas, characterized by rounded homogeneous cells with a perinuclear halo of cytoplasm (**Panel D**, Hematoxylin & Eosin), which also express nuclear T-antigen when tested by immunohistochemistry (**Panel E**). In the same cellular compartment there is strong immunoreactivity for p53 (**Panel F**). Another tumor developed by a line of JCV early transgenic mice is pituitary adenomas, characterized by numerous pleomorphic cells of different sizes and abundant eosinophilic cytoplasm (**Panel G**, Hematoxylin & Eosin). The neoplastic cells demonstrate intense nuclear positivity for T-antigen (**Panel H**), as well as p53 (**Panel I**). All panels original magnification x1000.

(MBP) and myelin associated glycoprotein which collectively make up the axonal myelin sheet although the mRNA message levels for those proteins appeared to be normal. The mechanism by which T antigen plays a critical role in the reduction of these respective protein levels in the brains of transgenic mice remains unknown, however, it is suggested that T antigen may alter the expression levels of both proteolipid and myelin basic protein at the protein levels or may inhibit the maturation process of oligodendrocytes thereby altering the level of myelin around the axons.

B. Detection of JCV in human tumors

In recent years, a widespread detection of JCV genome in variety of human tumors raised the possibility that JCV may induce tumors in humans. In fact, Richardson, who first described PML in 1961

(Richardson, 1961), reported the incidental detection of an oligodendroglioma in a patient with concomitant occurrences of chronic lymphatic leukemia and PML. Following this report, concomitant occurrences of PML with different human tumors was described in several more cases. Sima et al, reported the association of PML with multiple astrocytomas in 1983 (Sima, 1983). Similarly, Casteigne et al, (1974) described a case where a patient with long history of immunodeficiency syndrome, in addition to PML, showed numerous foci of anaplastic astrocytes. Microscopic analysis of the demyelinating lesions demonstrated the presence of viral particles in both oligodendrocytes and astrocytes within PML foci, but not in the neoplastic astrocytes (Casteigne, 1974). A more recent report by Shintaku and colleagues showed dysplastic ganglionic-like cells in a patient with PML (Shintaku et al, 2000). A large number of dysplastic or dysmorphic ganglionic-like cells were found in the cerebral

cortex that showed properties of neurons. Expression of JCV large T antigen was demonstrated in the infected neurons, however, the late gene products were not.

In addition to the cases described above, JCV genome has also been detected in human brain tumors in the absence of PML lesions. Boldorini et al, reported the detection of JCV DNA in the brain tumors of an immunocompetent patient with a pleomorphic xanthoastrocytoma (Boldorini et al, 1998). An earlier study by Rencic et al, demonstrated the presence of JCV viral DNA and expression of large T antigen in tumor tissue from an immunocompetent HIV-negative patient with oligoastrocytoma (Rencic et al, 1996). These two cases presented the experimental evidence for a possible association of JCV in brain tumors of immunocompetent

non-PML patients. Such findings further prompted the attempts to establish the association of JCV with different types of brain tumors in humans. In fact, Del Valle et al, (Del Valle et al., 2002; Del Valle et al., 2001) recently analyzed multiple brain tumors for the detection of JCV genome and showed that 62.5% of oligoastrocytomas, 83.3% of ependymomas, 80% of pilocytic astrocytomas, 57.1% of oligodendrogliomas, 76.9% of astrocytomas and 66% of anaplastic oligodendrogliomas contained JCV early gene sequence. **Figure 3** illustrates the detection of JCV early oncogenic protein, large T antigen, and cellular tumor suppressor protein, p53, in a variety of human tumors JCV genomic DNA has also been shown to be present in tumor tissue which is not neural origin. Recent reports indicate that the JCV genome was detected in.

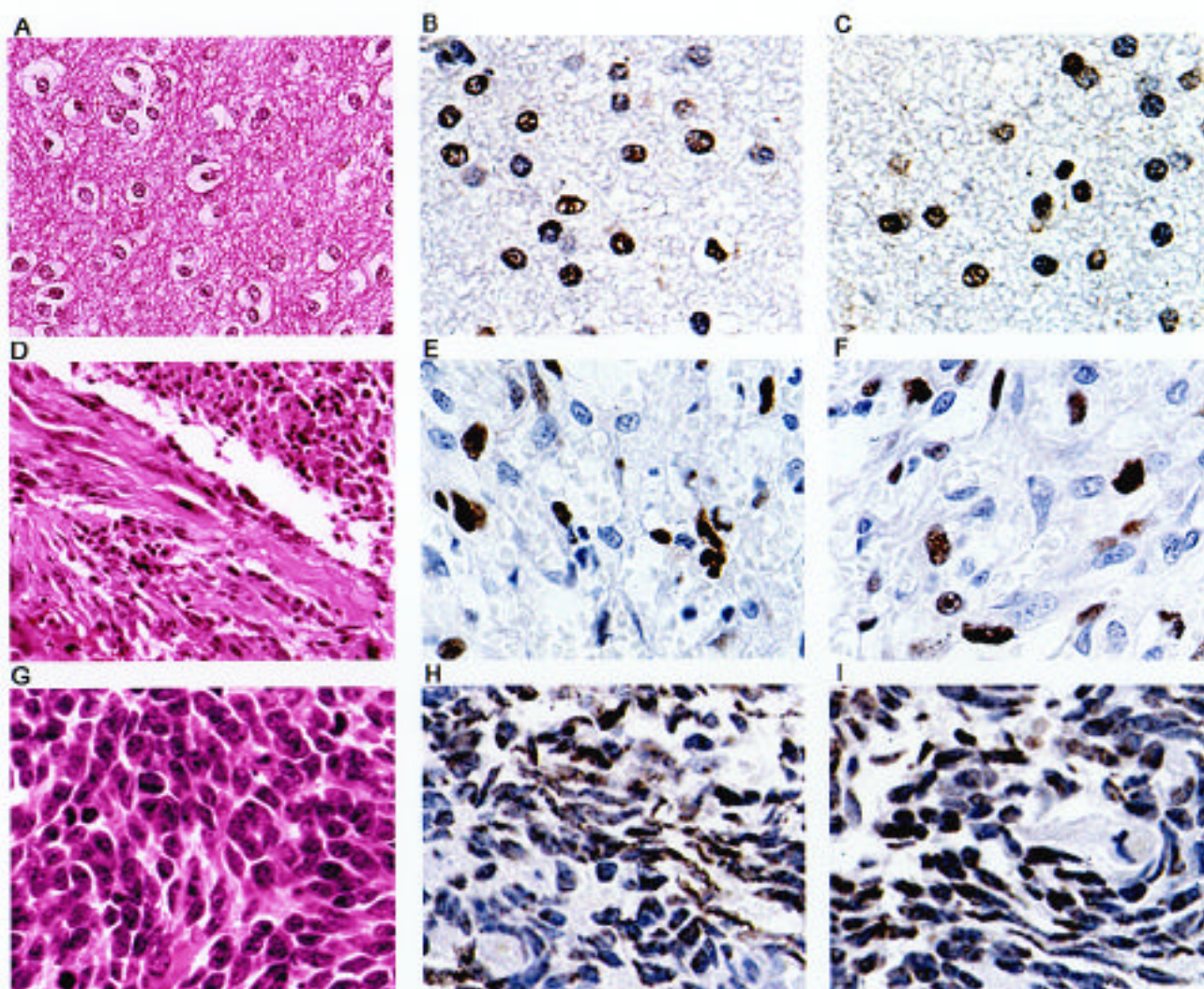


Figure 3. Detection of JCV proteins in human brain tumors. Expression of JCV early protein has been found in a wide variety of brain neoplasms, including low grade glial tumors, such as oligodendrogliomas (**Panel A**, Hematoxylin & Eosin), characterized by homogeneous cells with a clear halo surrounding their nuclei. Immunohistochemistry from T-antigen is positive in the nuclei of the majority of the neoplastic cells (**Panel B**), where the cell cycle regulator protein p53 is also found (**Panel C**). High-grade glial tumors such as glioblastoma multiforme (**Panel D**) characterized by extensive areas of necrosis and pleomorphic, atypical cells expressing T-antigen in their nuclei (**Panel E**). p53 is also present in the nuclei of the neoplastic cells (**Panel F**). Tumors of neural origin, such as medulloblastomas, characterized by numerous sheaths of homogeneous cells, with scant cytoplasm (**Panel G**, Hematoxylin & Eosin), demonstrate nuclear expression of the early JCV protein, T-antigen (**Panel H**), and also nuclear immunoreactivity for p53 (**Panel I**). All panels original magnification x1000.

gastrointestinal tract and solid non-neural tumors including colorectal cancers (Laghi et al, 1999; Ricciardiello et al, 2000, 2001; Enam et al, 2002). It should be however noted here that such studies explored the possibility of whether JCV genome or its expressed proteins could be detected by certain molecular biology techniques but does not provide information about the mechanism by which JCV could possibly induce tumors in humans

III. BK virus (BKV)

Another human polyomavirus which is classified within the Papovaviridae family is BK virus. This virus was first isolated in 1971 from the urine of a renal allograft recipient who developed ureteric stenosis (Gardner, 1971). Like JCV and SV40, the BKV early and late genomes code for six viral proteins, two from the early genome and four from the late genome. Early proteins are nonstructural regulatory proteins (small t and large T antigens), of which large T antigen is involved in regulation of the viral DNA replication and late gene expression. The function of small t antigen in this regard is not known. The viral late genome, in addition to encoding the structural proteins VP-1, VP2 and VP3, also encodes a small regulatory peptide, agnoprotein, whose function largely remains unclear in the viral lytic cycle. Recent evidence from JCV virus agnoprotein work, however, suggests that it plays a role in viral DNA replication, transcription (Safak et al, 2001, 2002), and cell cycle regulation (Darbinyan et al, 2002).

Like JCV, BKV has also a worldwide distribution in the human population. Primary infection by BKV takes place during early childhood and is subclinical although a mild respiratory illness or urinary track disease may occur (Goudsmit et al, 1982; Padgett et al, 1983). Little is known about the route of BKV transmission although induction of upper respiratory disease by BKV and detection of latent BKV DNA in tonsils suggests a possible oral or respiratory route of transmission (Goudsmit et al, 1982). During primary infection, viremia occurs and the virus spreads to a number of organs in the infected individuals including kidneys, bladder, prostate, uterine cervix, lips and tongue (Monini et al, 1995) where it remains in a latent state. Reactivation of the virus from latent state is mostly associated with the immunocompromised state of individuals. Reactivated virus was detected in the urine of renal and bone marrow transplant recipients undergoing immunosuppressive therapy (Gardner et al, 1984) as well as in the urine of pregnant women (Coleman et al, 1977). Upon reactivation, BKV may cause interstitial nephritis and ureteral obstruction in patients receiving renal transplants, and in some cases, it can cause viral-infection-induced transplant dysfunction and graft rejection (Howell et al, 1999). In addition, an association between hemorrhagic cystitis and BKV was shown in bone marrow transplant recipients (Azzi et al, 1994).

A. BKV genome is oncogenic in animal models

Like JCV, the oncogenic potential of BKV has been tested in experimental animals including young and newborn mice, rats, and hamsters by inoculation of live virus. (Chenciner et al, 1980; Corallini et al, 1982; Corallini et al, 1978; Corallini et al, 1977). The type of tumors induced by BKV was strictly dependent on the route of inoculation. It was observed that BKV is weakly oncogenic when inoculated subcutaneously (Nase et al, 1975; Shah et al, 1975) but induced tumors in high proportions when inoculated intracerebrally or intravenously (Uchida et al, 1976, 1979; Corallini et al, 1977, 1978, 1982). Tumors induced by BKV belong to a variety of histotypes including ependymoma, neuroblastoma, pineal gland tumors, fibrosarcoma, osteosarcoma and tumors of pancreatic islets (Nase et al, 1975; Dougherty, 1976; Uchida et al, 1976, 1979; van der Noordaa, 1976; Corallini et al, 1977, 1978, 1982; Greenlee et al, 1977; Watanabe et al, 1979; Noss and Stauch, 1981, 1984; Watanabe and Yoshiike, 1982). Rats inoculated with BKV developed fibrosarcoma, liposarcoma, osteosarcoma, nephroblastoma, and glioma. Mice, however, developed only choroids plexus papilloma in a similar setting (Noss et al, 1981; Noss and Stauch, 1984).

Transgenic mice were also used to test the oncogenicity of BKV large T antigen (T-Ag). Transgenic mice with BKV T-Ag developed renal tumors, hepatocellular carcinoma, and lymphoproliferative disease (Small et al, 1986a; Dalrymple and Beemon, 1990). In such studies, there appears to be differences among the strains of BKV in terms of oncogenicity. For example, Gardner's BKV strain seems to be more potent to induce tumors in transgenic mice than other isolates such as MM, BKV-IR or RF (Dougherty, 1976; Caputo et al, 1983).

The mechanism by which BKV causes tumors in experimental animals and cell transformation in tissue culture remains elusive. It was shown that like JCV and SV40 T-Ag, BKV T-Ag interacts with several key cell cycle regulatory proteins, including tumor suppressor proteins p53 and the family members of retinoblastoma proteins, pRb105 and Rb130. BKV T-Ag perhaps inactivates the function of these proteins and thereby contributes to the cell transformation (Dyson, 1990; Harris et al, 1996; Shivakumar and Das, 1996; Eggers et al, 1999). It was recently shown that the complex formation of SV40 T-Ag with mouse p53 completely blocks the transactivation function of p53 protein (Sheppard et al, 1999). Due to the high homology between BKV T-Ag and SV40 T-Ag, a similar mechanism may hold for the BKV T-Ag as well.

It is proposed that BKV T-Ag may also transform cells through a "hit and run" mechanism. In a study by Brunner et al, (1989) it was observed that although transfection of BKV DNA into human cells resulted in a transformed phenotype, viral DNA was absent in most of the clones. This suggested that transformed cells no longer require the expression of T-Ag after a certain stages in the transformation process.

BKV T-Ag was also shown to induce a number of structural chromosomal alterations characterized by

breaks, gaps, dicentric and ring chromosomes, deletions, duplications and translocations (Tognon et al, 1996). While the molecular mechanism of this clastogenic effect of BKV on host DNA is unknown, it is thought to reside in its ability to bind to topoisomerase I or in its helicase activity in which it may induce chromosome damage when unwinding the strands of cellular DNA. Moreover, since BKV binds to tumor suppressor protein p53 and inactivates its function, this may lead to survival of DNA-damaged cells and increase their probability to transform and acquire immortality. As a result, the clastogenic and mutagenic activities of BKV may disturb the crucial function of the genes that are important for the maintenance of genomic stability such as oncogenes, tumor suppressor genes and DNA repair genes.

B. Human tumors harbor BKV genome

Detection of BKV DNA in a variety of human tumors and tumor cell lines during the late 1970's prompted researchers to further investigate the possible association of BKV with the induction of a variety of human tumors (Fiori and Di Mayorca, 1976). Since BKV exhibits a specific oncogenic tropism for the ependymal tissue, endocrine pancreas, and osteosarcomas in rodents (Corallini et al, 1977, 1978, 1982; Uchida et al, 1979; Chenciner et al, 1980), investigators primarily focused on the characterization of such tumors in humans for the detection of BKV genome. Southern blot hybridization studies showed that 4 out of 9 (44%) human tumors of the pancreatic islets and 19 out of 74 (26%) of human brain tumors contained BKV DNA in a free, episomal state (Corallini et al, 1987). BKV was even rescued from some of the tumors by transfection of human embryonic fibroblasts with tumor DNA.

The detection of BKV DNA was also reported by Dorries et al, in 46% of brain tumors of the most common histotypes (Dorries et al, 1987). In this particular study, BKV DNA was found to be integrated into the chromosomal DNA. Human tumors associated with immunocompromised conditions were also analyzed by Southern blotting and it was shown that BKV DNA was associated with Kaposi's sarcoma with low frequencies (20%) (Barbanti-Brodano et al, 1987).

Recently, tumor cell lines, normal and neoplastic human tissues were investigated for the detection of BKV by PCR methods utilizing specific primers for the early region of BKV DNA. The nucleotide sequence analysis of PCR products from these studies revealed the presence of BKV specific sequences in several brain tumor samples: one osteocarcinoma, two glioblastoma cell lines, one normal brain tissue and one normal bone tissue specimen (De Mattei et al, 1995). Even the expression of the early region of the BKV was demonstrated by Northern blotting of RT-PCR studies in some of the samples in those studies. The presence of BKV DNA was also investigated in several different tumors including urinary track tumors, in carcinomas of the uterine cervix, vulva, lips and tongue (Monini et al, 1995, 1996). However, data obtained from such studies were inconclusive because the percentage of positive samples in these neoplastic tissues of the urinary

and genital tracks and of the oral cavity were similar to that detected in the corresponding normal tissues (Monini et al, 1996). BKV DNA was shown to be present in Kaposi's sarcoma (KS) cases in high percentages suggesting that BKV may be an important co-factor in KS (Peterman et al, 1993).

IV. Simian virus 40 (SV40)

SV40 is the most extensively studied polyomavirus. Its small genome size was exploited as a model system to study transcription and replication for more complex eukaryotic systems. Characteristic cytopathic vacuolization effects caused by SV40 in African green monkey cells led to the recognition and isolation of the virus in 1960 by Sweet and Hilleman, (1960). Apparently SV40 was introduced into the human population through widespread use of contaminated poliovaccines. Contamination occurred during the vaccine preparation process because the early poliovaccines were prepared in primary cultures of kidney cells derived from rhesus monkeys, which are often naturally infected with SV40. As described above, SV40 genome is very similar to the other polyomaviruses, BKV and JCV, in structure containing regulatory and coding regions. Coding regions encode regulatory (small t and large T antigens, agnoprotein) and structural capsid proteins (VP-1, VP-2 and VP-3). The regulatory region of SV40, like JCV and BKV, contains the origin of DNA replication and promoter/enhancer elements which are targets for transcription factors. SV40's genome shows significant sequence homology to BKV and JCV at the coding regions, however, more divergent sequences lie within its regulatory region.

A. Cell transformation and tumor induction by SV40

Following its discovery, SV40 was tested for its ability to induce tumors in experimental animals and to transform a variety of cell types from different species in tissue culture. Particularly, studies with Syrian hamsters showed the ability of SV40 to induce a variety of tumors in experimental animals (Eddy et al, 1962; Girardi et al, 1962; Butel et al, 1972). Such observations raised a question whether SV40 is involved in human carcinogenesis because SV40 was shown to establish infections in humans (Melnick and Stinebaugh, 1962). Injection of SV40 DNA into hamsters resulted in a variety of tumors depending on the site of injection. For example, injection of SV40-infected rhesus monkey kidney cells into newborn hamsters induced sarcomas at the site of inoculation (Eddy et al, 1962). Intravenous injection of SV40 into weanling hamsters resulted in lymphocytic leukemia, soft tissue sarcoma, osteosarcoma and lymphoma (Diamandopoulos, 1972). Intracranial injection of SV40 into both newborn hamsters and *Mastomys* produced ependymomas (Rabson et al, 1962). Mesotheliomas were induced upon injection of SV40 into the intrapleural region of weanling hamsters (Cicala et al, 1993).

A variety of cell types have been used to characterize

the transforming properties of SV40 including humans, hamsters, mice, rats, guinea pigs and cattle (Butel, 1972, 2000; Butel and Lednický, 1999). It turned out that not every cell is permissive to infection of SV40. Monkey cells are considered to be permissive to SV40 infection. Mouse cells are nonpermissive, and human cells are considered to be “semipermissive” to SV40 infection. It was observed that in nonpermissive cells, the viral genome is often found to be integrated into the host genome and the integration is not directed to any specific site (Grodzicker and Hopkins, 1980). The cellular transformation and immortalization are the consequence of nonlytic infection of the host cells. Viral oncogenic proteins are generally expressed continuously during that period perhaps to maintain the cells in the transformed state. The exact mechanism of cell transformation and immortalization is unknown. However, it appears that viral onco-protein, T-Ag, targets primarily tumor suppressor and key cell cycle regulator proteins, such as p53 and pRb, which inactivates their function and results in deregulation of cell cycle progression.

SV40 T-Ag is a multifunctional oncoprotein that possesses several defined functional domains and has been shown to play a critical role in cell transformation and tumor induction (Butel and Lednický, 1999). **Figure 4** schematically illustrates different functional domains of SV40 large T antigen. The amino terminus of the T-Ag contains two distinct domains important in cell transformation. The far amino terminus of T-Ag includes the J domain involved in proper folding of protein complexes. This region shares 82 amino acid residues with small t antigen. The second region of the amino terminus of T-Ag mediates the binding to pRb and the pRb family members p107 and p130 (Fanning, 1992; Fanning and Knippers, 1992). Although the function of p107 and p130 in cell cycle regulation remains unclear, the mechanism of action of tumor suppressor protein pRb at the G1 checkpoint has been well demonstrated. It forms an inactive complex with a transcription factor E2F and arrests cells at the G1 phase of cell cycle. When specific cyclin dependent kinases phosphorylate Rb, it releases transcription factor E2F which in turn transactivates S phase specific gene promoters and causes the cell to progress into S phase. When bound to Rb, T-Ag

inactivates the regulatory function of pRb which allows unscheduled S-phase entry thereby establishing favorable conditions for cellular transformation (Butel, 2000). T-Ag also targets another tumor suppressor protein, p53, which plays a critical role in cell cycle progression at the G1 checkpoint and induces apoptosis when overexpressed in cultured cells (Shaw et al, 1992; Amundson et al, 1998). A possible mechanism by which p53 regulates the genomic stability is through the induction of apoptosis in DNA damaged cells before potentially oncogenic events deregulate cell cycle progression. p53 is found mutated or lost in up to 50% of all human cancers which emphasizes the importance of its functional loss in carcinogenesis (Hollstein et al, 1996; Levine, 1997). SV40 T-Ag possesses two p53 binding sites near its carboxy-terminal end. By binding to p53 at these sites, T-Ag inhibits p53-mediated activities including arresting cells that have mild DNA damage in G1 or G2/M phases of the cell cycle for DNA repair and eliminating the cells that has extensive DNA damage by apoptosis. Under these circumstances, the cells with damaged DNA go through the cell cycle stages without DNA repair which results in accumulation of cellular mutation and increased genomic instability that can lead to cancer.

T-Ag, in addition to targeting cellular tumor suppressor proteins, also targets nuclear acetylases including CREB-binding protein (CBP), P/CAF and p300. These regulatory proteins function as cofactors and play important roles in transcription and posttranslational modification of cellular tumor suppressor proteins. T-Ag interacts with these proteins through multiple regions (Eckner et al, 1996; Srinivasan et al, 1997) and inactivates their important cellular functions. This is also thought to contribute to deregulation of cell cycle progression.

Small t antigen of SV40, which is produced by alternative splicing of early transcripts, was shown to form complexes with the regulatory subunit of PP2A. This association appears to inhibit the function PP2A (Pallas et al, 1990; Yang et al, 1991) which in turn leads to more phosphorylated and increased kinase activity of several cellular kinases including MAP kinase and its kinase ERK, Jun N-terminal kinase (JNK) and a key ion transporter, the Na/H antiporter (Sontag et al, 1993; Frost et al, 1994).

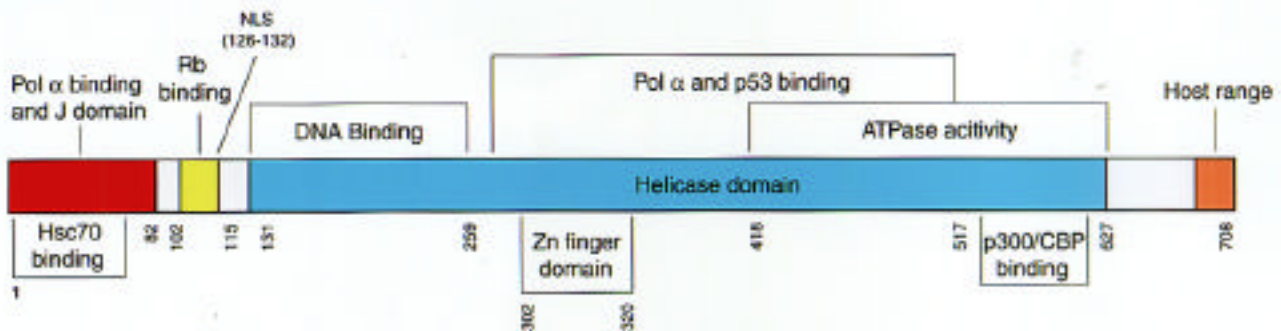


Figure 4. Schematic representation of functional domains of SV40 large T-antigen. Approximate minimal regions of T-antigen that retain binding activity to polymerase α -primase (Pol α), tumor suppressor proteins Rb and p53, human heat shock protein 70 (hsc70) and coactivators p300 and CBP are illustrated. DNA binding domain, ATPase activity domain, nuclear localization signal (NLS) domain, helicase domain, host range domain, Zn finger domain, and J domain are also depicted.

It is also believed that small t antigen antagonizes T-Ag-induced cellular apoptosis and thereby contributes to more efficient transformation of rat embryo fibroblasts (Kolzau et al, 1999). Transgenic animals created with a small t antigen deletion mutant of SV40 genome consistently developed tumors in highly mitotic tissues relative to wild-type virus (Carbone et al, 1989; Choi et al, 1988) indicating that small t antigen contributes to large T-Ag-mediated transformation of resting cells.

B. Human tumors and SV40

The detection of SV40 in a metastatic melanoma patient by Soriano et al, (1974) in 1974 was the first observation that links the association of SV40 with human cancers. The virus was isolated from a lung metastasis and viral T-Ag and capsid proteins were detected in lung, liver and muscle metastasis but not in normal tissue. Since then, numerous reports have been published regarding a possible link between SV40 and human tumors. SV40 genome and the expression of T-Ag were detected by PCR, DNA hybridization, DNA sequencing and immunofluorescence techniques in a variety of human tumors and nontumor tissues including mesotheliomas (Carbone et al, 1994; Griffiths, Nicholson, and Weiss, 1998; Rizzo et al, 1998, 1999; Testa et al, 1998; Shivapurkar et al, 2000), brain tumors (Weiss et al, 1975; Krieg et al, 1981; Bergsagel et al, 1992; Lednický et al, 1995; Martini et al, 1996), and other human tumors and nontumor tissues including osteosarcomas, AIDS-related lymphomas, peripheral blood cells, kidney tissue from pediatric renal transplant patients and non-Hodgkin's lymphomas (Carbone et al, 1996; Lednický and Butel, 1997; Butel et al, 1999; Rizzo et al, 1999; David et al, 2001).

A large number of reports have described the association of SV40 with malignant mesothelioma and yet asbestos, an environmental carcinogen is believed to be the predominant cause of mesotheliomas. Development of malignant mesotheliomas (up to 20%) in patients with no known asbestos exposure raised a controversial case of whether asbestos can be considered as the only causative agent of fatal mesotheliomas or there are other factors or co-factor, such as SV40, that play a role in the development of such tumors. Many studies have repeatedly linked the association of SV40 with mesothelioma. A recent multi-laboratory study by Testa et al, confirmed the presence of SV40 sequences in frozen mesothelioma samples by PCR, DNA hybridization and/or DNA sequencing (Testa et al, 1998). The complex formation between T-Ag with p53 and T-Ag (Carbone et al, 1997) with retinoblastoma family members, including pRb, p107 and p130, was also demonstrated by co-immunoprecipitation assays (De Luca et al, 1997). Some studies suggested that a relatively higher susceptibility of mesothelial cells to SV40 infection maybe a part of the determining factor in development of mesotheliomas. Bochetta et al, (2000) compared the rate of transformation of SV40-infected mesothelial cells with that of human fibroblasts in a tissue culture system and the results were striking (Ozer et al, 1996). Mesothelial cells were found to be 1000 times more susceptible to transformation upon

SV40 infection than human fibroblast cells. This may partially offer an explanation for the relationship between SV40 and human mesotheliomas.

There are also now a number of studies describing the association of SV40 with human brain tumors. Experimental animal studies showed that SV40 is oncogenic in neural tissues when injected, for example, into the newborn hamsters (Eddy et al, 1962; Girardi et al, 1962) and SV40 was shown to be capable of transforming primary human astrocytes in culture (Shein, 1967). SV40 genome and its gene products were detected by PCR or Western blotting in a variety of brain tumors including glioblastomas, gliomas, gliosarcomas, medullablastomas, meningiomas, pituitary adenomas and oligodendromas (Weiss et al, 1975; Krieg et al, 1981; Bergsagel et al, 1992; Lednický et al, 1995; Martini et al, 1996). Even a complex formation of T-Ag with p53 and T-Ag with pRb was demonstrated by co-immunoprecipitation assays (Zhen et al, 1999) suggesting that T-Ag targets common pathways in different tumors.

V. Concluding remarks

We have briefly reviewed recent developments regarding the tumor inducing aspects of polyomaviruses JCV, BKV and SV40. We have learned much about the molecular mechanisms underlying the cell transformation process induced by the oncogenic protein of each virus, large T antigen. However, many questions still remain unanswered as to how large T antigen can perturb the normal cell cycle progression and eventually cause cell transformation and immortalization. Further research is required to understand the molecular mechanism(s) of cell transformation, and polyomaviruses offer an excellent model system to study many aspects of this process. This in turn may help us to understand the foundation of human cancers.

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References

- Aksamit AJ, Gendelman HE, Orenstein JM, and Pezeshkpour GH (1990) AIDS-associated progressive multifocal leukoencephalopathy (PML): comparison to non-AIDS PML with in situ hybridization and immunohistochemistry. *Neurology* 40, 1073-8.
- Aksamit AJ, Jr (1995) Progressive multifocal leukoencephalopathy: a review of the pathology and pathogenesis. *Microsc Res Tech* 32, 302-11.
- Amundson SA, Myers TG, and Fornace AJ, Jr (1998) Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. *Oncogene* 17, 3287-99.

- Atwood WJ, Amemiya K, Traub R, Harms J, and Major EO (1992) Interaction of the human polyomavirus JCV with human B-lymphocytes. **Virology** 190, 716-23.
- Azzi A, Fanci R, Bosi A, Ciappi S, Zakrzewska K, de Santis R, Laszlo D, Guidi S, Saccardi R, Vannucchi AM, and et al (1994) Monitoring of polyomavirus BK viruria in bone marrow transplantation patients by DNA hybridization assay and by polymerase chain reaction: an approach to assess the relationship between BK viruria and hemorrhagic cystitis. **Bone Marrow Transplant** 14, 235-40.
- Barbanti-Brodano G, Pagnani M, Viadana P, Beth-Giraldo E, Giraldo G, and Corallini A (1987) BK virus DNA in Kaposi's sarcoma. **Antibiot Chemother** 38, 113-20.
- Baserga R (1999) The IGF-I receptor in cancer research. **Exp Cell Res** 253, 1-6.
- Berger JR, and Concha M (1995) Progressive multifocal leukoencephalopathy: the evolution of a disease once considered rare. **J Neurovirol** 1, 5-18.
- Berger JR, and Major EO (1999) Progressive multifocal leukoencephalopathy. **Semin Neurol** 19, 193-200.
- Berger JR, Chauhan A, Galey D, and Nath A (2001) Epidemiological evidence and molecular basis of interactions between HIV and JC virus. **J Neurovirol** 7, 329-38.
- Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, and Garcea RL (1992) DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. **N Engl J Med** 326, 988-93.
- Bocchetta M, Di Resta I, Powers A, Fresco R, Tosolini A, Testa JR, Pass HI, Rizzo P, and Carbone M (2000) Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. **Proc Natl Acad Sci USA** 97, 10214-9.
- Boldorini R, Caldarelli-Stefano R, Monga G, Zocchi M, Mediati M, Tosoni A, and Ferrante P (1998) PCR detection of JC virus DNA in the brain tissue of a 9-year-old child with pleomorphic xanthoastrocytoma. **J Neurovirol** 4, 242-5.
- Bollag B, Prins C, Snyder EL, and Frisque RJ (2000) Purified JC virus T and T' proteins differentially interact with the retinoblastoma family of tumor suppressor proteins. **Virology** 274, 165-78.
- Brunner M, di Mayorca G, and Goldman E (1989) Absence of BK virus sequences in transformed hamster cells transfected by human tumor DNA. **Virus Res** 12, 315-30.
- Butel JS, Tevethia SS, and Melnick JL (1972) Oncogenicity and cell transformation by papovavirus SV40: the role of the viral genome. **Adv Cancer Res** 15, 1-55.
- Butel JS, Arrington AS, Wong C, Lednicki JA, and Finegold MJ (1999) Molecular evidence of simian virus 40 infections in children. **J Infect Dis** 180, 884-7.
- Butel JS, and Lednicki JA (1999) Cell and molecular biology of simian virus 40: implications for human infections and disease. **J Natl Cancer Inst** 91, 119-34.
- Butel JS (2000) Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. **Carcinogenesis** 21, 405-26.
- Caputo A, Corallini A, Grossi MP, Carra L, Balboni PG, Negrini M, Milanese G, Federspil G, and Barbanti-Brodano G (1983) Episomal DNA of a BK virus variant in a human insulinoma. **J Med Virol** 12, 37-49.
- Carbone M, Lewis AM, Jr, Matthews BJ, Levine AS, and Dixon K (1989) Characterization of hamster tumors induced by simian virus 40 small t deletion mutants as true histiocytic lymphomas. **Cancer Res** 49, 1565-71.
- Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJ, Levine AS, and Procopio A (1994) Simian virus 40-like DNA sequences in human pleural mesothelioma. **Oncogene** 9, 1781-90.
- Carbone M, Rizzo P, Procopio A, Giuliano M, Pass HI, Gebhardt MC, Mangham C, Hansen M, Malkin DF, Bushart G, Pompetti F, Picci P, Levine AS, Bergsagel JD, and Garcea RL (1996) SV40-like sequences in human bone tumors. **Oncogene** 13, 527-35.
- Carbone M, Rizzo P, Grimley PM, Procopio A, Mew DJ, Shridhar V, de Bartolomeis A, Esposito V, Giuliano MT, Steinberg SM, Levine AS, Giordano A, and Pass HI (1997) Simian virus-40 large-T antigen binds p53 in human mesotheliomas. **Nat Med** 3, 908-12.
- Casteigne PR, Escourolle P, Ribadeau R, Cathala DJL, Hauw JJF (1974) Progressive multifocal leukoencephalopathy and multiple gliomas. **Rev Neuro Paris** 9-10 379-392.
- Chenciner N, Grossi MP, Meneguzzi G, Corallini A, Manservigi R, Barbanti-Brodano G, and Milanese G (1980) State of viral DNA in BK virus-transformed rabbit cells. **Virology** 103, 138-48.
- Choi YW, Lee IC, and Ross SR (1988) Requirement for the simian virus 40 small tumor antigen in tumorigenesis in transgenic mice. **Mol Cell Biol** 8, 3382-90.
- Cicala C, Pompetti F, and Carbone M (1993) SV40 induces mesotheliomas in hamsters. **Am J Pathol** 142, 1524-33.
- Coleman DV, Daniel RA, Gardner SD, Field AM, and Gibson PE (1977) Polyomavirus in urine during pregnancy. **Lancet** 2, 709-710.
- Corallini A, Barbanti-Brodano G, Bortoloni W, Nenci I, Cassai E, Tampieri M, Portolani M, and Borgatti M (1977) High incidence of ependymomas induced by BK virus a human papovavirus: brief communication. **J Natl Cancer Inst** 59, 1561-4.
- Corallini A, Altavilla G, Cecchetti MG, Fabris G, Grossi MP, Balboni PG, Lanza G, and Barbanti-Brodano G (1978) Ependymomas malignant tumors of pancreatic islets and osteosarcomas induced in hamsters by BK virus a human papovavirus. **J Natl Cancer Inst** 61, 875-83.
- Corallini A, Altavilla G, Carra L, Grossi MP, Federspil G, Caputo A, Negrini M, and Barbanti-Brodano G (1982) Oncogenicity of BK virus for immunosuppressed hamsters. **Arch Virol** 73, 243-53.
- Corallini A, Pagnani M, Viadana P, Silini E, Mottes M, Milanese G, Gerna G, Vettor R, Trapella G, Silvani V, and et al (1987) Association of BK virus with human brain tumors and tumors of pancreatic islets. **Int J Cancer** 39, 60-7.
- Dalrymple SA, and Beemon KL (1990) BK virus T antigens induce kidney carcinomas and thymoproliferative disorders in transgenic mice. **J Virol** 64, 1182-91.
- Darbinyan A, Darbinian N, Safak M, Radhakrishnan S, Giordano A, and Khalili K (2002) Evidence for dysregulation of cell cycle by human polyomavirus JCV late auxiliary protein. **Oncogene** 21, 5574-81.
- David H, Mendoza S, Konishi T, and Miller CW (2001) Simian virus 40 is present in human lymphomas and normal blood. **Cancer Lett** 162, 57-64.
- De Luca A, Baldi A, Esposito V, Howard CM, Bagella L, Rizzo P, Caputo M, Pass HI, Giordano GG, Baldi F, Carbone M, and Giordano A (1997) The retinoblastoma gene family pRb/p105 p107 pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. **Nat Med** 3, 913-6.
- De Mattei M, Martini F, Corallini A, Gerosa M, Scotlandi K, Carinci P, Barbanti-Brodano G, and Tognon M (1995) High incidence of BK virus large-T-antigen-coding sequences in normal human tissues and tumors of different histotypes. **Int J Cancer** 61, 756-60.
- Del Valle L, Gordon J, Assimakopoulou M, Enam S, Geddes JF, Varakis JN, Katsetos CD, Croul S, and Khalili K (2001) Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. **Cancer Res** 61, 4287-93.

- Del Valle L, Delbue S, Gordon J, Enam S, Croul S, Ferrante P, and Khalili K (2002) Expression of JC virus T-antigen in a patient with MS and glioblastoma multiforme. **Neurology** 58, 895-900.
- Diamandopoulos GT (1972) Leukemia lymphoma and osteosarcoma induced in the Syrian golden hamster by simian virus 40. **Science** 176, 173-5.
- Dorries K, Loeber G, and Meixensberger J (1987) Association of polyomaviruses JC SV40 and BK with human brain tumors. **Virology** 160, 268-70.
- Dougherty RM (1976) A comparison of human papovavirus T antigens. **J Gen Virol** 33, 61-70.
- Dyson NB, RFriend SH, Gooding LR, Hassell J, Major EO (1990) Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. **J Virol** 64, 1353-6.
- Eckner R, Ludlow JW, Lill NL, Oldread E, Arany Z, Modjtahedi N, DeCaprio JA, Livingston DM, and Morgan JA (1996) Association of p300 and CBP with simian virus 40 large T antigen. **Mol Cell Biol** 16, 3454-64.
- Eddy BE, Borman GS, Grubbs GE, and Young RD (1962) Identification of the oncogenic substance in rhesus monkey cell cultures as simian virus 40. **Virology** 17, 65-75.
- Eggers C, Stellbrink HJ, Buhk T, and Dorries K (1999) Quantification of JC virus DNA in the cerebrospinal fluid of patients with human immunodeficiency virus-associated progressive multifocal leukoencephalopathy—a longitudinal study. **J Infect Dis** 180, 1690-4.
- Enam S, Del Valle L, Lara C, Gan DD, Ortiz-Hidalgo C, Palazzo JP, and Khalili K (2002) Association of Human Polyomavirus JCV with Colon Cancer: Evidence for Interaction of Viral T-Antigen and beta-Catenin. **Cancer Res** 62, 7093-101.
- Fanning E (1992) Simian virus 40 large T antigen: the puzzle the pieces and the emerging picture. **J Virol** 66, 1289-93.
- Fanning E, and Knippers R (1992) Structure and function of simian virus 40 large tumor antigen. **Annu Rev Biochem** 61, 55-85.
- Fiori M, and Di Mayorca G (1976) Occurrence of BK virus DNA in DNA obtained from certain human tumors. **Proc Natl Acad Sci U S A** 73, 4662-6.
- Franks RR, Rencic A, Gordon J, Zoltick PW, Curtis M, Knobler RL, and Khalili K (1996) Formation of undifferentiated mesenteric tumors in transgenic mice expressing human neurotropic polyomavirus early protein. **Oncogene** 12, 2573-8.
- Frisque RJ, Bream GL, and Cannella MT (1984) Human polyomavirus JC virus genome. **J Virol** 51, 458-69.
- Frisque RJ, and FAWhite (1992) "The molecular biology of JC virus causative agent of progressive multifocal leukoencephalopathy." (ERPR (ed. Ed.) Humana Press Inc, Totowa NJ.
- Frisque RJ (1998) Rearranged and chimaeric primate polyomavirus genomes. **Dev Biol Stand**, 103-13.
- Frost JA, Alberts AS, Sontag E, Guan K, Mumby MC, and Feramisco JR (1994) Simian virus 40 small t antigen cooperates with mitogen-activated kinases to stimulate AP-1 activity. **Mol Cell Biol** 14, 6244-52.
- Gallia GL, Safak M, and Khalili K (1998) Interaction of the single-stranded DNA-binding protein Puralpha with the human polyomavirus JC virus early protein T-antigen. **J Biol Chem** 273, 32662-9.
- Gan DD, Reiss K, Carrill T, Del Valle L, Croul S, Giordano A, Fishman P, and Khalili K (2001) Involvement of Wnt signaling pathway in murine medulloblastoma induced by human neurotropic JC virus. **Oncogene** 20, 4864-70.
- Gardner SD, AMFeild DV, Coleman and BHulme (1971) New human papovavirus (BK) isolated from urine after renal transplantation. **Lancet**, 1253-1257.
- Gardner SD, MacKenzie EF, Smith C, and Porter AA (1984) Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. **J Clin Pathol** 37, 578-86.
- Girardi AJ, Sweet BH, Sotnick VB, and Hilleman MR (1962) Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus SV40. **Proc Soc Exp Biol Med** 109, 649-660.
- Gordon J, Del Valle L, Otte J, and Khalili K (2000) Pituitary neoplasia induced by expression of human neurotropic polyomavirus JCV early genome in transgenic mice. **Oncogene** 19, 4840-6.
- Goudsmit J, Wetheim-van Dillen P, van Strien A, and van der Noordaa J (1982) The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. **J Med Virol** 10, 91-99.
- Greenlee JE, Narayan O, Johnson RT, and Herndon RM (1977) Induction of brain tumors in hamsters with BK virus a human papovavirus. **Lab Invest** 36, 636-41.
- Griffiths DJ, Nicholson AG, and Weiss RA (1998) Detection of SV40 sequences in human mesothelioma. **Dev Biol Stand** 94, 127-36.
- Grodzicker T, and Hopkins N, Eds (1980) Origins of contemporary DNA tumor virus research 2nd ed DNA tumor viruses Edited by JTooze Cold Spring Harbor: Cold Spring Harbor Laboratory.
- Harris KF, Christensen JB, and Imperiale MJ (1996) BK virus large T antigen: interactions with the retinoblastoma family of tumor suppressor proteins and effects on cellular growth control. **J Virol** 70, 2378-86.
- Hollstein M, Shomer B, Greenblatt M, Soussi T, Hovig E, Montesano R, and Harris CC (1996) Somatic point mutations in the p53 gene of human tumors and cell lines: updated compilation. **Nucleic Acids Res** 24, 141-6.
- Howell DN, Smith SR, Butterly DW, Klassen PS, Krigman HR, Burchette JL, Jr, and Miller SE (1999) Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. **Transplantation** 68, 1279-88.
- Kolzau T, Hansen RS, Zahra D, Reddel RR, and Braithwaite AW (1999) Inhibition of SV40 large T antigen induced apoptosis by small T antigen. **Oncogene** 18, 5598-603.
- Krieg P, Amtmann E, Jonas D, Fischer H, Zang K, and Sauer G (1981) Episomal simian virus 40 genomes in human brain tumors. **Proc Natl Acad Sci U S A** 78, 6446-50.
- Krynska B, Otte J, Franks R, Khalili K, and Croul S (1999) Human ubiquitous JCV(CY) T-antigen gene induces brain tumors in experimental animals. **Oncogene** 18, 39-46.
- Laghi L, Randolph AE, Chauhan DP, Marra G, Major EO, Neel JV, and Boland CR (1999) JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. **Proc Natl Acad Sci U S A** 96, 7484-9.
- Lassak A, Del Valle L, Peruzzi F, Wang JY, Enam S, Croul S, Khalili K, and Reiss K (2002) Insulin receptor substrate 1 translocation to the nucleus by the human JC virus T-antigen. **J Biol Chem** 277, 17231-8.
- Lednický JA, Garcea RL, Bergsagel DJ, and Butel JS (1995) Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. **Virology** 212, 710-7.
- Lednický JA, and Butel JS (1997) A coupled PCR and restriction digest method for the detection and analysis of the SV40 regulatory region in infected-cell lysates and clinical samples. **J Virol Methods** 64, 1-9.
- Levine AJ (1997) p53 the cellular gatekeeper for growth and division. **Cell** 88, 323-331.

- London WT, Houff SA, Madden DL, Fuccillo DA, Gravell M, Wallen WC, Palmer AE, Sever JL, Padgett BL, Walker DL, ZuRhein GM, and Ohashi T (1978) Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). **Science** 201, 1246-9.
- London WT, Houff SA, McKeever PE, Wallen WC, Sever JL, Padgett BL, and Walker DL (1983) Viral-induced astrocytomas in squirrel monkeys. **Prog Clin Biol Res** 105, 227-37.
- Major EO, Amemiya K, Tornatore CS, Houff SA, and Berger JR (1992) Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy the JC virus-induced demyelinating disease of the human brain. **Clin Microbiol Rev** 5, 49-73.
- Major OM, Amemiya K, Tornatore CS, Houff SA, and Berger JR, (1992) Pathogenesis and molecular biology of progressive multifocal encephalopathy The JC virus-induced demyelinating disease of the human brain. **Clin Microbiol Rew** 5, 49-73.
- Martini F, Iaccheri L, Lazzarin L, Carinci P, Corallini A, Gerosa M, Iuzzolino P, Barbanti-Brodano G, and Tognon M (1996) SV40 early region and large T antigen in human brain tumors peripheral blood cells and sperm fluids from healthy individuals. **Cancer Res** 56, 4820-5.
- Melnick JL, and Stinebaugh S (1962) Excretion of vacuolating SV-40 virus (papova virus group) after ingestion as a contaminant of oral poliovaccine. **Proc Soc Exp Biol Med** 109, 965-968.
- Monaco MC, Atwood WJ, Gravell M, Tornatore CS, and Major EO (1996) JC virus infection of hematopoietic progenitor cells primary B lymphocytes and tonsillar stromal cells: implications for viral latency. **J Virol** 70, 7004-12.
- Monaco MC, Jensen PN, Hou J, Durham LC, and Major EO (1998a) Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection. **J Virol** 72, 9918-23.
- Monaco MC, Shin J, and Major EO (1998b) JC virus infection in cells from lymphoid tissue. **Dev Biol Stand** 94, 115-22.
- Monaco MC, Sabath BF, Durham LC, and Major EO (2001) JC virus multiplication in human hematopoietic progenitor cells requires the NF-1 class D transcription factor. **J Virol** 75, 9687-95.
- Monier R (1986) Transformation by SV40 and polyomaviruses In "The polyomaviruses" (NPSalzman Ed. Vol1 Plenum Press New York.
- Monini P, De Lellis L, and Barbanti-Brodano G (1995) Association of BK and JC human polyomaviruses and SV40 with human tumors In "DNA tumor viruses: Oncogenic mechanisms" (GBarbanti-Brodano Bendinelli MFriedman H, Ed. pp51-73 Plenum Press: New York.
- Monini P, Rotola A, de Lellis L, Corallini A, Secchiero P, Albini A, Benelli R, Parravicini C, Barbanti-Brodano G, and Cassai E (1996) Latent BK virus infection and Kaposi's sarcoma pathogenesis. **Int J Cancer** 66, 717-22.
- Nase LM, Karkkainen M, and Mantyjarvi RA (1975) Transplantable hamster tumors induced with the BK virus. **Acta Pathol Microbiol Scand [B]** 83, 347-52.
- Noss G, Stauch G, Mehraein P, and Georgii A (1981) Oncogenic activity of the BK type of human papova virus in newborn Wistar rats. **Arch Virol** 69, 239-51.
- Noss G, and Stauch G (1984) Oncogenic activity of the BK type of human papova virus in inbred rat strains. **Arch Virol** 81, - 2 41-51.
- Ozer HL, Banga SS, Dasgupta T, Houghton J, Hubbard K, Jha KK, Kim SH, Lenahan M, Pang Z, Pardinias JR, and Patsalis PC (1996) SV40-mediated immortalization of human fibroblasts. **Exp Gerontol** 31, 303-10.
- Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. (1971) Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. **Lancet** 1,1257-1260.
- Padgett BL, Walker DL, Desquitado MM, and Kim DU (1983) BK virus and non-haemorrhagic cystitis in a child. **Lancet** 1, 770.
- Pallas DC, Shahrik LK, Martin BL, Jaspers S, Miller TB, Brautigam DL, and Roberts TM (1990) Polyoma small and middle T antigens and SV40 small t antigen form stable complexes with protein phosphatase 2A. **Cell** 60, 167-76.
- Peterman TA, Jaffe HW, and Beral V (1993) Epidemiologic clues to the etiology of Kaposi's sarcoma. **Aids** 7, 605-11.
- Rabson AS, O'Connor GT, Kirschstein RL, and Branigan WJ (1962) Papillary ependymomas produced in Rattus (Mastomys) natalensis inoculated with vacuolating virus (SV40). **J Natl Cancer Inst** 29, 765-787.
- Rencic A, Gordon J, Otte J, Curtis M, Kovatich A, Zoltick P, Khalili K, and Andrews D (1996) Detection of JC virus DNA sequence and expression of the viral oncoprotein tumor antigen in brain of immunocompetent patient with oligoastrocytoma. **Proc Natl Acad Sci U S A** 93, 7352-7.
- Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, and Boland CR (2001) Mad-1 is the exclusive JC virus strain present in the human colon and its transcriptional control region has a deleted 98-base-pair sequence in colon cancer tissues. **J Virol** 75, 1996-2001.
- Ricciardiello L, Laghi L, Ramamirtham P, Chang CL, Chang DK, Randolph AE, and Boland CR (2000) JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. **Gastroenterology** 119, 1228-35.
- Richardson EP (1961) Progressive multifocal encephalopathy. **New Engl J Med** 265, 815-823.
- Rizzo P, Di Resta I, Powers A, Matker CM, Zhang A, Mutti L, Kast WM, Pass H, and Carbone M (1998) The detection of simian virus 40 in human tumors by polymerase chain reaction. **Monaldi Arch Chest Dis** 53, 202-10.
- Rizzo P, Carbone M, Fisher SG, Matker C, Swinnen LJ, Powers A, Di Resta I, Alkan S, Pass HI, and Fisher RI (1999) Simian virus 40 is present in most United States human mesotheliomas but it is rarely present in non-Hodgkin's lymphoma. **Chest** 116, 470S-473S.
- Safak M, Gallia GL, Ansari SA, and Khalili K (1999) Physical and functional interaction between the Y-box binding protein YB-1 and human polyomavirus JC virus large T antigen. **J Virol** 73, 10146-57.
- Safak M, Barrucco R, Darbinyan A, Okada Y, Nagashima K, and Khalili K (2001) Interaction of JC virus agno protein with T antigen modulates transcription and replication of the viral genome in glial cells. **J Virol** 75, 1476-86.
- Safak M, Sadowska B, Barrucco R, and Khalili K (2002) Functional interaction between JC virus late regulatory agnoprotein and cellular Y-box binding transcription factor YB-1. **J Virol** 76, 3828-38.
- Shah KV, Daniel RW, and Strandberg JD (1975) Sarcoma in a hamster inoculated with BK virus a human papovavirus. **J Natl Cancer Inst** 54, 945-50.
- Shaw P, Bovey R, Tardy S, Sahli R, Sodat B, and Costa J (1992) Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. **Proc Natl Acad Sci U S A** 89, 4495-4499.
- Shein HM (1967) Transformation of astrocytes and destruction of spongioblasts induced by a simian tumor virus (SV40) in cultures of human fetal neuroglia. **J Neuropathol Exp Neurol** 26, 60-76.
- Sheppard HM, Corneillie SI, Espiritu C, Gatti A, and Liu X (1999) New insights into the mechanism of inhibition of p53

- by simian virus 40 large T antigen. **Mol Cell Biol** 19, 2746-53.
- Shintaku M, Matsumoto R, Sawa H, and Nagashima K (2000) Infection with JC virus and possible dysplastic ganglion-like transformation of the cerebral cortical neurons in a case of progressive multifocal leukoencephalopathy. **J Neuropathol Exp Neurol** 59, 921-9.
- Shivakumar CV, and Das GC (1996) Interaction of human polyomavirus BK with the tumor-suppressor protein p53. **Oncogene** 13, 323-32.
- Shivapurkar N, Wiethage T, Wistuba II Milchgrub S, Muller KM, and Gazdar AF (2000) Presence of simian virus 40 sequences in malignant pleural peritoneal and noninvasive mesotheliomas. **Int J Cancer** 85, 743-5.
- Sima AAF, SDMclachlan DR (1983) Multiple malignant astrocytomas in a patient with spontaneous progressive multifocal leukoencephalopathy. **Ann Neurol** 14, 183-188.
- Small JA, Khoury G, Jay G, Howley PM, and Scangos GA (1986a) Early regions of JC virus and BK virus induce distinct and tissue- specific tumors in transgenic mice. **Proc Natl Acad Sci U S A** 83, 8288-92.
- Small JA, Scangos GA, Cork L, Jay G, and Khoury G (1986b) The early region of human papovavirus JC induces dysmyelination in transgenic mice. **Cell** 46, 13-8.
- Sontag E, Fedorov S, Kamibayashi C, Robbins D, Cobb M, and Mumby M (1993) The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. **Cell** 75, 887-97.
- Soriano F, Shelburne CE, and Gokcen M (1974) Simian virus 40 in a human cancer. **Nature** 249, 421-4.
- Srinivasan A, McClellan AJ, Vartikar J, Marks I, Cantalupo P, Li Y, Whyte P, Rundell K, Brodsky JL, and Pipas JM (1997) The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. **Mol Cell Biol** 17, 4761-73.
- Sweet BH, and Hilleman MR (1960) The vacuolating virus S.V.40. **Proc Soc Exp Biol Med** 105, 420-427.
- Testa JR, Carbone M, Hirvonen A, Khalili K, Krynska B, Linnainmaa K, Pooley FD, Rizzo P, Rusch V, and Xiao GH (1998) A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. **Cancer Res** 58, 4505-9.
- Tognon M, Casalone R, Martini F, De Mattei M, Granata P, Minelli E, Arcuri C, Collini P, and Bocchini V (1996) Large T antigen coding sequences of two DNA tumor viruses BK and SV40 and nonrandom chromosome changes in two glioblastoma cell lines. **Cancer Genet Cytogenet** 90, 17-23.
- Trapp BD, Small JA, Pulley M, Khoury G, and Scangos GA (1988) Dysmyelination in transgenic mice containing JC virus early region. **Ann Neurol** 23, 38-48.
- Uchida S, Watanabe S, Aizawa T, Furuno A, and Muto T (1979) Polyoncogenicity and insulinoma-inducing ability of BK Virus a human Papovavirus in Syrian golden hamsters. **J Natl Cancer Inst** 63, 119-26.
- Uchida S, Watanabe S, Aizawa T, Kato K, and Furuno A (1976) Induction of papillary ependymomas and insulinomas in the Syrian golden hamster by BK virus a human papovavirus. **Gann** 67, 857-65.
- van der Noordaa J (1976) Infectivity oncogenicity and transforming ability of BK virus and BK virus DNA. **J Gen Virol** 30, 371-3.
- Varakis J, ZuRhein GM, Padgett BL, and Walker DL (1978) Induction of peripheral neuroblastomas in Syrian hamsters after injection as neonates with JC virus a human polyoma virus. **Cancer Res** 38, 1718-22.
- Walker DL, Padgett BL, ZuRhein GM, Albert AE, and Marsh RF (1973) Human papovavirus (JC): induction of brain tumors in hamsters. **Science** 181, 674-6.
- Watanabe S, Yoshiike K, Nozawa A, Yuasa Y, and Uchida S (1979) Viable deletion mutant of human papovavirus BK that induces insulinomas in hamsters. **J Virol** 32, 934-42.
- Watanabe S, and Yoshiike K (1982) Change of DNA near the origin of replication enhances the transforming capacity of human papovavirus BK. **J Virol** 42, 978-85.
- Weiss AF, Portmann R, Fischer H, Simon J, and Zang KD (1975) Simian virus 40-related antigens in three human meningiomas with defined chromosome loss. **Proc Natl Acad Sci U S A** 72, 609-13.
- Yang SI, Lickteig RL, Estes R, Rundell K, Walter G, and Mumby MC (1991) Control of protein phosphatase 2A by simian virus 40 small-t antigen. **Mol Cell Biol** 11, 1988-95.
- Zhen HN, Zhang X, Bu XY, Zhang ZW, Huang WJ, Zhang P, Liang JW, and Wang XL (1999) Expression of the simian virus 40 large tumor antigen (Tag) and formation of Tag-p53 and Tag-pRb complexes in human brain tumors. **Cancer** 86, 2124-32.



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