Minireview

Polyomaviruses and human cancer: molecular mechanisms underlying patterns of tumorigenesis

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Abstract

Polyomaviruses are DNA tumor viruses with small circular genomes encoding only six proteins including three structural capsid proteins. Despite this simplicity, our understanding of the mechanisms of polyomavirus-mediated tumorigenesis is far from complete. The archetypal primate polyomavirus, SV40, was isolated more than 40 years ago and has been used extensively as a model system for the study of basic eukaryotic cellular processes such as DNA replication and transcription. Two human polyomaviruses have been isolated from clinical samples: JC virus (JCV) and BK virus (BKV). In this review, SV40, JCV, and BKV will be compared based on what is known about their molecular biology from experiments performed in vitro, in cell culture and in laboratory animals. The association of these viruses with clinical tumors is discussed along with the possible roles of these polyomaviruses in the etiology of human malignant disease.

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Introduction

Polyomaviruses are a genus of non-enveloped DNA viruses with icosahedral capsids containing small, circular, double-stranded DNA genomes. These viruses have been isolated from humans, monkeys, rodents, and birds. Each polyomavirus has a limited host range and does not usually produce productive infections in other species (Cole, 1996; Imperiale, 2001).

Mouse polyoma virus (Stewart et al., 1958) and simian vacuolating virus 40 (SV40) (Sweet and Hilleman, 1960) were the first polyomaviruses to be discovered. As its name implies, mouse polyoma virus is able to induce a variety of different tumors when it is inoculated into newborn mice. These two archetypal polyomaviruses have been intensively studied and have provided a model system for the study of basic eukaryotic cell processes including DNA replication, transcription, malignant transformation, and signal transduction.

In 1971, two human polyomaviruses were discovered. BK virus was isolated from the urine of a kidney allograft recipient with chronic pyelonephritis and advanced renal failure (Gardner et al., 1971). JC virus was isolated from the brain of a patient suffering from progressive multifocal leukoencephalopathy (Padgett et al., 1971). The designations JC and BK were derived from the initials of the patients involved.

Both BK and JC viruses are very similar to SV40 with respect to size (~5.2 kbp), genome organization, and DNA sequence. The circular genome of these polyomaviruses contains two regions of approximately equal size known as the early and late transcription units. Transcription of both units is initiated from a common regulatory region at the origin of DNA replication (ori) with early transcription proceeding in a counterclockwise direction. Late transcription proceeds from ori in the opposite direction and goes around the genome in a clockwise direction (Cole, 1996; Imperiale, 2001). The late region encodes the capsid structural proteins VP1, VP2, and VP3 that are encoded by alternatively spliced mRNAs derived from the same primary late transcript and a small regulatory protein known as Agnoprotein that is discussed below. The early region encodes the alternatively spliced transforming proteins: large T antigen (T-Ag) and small t antigen (t-Ag). These proteins are important in promoting transformation of cells in culture and oncogenesis in vivo, as discussed below. The polyomavirus virion consists of a capsid containing VP1, VP2, and VP3 surrounding a single molecule of supercoiled DNA.
viral DNA complexed with the four cellular nucleosomal histones, H2A, H2B, H3, and H4, which is often referred to as the viral minichromosome (Chen et al., 1979; Tan, 1977). After infection, the viral minichromosome facilitates viral transcription and contains histone H1.

There are two possible outcomes to infection of a cell by a polyomavirus. Permissive cells are cells that are able to support viral DNA replication and the result of polyomavirus entry is a lytic infection with viral amplification. Nonpermissive cells are cells that do not support viral DNA replication and the result of viral entry is an abortive infection or cell transformation (oncogenesis). Two examples illustrate this distinction. Firstly, when SV40 infects nonpermissive mouse cells, for example, 3T3 fibroblasts, species-related differences block viral DNA replication and cell transformation occurs (Todaro and Green, 1966). Experiments with cell extract suggest that the species-related block in SV40 replication is caused by an inability of the primate virus to use the mouse host DNA polymerase (Murakami et al., 1986). Secondly, when SV40 infects permissive monkey kidney cell lines, the virus replicates and cell lysis occurs.

Most human cells represent a unique intermediate status with respect to SV40 infection. When SV40 infects human fibroblasts, DNA replication is only partially effective and the virus is amplified and cell transformation occurs (discussed below). Another example of the intermediate status of human cells with respect to SV40 is afforded by human lymphoblastoid B-cell lines that grow in culture, yet produce SV40 at a low level (Dolcetti et al., 2003).

However, in human mesothelial cells, cell-type-specific differences completely block viral replication, and in this nonpermissive situation, cells are particularly amenable to transformation. This may have important implications in human mesothelioma, as discussed below (Bocchetta et al., 2000). The purpose of this review is to explore what is known about the molecular biology of the SV40, BK, and JCV polyomaviruses and how this may be relevant to their relative involvement in human cancer.

Life cycle of polyomaviruses

The infection of cells by polyomaviruses is initiated by the binding of the virion to a receptor on the outside of the cell membrane. In the case of SV40, this receptor has been identified as the major histocompatibility complex (MHC) (Norkin, 1999). Because nearly every cell type expresses MHC molecules, it would be expected that SV40 should be able to infect most cells. In the case of BKV and JCV, the cell receptors are not fully understood. It has been reported that JCV binds specifically to certain cell types (glial cells, tonsillar stromal cells, and B but not T lymphocytes) that reflect JCV’s cell tropism (Wei et al., 2000). However, more recent reports indicate that JCV can enter a broad spectrum of mammalian cell types through binding to oligosaccharides ubiquitously present on glycoproteins and glycolipids on the cell membrane. After the viral DNA enters the nucleus, cell-specific intranuclear mechanisms suppressed JCV DNA replication in all cells except human neuroblastoma cell lines in these experiments (Komagome et al., 2002; Suzuki et al., 2001). In the human body, it is thought that JCV can infect cells in the tonsils and spread from there by replication in lymphoid cells (Monaco et al., 1998). BKV can also replicate in human lymphocytes (Portolani et al., 1985). JCV and BKV can infect cells in the kidney, which is the major organ of JCV and BKV persistence during latency (Chesters et al., 1983). In monkeys, initial lytic infection by SV40 is controlled by the immune system with virus then persisting in the kidney where it may be reactivated by immunosuppression (Horvath et al., 1992). The life cycle of SV40 in humans is poorly understood (Garcea and Imperiale, 2003).

After binding to the cell surface, polyomavirus capsids undergo endocytosis and are transported to the nucleus where the viral DNA is uncoated and transcription of the early region begins. Interestingly, SV40 enters cells by caveola-mediated endocytosis (Anderson et al., 1996) although JCV employs a clathrin-dependent mechanism that is blocked by tyrosine-specific protein kinase inhibition (Querbes et al., 2004).

The primary transcript from the early region is alternatively spliced to give two mRNAs that encode T-Ag and t-Ag. T-Ag is a large nuclear phosphoprotein and is an essential factor for viral DNA replication. It binds to the viral origin of replication region (ori) where it promotes unwinding of the double helix and recruitment of cellular proteins that are required for DNA synthesis including DNA polymerase-α and replication protein A (Dean et al., 1987; Dornreiter et al., 1990; Melendy and Stillman, 1993; Stahl et al., 1986).

Polyomaviruses rely on cellular enzymes and cofactors for DNA replication and these proteins are expressed in S phase. Another major function of T-Ag is to modulate cellular signaling pathways to induce cells to enter S phase and this accounts for the ability of T-Ag to transform cells. T-Ag is thought to stimulate the cell cycle through its ability to bind to several cellular proteins that are involved and in crucial signal transduction pathways that control cell cycle progression and apoptosis. These functions of T-Ag are discussed below. What is known about T-Ag function for each polyomavirus is summarized in Table 1.

The role of the small t-Ag in the polyomavirus life cycle is less clear. Analysis of SV40 deletion mutants revealed that t-Ag is not essential for lytic infection in culture (Shenk et al., 1976). However, t-Ag cooperates with T-Ag in the transformation of cells by SV40 (Martin et al., 1979; Sleigh et al., 1978) and increases virus yield in permissive cell infections (Rundell and Parakati, 2001). The mechanism of action of t-Ag is discussed below.

As viral replication proceeds, the late genes begin to be expressed. T-Ag acts to stimulate transcription from the
late promoter and repress transcription from the early promoter. The gene products of the late region are the capsid proteins VP1, VP2, and VP3, which assemble with the replicated viral DNA to form virions, which are released upon cell lysis.

Polyomavirus DNA can become integrated into the chromosomal DNA of the cell especially upon nonpermissive infection. This was first described for SV40 (Hirai et al., 1971) and is also the case for JCV (Mandl and Frisque, 1980) and BKV (Chenciner et al., 1980). Integration occurs at random both in terms of the site in the cellular chromosomal DNA of the cell especially upon nonpermissive infection (Mandl and Frisque, 1971) and is also the case for JCV (Mandl and Frisque, 1980) and BKV (Chenciner et al., 1980). Integration occurs at random both in terms of the site in the cellular genome where integration occurs and the point in the viral genome where recombination occurs (Hara and Kaji, 1987).

Infections in humans by polyomaviruses are usually restricted by the actions of the immune system, particularly cell-mediated immunity. For JCV, a general impairment of the Th1-type T-helper cell function of cell-mediated immunity has been found in the disease progressive multifocal leukoencephalopathy (PML) (Weber et al., 2001). PML is discussed below. Cell-mediated immunity is also important in BKV infection (Drummond et al., 1985).

### Mechanisms of oncogenic transformation by polyomaviruses

Polyomavirus genomes do not encode replication proteins and so they must drive cells into S phase where host DNA replication proteins are produced. A major mechanism by which this is achieved is through the action of the early proteins large T-Ag and small t-Ag (discussed below). T-Ag interferes with two tumor suppressor proteins that regulate cell cycle progression, pRb and p53. This aberrant stimulation of the cell cycle is a driving force for oncogenic transformation.

pRb sequesters the E2F family of transcription factors that promote the G1/S phase transition thereby preventing expression of E2F-dependent genes such as $c-fos$ and $c-myc$ whose products are needed for entry into S phase. During the cell cycle, CDK4 and CDK6 cause phosphorylation of pRb in late G1 allowing release of the E2F transcription factors allowing S phase progression (Weinberg, 1995, 1996). The T-Ags of all three polyomaviruses bind to pRb and displace E2F thereby promoting cell cycle progression. This is a major mechanism whereby T-Ag promotes the inappropriate cell proliferation characteristic of oncogenically transformed cells (Cress and Nevins, 1996; DeCaprio et al., 1988; Harris et al., 1996; Krynska et al., 1997). The Rb-binding domain of T-Ag is highly conserved between JCV and BKV but not between JCV/BKV and SV40 (Table 2) perhaps explaining the lower affinity for pRb exhibited by JCV and BKV T-Ags compared to SV40 T-Ag (Harris et al., 1998; Tavis et al., 1994).

p53 protein is a tumor suppressor encoded by a gene whose disruption is associated with ~50–55% of all human cancers. p53 protein acts as a checkpoint in the cell cycle and regulates apoptosis. The release of E2F from pRb by T-Ag activates p14ARF expression that leads to the stabilization of p53. However, polyomavirus T-Ags bind to and inactivate p53 and thus prevent inhibition of the cell cycle or apoptosis (Fig. 1). This is important for providing an optimal cell environment for viral replication and packaging during polyomavirus lytic infection and facilitates transformation in nonpermissive cells (Levine, 1997; Vogelstein et al., 2000). Interaction of T-Ag with p53 has been demonstrated for SV40 (Pipas and Levine, 2001) and for JCV and BKV (Bollag et al., 1989). It has been reported that the subpopulation of T antigen that forms a stable complex with cellular p53 protein was smaller in JCV-transformed cells than in SV40- or BKV-transformed cells (Bollag et al., 1989).

### Table 1

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Virus</th>
<th>Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large T-Ag Binding to pRb</td>
<td>SV40</td>
<td>/</td>
<td>DeCaprio et al., 1988</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Krynska et al., 1997</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>/</td>
<td>Harris et al., 1996</td>
</tr>
<tr>
<td>Binding to p53</td>
<td>SV40</td>
<td>/</td>
<td>Pipas and Levine, 2001</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Bollag et al., 1989</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>/</td>
<td>Bollag et al., 1989</td>
</tr>
<tr>
<td>J domain</td>
<td>SV40</td>
<td>/</td>
<td>Sullivan and Pipas, 2002</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Sullivan et al., 2000</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Binding to IRS-1</td>
<td>SV40</td>
<td>/</td>
<td>Fei et al., 1995; Prisco et al., 2002</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Khalili et al., 2003a, 2003b; Lassak et al., 2002</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Binding to β-catenin</td>
<td>SV40</td>
<td>/</td>
<td>Enam et al., 2002; Gan et al., 2001</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Theile and Grabowski, 1990; Ray et al., 1990; Neel et al., 1996; Ricciardiello et al., 2003; Theile and Grabowski, 1990</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td>Theile and Grabowski, 1990; Trabaneli et al., 1998</td>
</tr>
<tr>
<td>Induction of genetic instability</td>
<td>SV40</td>
<td>/</td>
<td>Theile and Grabowski, 1990; Ray et al., 1992; Neel et al., 1996; Ricciardiello et al., 2003; Theile and Grabowski, 1990</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>/</td>
<td>Theile and Grabowski, 1990; Trabaneli et al., 1998</td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Agnoprotein</td>
<td>SV40</td>
<td>?</td>
<td>Del Valle et al., 2002</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Small t-Ag Inactivation of PP2A</td>
<td>SV40</td>
<td>/</td>
<td>Sontag et al., 1993</td>
</tr>
<tr>
<td></td>
<td>JCV</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BKV</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

The three polyomavirus proteins that have been shown to be involved in transformation are listed on the left together with their known mechanisms of action. For each virus, a “/” in the status column indicates that this mechanism has been demonstrated to occur. A “?” in the status column indicates that it is presently unknown whether the mechanism is utilized by the particular virus.
reviewed recently (Khalili et al., 2003a, 2003b). The con-
mation in childhood medulloblastomas and this has been
with IRS-1 contributes to the process of malignant transfor-
There is growing evidence that the interaction of JCV T-Ag
Table 2
Conservation of amino acid sequences between polyomavirus oncoproteins

<table>
<thead>
<tr>
<th></th>
<th>JC/BK (%)</th>
<th>JC/SV40 (%)</th>
<th>BK/SV40 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large T</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>(1–708)</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>J domain</td>
<td>(1–100)</td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>Rb domain</td>
<td>(101–118)</td>
<td>88</td>
<td>71</td>
</tr>
<tr>
<td>DNA binding</td>
<td>(131–259)</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>p53/ATPase</td>
<td>(350–610)</td>
<td>85</td>
<td>79</td>
</tr>
<tr>
<td>C terminus</td>
<td>(620–708)</td>
<td>65</td>
<td>47</td>
</tr>
<tr>
<td><strong>Small t</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>(1–172)</td>
<td>78</td>
<td>67</td>
</tr>
<tr>
<td>J domain</td>
<td>(1–83)</td>
<td>89</td>
<td>82</td>
</tr>
<tr>
<td>PP2A domain</td>
<td>(84–172)</td>
<td>69</td>
<td>56</td>
</tr>
<tr>
<td><strong>Agnoprotein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>(1–62)</td>
<td>80</td>
<td>59</td>
</tr>
</tbody>
</table>

The table shows the percentage of amino acid (aa) sequence identity between
the oncogenes of the primate polyomaviruses and selected subregions. Sequences were extracted from GenBank for the Mad-1 strain of JCV (J02226), the Dunlop strain of BKV (V01108) and the 776 strain of SV40 (AF316139). Large T antigens were 688, 695, and 708 aas long for JCV, BKV, and SV40, respectively: for small t, 172, 172, and 174 aas: for Agnoproteins, 66, 71, and 62 aas. The numbers in brackets show the position relative to the N terminus of the SV40 protein. The position of the domains within SV40 T/ t antigens was taken from information presented in two recent reviews (Frisque and White, 1992; Saenz-Robles et al., 2001).

Overall T-Ag is highly conserved with functional domains showing even higher degrees of homology with two exceptions. The C terminus of T-Ag (which determines host range) is much less conserved. The Rb-binding domain of T-Ag is highly conserved between JCV and BKV (88%) but not between JCV/BKV and SV40 (71/67%). This may provide an explanation for the observation that JCV and BKV T-Ags do not bind pRb as well as SV40 T-Ag (Tavis et al., 1994; Harris et al., 1998).

Small t can be divided into two regions based on the position of occurrence of alternative splicing (between aa 83 and aa 84). The N terminus is identical to aas 1–83 in the J domain of T-Ag and similarly shows a high degree of conservation. The C terminus which, in SV40, binds PP2A (84–628), and the C terminus of T-Ag spanning residues 82–628, and the C terminus of JCV within SV40 T/ t antigens was taken from information presented in two recent reviews (Frisque and White, 1992; Saenz-Robles et al., 2001).

Recently, it has become apparent that T-Ag can modulate other signaling proteins besides pRb and p53. JCV T-Ag has been shown to directly bind to insulin receptor substrate 1 (IRS-1) and cause it to be translocated to the nucleus. A dominant negative mutant of IRS-1 inhibited growth and survival of JCV T-Ag-transformed cells in anchorage-independent culture conditions demonstrating the importance of this interaction in cell transformation (Lassak et al., 2002). There is growing evidence that the interaction of JCV T-Ag with IRS-1 contributes to the process of malignant transformation in childhood medulloblastomas and this has been reviewed recently (Khalili et al., 2003a, 2003b). The consequences of IRS-1 nuclear translocation are still being characterized but one possibility is that it may be important in Rad51 trafficking and homologous recombination-directed DNA repair (Trojanek et al., 2003). There is also evidence that interaction with IRS-1 is involved in transfor-
mation by SV40 T-Ag (Fei et al., 1995; Prisco et al., 2002), but a role for IRS-1 in BKV T-Ag transformation has not been explored (Table 1).

JCV T-Ag has also been shown to directly bind to β-catenin (Enam et al., 2002; Gan et al., 2001). The interaction of T-Ag with β-catenin occurs through the central domain of T-Ag spanning residues 82–628, and the C terminus of β-catenin located between amino acids 695 and 781. The association of T-Ag with β-catenin increases the level of β-catenin in the cell due to increased protein stability (Gan et al., 2001). This interaction causes β-catenin to translocate to the nucleus where it enhances expression of genes such as c-myc and cyclin D1. These observations ascribe a new mechanism for the deregulation of the Wnt pathway through stabilization of β-catenin. The Wnt pathway signals to the downstream transcription factors TCF-4 and LEF-1 (Wong and Pignatelli, 2002). These signaling events may be important in certain human cancers that are associated with JCV such as medulloblastoma and colon cancer (Coyle-Rink et al., 2002; Enam et al., 2002; Gan et al., 2001).

Recently, it has been shown that Epstein–Barr virus also activates Wnt pathway signaling in epithelial cells (Morri-

![Fig. 1. Functions of large T antigen. Large T antigen (T-Ag) binds to the retino-
An important conserved motif known as the J domain is found at the N terminus of T-Ag. This was discovered from sequence similarity between the N terminus of T-Ag and the J class of chaperonins (Sullivan and Pipas, 2002). Domain-swapping experiments have shown that the N terminus of T-Ag can functionally substitute for the J domain of E. coli DnaJ (Kelley and Georgopoulos, 1997). J proteins are cochaperonins for the DnaK (Hsp70) family of proteins (Sullivan and Pipas, 2002). The N terminus of T-Ag functions as a J protein in assays in vitro, for example, stimulation of bovine Hsp70 ATP-ase activity (Srinivasan et al., 1997). The J domain is essential for many of the functions of T-Ag including DNA replication. In addition to the Rb-binding domain (amino acid residues 101–118), the J domain of T-Ag is required in cis for binding to the Rb family of proteins. The role of the J domain in other aspects of transformation is complex and has been reviewed recently (Sullivan and Pipas, 2002). Although much remains to be elucidated about the exact biophysical nature of the interactions of T-Ag and cellular proteins, it seems likely that the chaperonin functionality of T-Ag will turn out be an important determinant in the ability of T-Ag to bind to many of its cellular targets. Thus, T-Ag is a highly multifunctional protein that can bind a plethora of cellular proteins and affect many cellular pathways of growth control.

As well as being able to transform cells by virtue of interacting with cellular signaling proteins, the T-Ag of primate polyomaviruses has a mutagenic effect on cellular DNA. It seems likely that secondary mutations induced by T-Ag contribute to the tumorigenicity of infected cells. Theile and Grabowski (1990) reported increases in spontaneous mutation frequencies up to 100-fold when cultured cells were infected with BKV. The variant strain BKV-IR, which does not express small t-Ag, was also mutagenic. Besides cultured cells, BKV was also able to induce gene mutations in human peripheral blood lymphocytes. These studies also compared the mutagenicities of DNAs from BKV, JCV, and the mutagenic effects of the three viruses were essentially the same.

Primate polyomavirus T antigen also induces karyotypic changes. Human fibroblasts transfected with plasmid containing the BKV early region exhibited cytogenetic damage including deletions and translocations. This damage preceded morphological transformation (Trabanelli et al., 1998). Ricciardiello et al. (2003) showed that JCV caused chromosomal instability in human colonic cells. (A role for JCV in colon cancer is discussed below.) Ray et al. (1990, 1992) constructed a plasmid containing SV40 large T-Ag driven by the Rous sarcoma virus promoter but lacking small t-Ag and the SV40 origin. When this was transfected into human fibroblasts, 99% of T-Ag-positive clones exhibited numerical or structural chromosome aberrations. These changes were evident before the transformation indicators of the clones were positive and continued throughout neoplastic progression. Antibody titers to BKV and JCV have been correlated with the occurrence of “rogue cells”—lymphocytes with multiple chromosome aberrations (Lazutka et al., 1996; Neel et al., 1996). Recently, it has been reported that SV40 T-Ag interferes with DNA repair (Digweed et al., 2002).

In the case of SV40 T-Ag, recent research has revealed a possible molecular mechanism for the induction of chromosomal instability. It is known that karyotypic changes are induced by large T-Ag but not small t-Ag in human diploid fibroblasts and embryonic kidney cells (Stewart and Bachetti, 1991), and this function maps to the N-terminal 147 amino acid residues of T-Ag (Woods et al., 1994). Using the yeast two-hybrid system with the N terminus of SV40 T-Ag as bait, it has recently been shown that T-Ag binds to the mitotic checkpoint protein Bub-1 (Cotsiki et al., 2004). T-Ag is known to disrupt the mitotic checkpoint and attenuate radiation-induced mitotic delay (Chang et al., 1997), suggesting that this is a mechanism of T-Ag induction of chromosome aberrations and aneuploidy.

Taken together, these data indicate that genetic instability induced by T-Ag is likely an important contributor to polyomavirus-induced oncogenesis. Because p53 is inactivated by T-Ag, the elimination of mutated cells by p53-mediated apoptosis would be expected to be impaired. This is likely to be of central importance in the etiology of cancer after polyomavirus infection. Although the inactivation of pRb, p53, and other cellular proteins by T-Ag is enough to dysregulate the cell cycle and produce a “transformed phenotype” in cultured cells, apparently, acquisition of other mutations is necessary for cells to be able to grow progressively in vivo (tumorigenicity). In this regard, transformation can clearly be separated from tumorigenicity in somatic cell genetic experiments (Gee and Harris, 1979). There are many phenotypic cellular and biochemical changes that are associated with transformation by T-Ag, and these include growth to high saturation density, focus formation, growth in soft agar, serum-independence, morphological changes, elevated glucose uptake, plasminogen activator production, and so forth (Saenz-Robles et al., 2001). The known molecular mechanisms of transformation for each polyomavirus are summarized in Table 1.

The second of the two proteins encoded by the early region of primate polyomaviruses is small t antigen (t-Ag). The N-terminal 82 amino acids are the same as the N terminus of large T-Ag but the C terminus is a unique domain that is incorporated by alternative splicing of the early region primary transcript. Published research on primate polyomavirus small t-Ag has concentrated almost exclusively on SV40. SV40 t-Ag has a mitogenic role in the transformation of cells (Martin et al., 1979; Sleigh et al., 1978). Protein phosphatase 2A (PP2A) is bound and inhibited by SV40 small t-Ag (Pallas et al., 1990). Phosphatases are negative regulators of growth promoting signal transduction pathways. PP2A is the major serine-
or threonine-specific protein phosphatase of eukaryotic cells (Cohen, 1997). By inactivating the negative regulator PP2A, t-Ag is able to activate several pathways that promote cell proliferation including the centrally important mitogen-activated protein kinase (MAPK) pathway (Sontag et al., 1993). It is not clear if this is true for JCV and BKV (Table 1), and indeed the C terminus of t-Ag (where PP2A binds) for these viruses is only moderately homologous to that of SV40 (Table 2). However, the t-Ag of all three viruses contains two conserved cysteine cluster motifs (CXCXXC) (Pipas, 1992) that are thought to be involved in the interaction of t-Ag with PP2A (Mateer et al., 1998). Indeed, two cellular proteins were specifically co-immunoprecipitated with BK virus t-Ag that may be subunits of PP2A (Rundell et al., 1981).

In addition to T-Ag and t-Ag, there is emerging evidence for a role in cell transformation for a third viral protein. A small protein known as Agnoprotein is encoded near the 5′ end of the late region. Agnoprotein is 62, 71, and 66 amino acid residues long for SV40, JCV, and BKV, respectively (Cole, 1996), and is produced late in the infectious cycle although it is not incorporated into virions (Cole, 1996; Jay et al., 1981). The predominant intracellular localization of Agnoprotein is in the cytoplasm and especially the perinuclear region in association with the outer nuclear membrane for all three primate polyomaviruses (Nomura et al., 1983; Okada et al., 2001; Rinaldo et al., 1998). A small amount Agnoprotein is also found in the nucleus for JCV and SV40 (Nomura et al., 1983; Okada et al., 2001), but not for BKV (Rinaldo et al., 1998). Interestingly, BKV Agnoprotein is phosphorylated and associates with three cellular proteins (Rinaldo et al., 1998). Agnoprotein has a role in the lytic cycle because SV40 mutants in which the Agnoprotein is deleted produce virions more slowly than wild-type virus (Ng et al., 1985). Agnoprotein may have regulatory roles in viral transcription, translation, as well as in virion assembly and maturation. It has been reported that Agnoprotein serves to localize VP1 to the perinuclear space during lytic infection (Carswell and Alwine, 1985; Resnick and Shenk, 1986). Functions of Agnoprotein have been reviewed recently (Safak and Khalili, 2003). The Agnoprotein of JCV can interact with the cellular YB-1 transcription factor to regulate JCV transcription (Safak and Khalili, 2001; Safak et al., 2002) and also can cause dysregulation of the cell cycle (Darbinyan et al., 2002). At least in the case of JCV, Agnoprotein binds directly to p53 (Darbinyan et al., 2002). JCV Agnoprotein dysregulates cell cycle progression when expressed in the absence of other viral proteins (Darbinyan et al., 2002). Constitutive expression of JCV Agnoprotein causes cells to accumulate at the G2/M stage of the cell cycle with a decline in cyclins A and B-associated kinase activity. Agnoprotein augments the activity of the p21/WAF-1 promoter and the level of p21/WAF-1. Activation of p21/WAF-1 gene expression is mediated, at least in part, through cooperation with p53 (Darbinyan et al., 2002). JCV Agnoprotein may also be important in malignancy (discussed below).

Involvement of polyomaviruses in human cancers

In this section, evidence of a role in neoplasia will be examined for the three primate polyomaviruses: JCV, BKV, and SV40. Where possible, molecular correlates will be elaborated for the observed differences in the patterns of tumor formation between these three viruses. Essentially, data can be divided into three categories: transformation of cells in culture, oncogenic potential in laboratory animals, and association with clinical human tumors.

JCV

JCV is widespread throughout the human population with ~80% of adults exhibiting antibodies specific for JCV. Infection is thought to occur during early childhood and is usually subclinical. However, under immunosuppressive conditions, for example, in patients with acquired immunodeficiency syndrome (AIDS), JCV can emerge from latency and cause progressive multifocal leukoencephalopathy (PML) (Gordon and Khalili, 1998; Safak and Khalili, 2003). PML is a neurodegenerative disease of the central nervous system characterized by multiple regions of demyelination. Lytic infection of oligodendrocytes by JCV destroys myelin-producing cells leading to brain lesions and death (Berger, 2003). PML used to be considered a rare disease, but since the advent of the AIDS pandemic, it is much more prevalent and affects ~5% of HIV-infected persons (Berger, 2003).

JCV is able to transform cells in culture although not as efficiently as SV40. JCV can transform human fetal glial cells and primary hamster brain cells. JCV-transformed cells exhibit the phenotypic properties associated with transformation including growth in soft agar, serum-independence, morphology, plasminogen activator production, and so forth. These studies have been reviewed recently (Del Valle et al., 2001a).

The transforming ability of JCV appears to be limited to cells of neural origin and this property maps to the viral regulatory region at the origin of replication. Specifically, the JCV early promoter (JCVE) directs glial cell-specific transcription of the large T antigen (T-Ag). Glial cells-specific transcriptional regulation of JCV-T-Ag expression by JCVE is believed to be a major determinant of the neural tropism of JCV in PML (Henson et al., 1995; Kim et al., 2001).

The following lines of evidence indicate that JCVE is glial-specific. JCVE:reporter gene constructs are expressed at higher levels in glial cells than in nonglial cells (Kenny et al., 1984). Transgenic mice with the JCV early region selectively express T-Ag in oligodendrocytes leading to a phenotype of dysmyelination (Trapp et al., 1988). Transgenic mice with constructs in which the early promoters and T-Ag regions of JCV and SV40 were exchanged showed patterns of expression that demonstrated that the JCVE promoter was responsible for glial-specific expres-
sion (Feigenbaum et al., 1992). In vitro transcription assays showed that hamster glial cell extract stimulated JCV<sub>E</sub> whereas HeLa cell extract reduced production of nuclear run-off transcripts. This suggests that glial cells contain an activating factor(s) for JCV<sub>E</sub> transcription although nonglial (HeLa) cells contain a repressor(s) (Ahmed et al., 1990a, 1990b).

Detailed analyses of the JCV<sub>E</sub> promoter have shown that transcription is regulated in a complex fashion. Multiple transcription factors, both general and cell-specific, regulate JCV<sub>E</sub> including Jun, NF-1, GF-1, Sp1, S<sub>jbbp-2</sub>, Pur<sub>c</sub>, and YB-1 (reviewed recently (Kim et al., 2001; Raj and Khalili, 1995)).

Thus, in JCV, we have compelling molecular evidence for the basis for the tissue-specificity of viral tropism in cell culture and in vivo (discussed below).

Many studies have established the highly oncogenic potential of JCV in laboratory animals, for example, JCV induced multiple types of brain tumors when injected into the brains of newborn Golden Syrian hamsters. JCV is the only polyomavirus that induces solid tumors in nonhuman primates. JCV caused the development of astrocytomas, glioblastomas, and neuroblastomas in owl and squirrel monkeys that occurred 16–24 months after inoculation of JCV intracerebrally, subcutaneously, or intravenously (Lon- don et al., 1978, 1983). Study of monkey tissue revealed T-Ag expression but no capsid protein or infectious virion (Miller et al., 1984). These animal studies have been reviewed recently (Del Valle et al., 2001a; Khalili et al., 2002; Gan et al., 2001). The involvement of JCV in carcinomas/renal tumors and thymomas or lymphoma is discussed below. In another study on JCV, Agnoprotein DNA was detected in 11 of 16 medulloblastoma samples and Agnoprotein expression in 11 of 20 samples. Because some of the Agnoprotein positive medulloblastoma samples were negative for concomitant T-Ag expression, Agnoprotein may have a role in the development of JCV-associated medulloblastoma (Del Valle et al., 2002). Many other studies that have employed PCR-mediated DNA amplification or immunohistochemistry of various brain samples provide support for an association of JCV with a wide variety of tumors of the central nervous system (CNS) and in other tumors such as colon cancer.

JCV DNA sequences have been found in a high percentage of normal tissue samples taken from the upper and lower human gastrointestinal tract (Ricciardiello et al., 2000) and in sewage (Bofill-Mas and Girones, 2001). In a study of epithelial malignant tumors of the large intestine, 22 of 27 samples tested positive for JCV DNA sequences. Expression of JCV T-Ag and Agnoprotein was observed in >50% of the samples (Enam et al., 2002). No expression of JCV proteins was detected in normal gastrointestinal epithelial tissue. Thus, JCV is associated with some colon cancers and evidence has been presented that dysregulation of β-catenin signaling by JCV T-Ag is involved (Enam et al., 2002; Gan et al., 2001). The involvement of JCV in brain and nonbrain tumors has recently been reviewed (Del Valle et al., 2001a).

Studies of JCV have provided us with a consistent story for the pattern of oncogenesis of this virus. Molecular studies show a glial cell-specific requirement for transcription from the JCV<sub>E</sub> promoter and hence JCV T-Ag expression. Transgenic mice with a JCV<sub>E</sub> promoter driving JCV-T-Ag transgene

<table>
<thead>
<tr>
<th>T antigen</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCV</td>
<td>adrenal neuroblastoma neuroectodermal tumors primitive tumors originating from the cerebellum pituitary neoplasia</td>
<td>Small et al., 1986a Franks et al., 1996 Krynska et al., 1999b</td>
</tr>
<tr>
<td>BKV</td>
<td>hepatocellular carcinomas/renal tumors renal tumors and thymomas or lymphoma choroid plexus tumors</td>
<td>Gordon et al., 2000 Small et al., 1986a Dalrymple and Beemon, 1990 Brinster et al., 1984; Messing et al., 1985</td>
</tr>
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The table lists the types of tumors induced in transgenic mice containing T antigen from each of the primate polyomaviruses. In each case, T-antigen expression is driven by its own promoter. Experiments in which T-Ag expression is driven by heterologous promoters have been reviewed recently (Saenz-Robles et al., 2001).
expression exhibit dysmyelination or develop neural tumors (Table 3). In humans, JCV is associated with the brain disease PML and with tumors of the CNS. For JCV and brain tumors, there is an impressive array of evidence that fits biologically and does not rely only on PCR.

BKV

Like JCV, BKV is widespread in the human population with more than 63% of individuals testing serologically positive. Primary infection occurs during childhood and is usually subclinical. However, BKV can reactivate from latency under conditions of immunocompromization, including pregnancy, transplantation, and AIDS, to cause nephropathy. Notably, kidney transplant recipients who receive highly immunosuppressive drugs may develop BKV-associated nephropathy that is a leading cause of allograft failure (Hirsch and Steiger, 2003; Moens and Rekvig, 2001; Nickeleit et al., 2002).

BKV, BKV complete genomic DNA, or BKV genomic fragments that include the early region are able to transform embryonic fibroblasts and cells cultured from the kidney or brain of mouse, rat, hamster, rabbit, and monkey (Corallini et al., 2001; Tognon et al., 2003). The efficiency of transformation is variable and dependent upon genetic features of the viral strain that is used. The efficiency of BKV T-Ag-induced transformation of hamster cells was improved by the co-introduction of the human c-Harvey-ras oncogene (Pagnani et al., 1988). In the case of human cells, transformation of human embryonic kidney (HEK) cells by BKV is not efficient and often abortive. Features of the transformed phenotype are not fully displayed. However, a fully transformed phenotype can be achieved in cooperation with other oncogenes such as adenovirus E1A, e-rasA, or e-myc (Corallini et al., 2001).

BK virus is highly oncogenic in young or newborn mice, rats, and hamsters. The efficiency of tumorigenesis by BKV in hamsters is dependent upon the route of injection. BKV is only weakly oncogenic when injected subcutaneously but gives a high incidence of tumors when injected intracerebrally or intravenously. The types of tumors induced include ependymoma, neuroblastoma, pineal gland tumors, pancreatic islet tumors, fibrosarcoma, and osteosarcoma. This suggests that BKV has a tropism for certain cell types (Corallini et al., 2001).

In studies that were conducted in parallel with the experiments analyzing the JCV early region (see JCV section), Small et al. (1986a) also established transgenic mice containing the BKV early region. As was the case with JCV, a highly tissue-specific production of tumors was observed but the tissue distribution of BKV-induced tumors differed from that of JCV (Table 3). BKV-containing mice developed primary hepatocellular carcinomas and renal tumors. Dalrymple and Beemon (1990) also established mice containing the BKV early region. They observed two types of pathology: enlarged thymuses (ranging from hypoplasia to thymomas and lymphomas) and renal adenocarcinoma. Expression of BKV T-Ag in these mice was restricted to epithelial cells of kidney tumors and the enlarged thymuses. No expression was detected in any normal tissues.

Does BKV have a role in human cancer? BKV DNA was detected by Southern blot hybridization in 19 out of 74 brain tumors and in 4 out 9 pancreatic islet tumors. In some of these tumors, BKV RNA and T-Ag expression could be detected (Corallini et al., 1987). Interestingly, BKV was found to be in an episomal state at a copy number of 0.2–2 genomes/cell. (Occasionally, tumors had 10–20 genome copies per cell.) Dorries et al. (1987) reported BK DNA by Southern blot hybridization in 46% of 11 common histotypes. In this report, BKV DNA sequences were found to be integrated into chromosomal DNA. Flaggstad et al. (1999) reported BKV DNA detected by PCR in 17 of 18 neuroblastomas and T-Ag expression by immunohistochemistry in 16 of 18. For two samples in this study, T-Ag was also detected by Western blotting after co-immunoprecipitation with p53. However, Arthur et al. (1994) reported a lack of association of BKV DNA with brain tumors (primarily, glioblastoma multiforme) using PCR. Whether these are real differences or due to differences in PCR sensitivity is unclear. This issue is discussed in the next section. More studies are warranted to look at the association of BKV with brain tumors.

BKV was detected in a patient with an adenoma of the pancreatic islets (insulinoma), and a variant of BKV (BKV-IR) was isolated by transfecting human fibroblasts with DNA isolated from the tumor (Caputo et al., 1983). The association of BKV with pancreatic islet tumors is consistent with its pattern of tumorigenesis in laboratory rodents (discussed above). There have been many other reports of the detection of BKV DNA in human tumors by PCR and Southern blotting. These include Kaposi’s sarcoma, brain tumors, and tumors of the urinary tract. These reports have been reviewed recently (Corallini et al., 2001).

Therefore, BKV shows important differences from JCV including reduced ability to transform cells in culture, a distinct pattern of tumor formation in laboratory animals, and a different clinical profile in humans. It is possible that these properties of BKV are related to the cell-type specificities of the early promoter as was described above for JCV. However, less is known about the molecular mechanisms of oncogenesis for BKV than for JCV. The relative roles of the intrinsic oncoprotein activity of T-Ag versus the specific characteristics of the BKV early promoter in determining BKV pathology have not been determined. In this regard, it would be interesting to investigate hybrid constructs where the BKV early promoter and the BKV T-Ag coding region have been swapped with the corresponding regions of other polyomaviruses as has been done for JCV (Feigenbaum et al., 1992).

The role of BKV in human malignancy is uncertain. From the standpoint of establishing a firmer verification of
an association, further investigations are needed using more recent technologies, for example, the application of laser capture microdissection of tumor samples to verify the presence and specificity of BKV DNA and expression of viral proteins.

**SV40**

Unlike JCV and BKV, it is widely accepted that the natural host for SV40 is the rhesus monkey and not the human. However, nearly 100 million people in the United States alone are estimated to have been exposed to SV40 inadvertently through poliovirus vaccinations between 1955 and 1963. This was because primary cultures of rhesus monkey kidney cells that were used to grow the poliovirus were contaminated with SV40. SV40 is a potent transforming agent for cell cultures from species that are non-permissive for viral replication including hamsters, mice, rats, cattle, and guinea pigs (Arrington and Butel, 2001). Most of the early studies on SV40 transformation were done with established rodent cell lines such as Balb-3T3 cells. As was described above, SV40 can enter these cells and express the early region (T-Ag) but does not replicate or express the late genes. Cells undergo many cellular and biochemical changes after SV40 transformation as described above (Saenz-Robles et al., 2001).

SV40 is also able to induce tumor formation in laboratory animals. Soon after its discovery, the oncogenic potential of SV40 was demonstrated in that it induced sarcoma formation after subcutaneous injection into hamsters (Eddy et al., 1962). Like BKV (see above), tumorigenesis by SV40 is dependent upon the route of injection. Hamsters injected intravenously with SV40 develop leukemia, lymphoma, and osteosarcoma (Diamandopoulos, 1972), whereas hamsters injected intracranially develop choroid plexus tumors and ependymomas (Kirschstein and Gerber, 1962), and hamsters injected intraperitoneally develop mesothelioma (Cicala et al., 1993).

Transgenic mice bearing the SV40 T-Ag develop characteristic brain tumors localized to the choroid plexus (Table 3). Deletion analysis showed that localization of tumors to the choroid plexus region is determined by the SV40 noncoding regulatory region (Brinster et al., 1984; Messing et al., 1985). Replacing the SV40 early promoter with another tissue-specific promoter can direct T-Ag to a particular tissue and promotes its oncogenesis. For example, mice bearing an elastase-1::SV40 T-Ag transgene develop pancreatic neoplasia (Ornitz et al., 1987). This is reminiscent of the findings for JCV that viral tropism depends on the T-Ag promoter. However, many other experiments of this type have been performed for different tissues and cell types (reviewed by Saenz-Robles et al., 2001), and variability has been found depending on the promoter used and the cell type targeted. Results range from hyperplasia and carcinoma to no apparent pathology at all. It is therefore an oversimplification to suggest that promoter-specific elements controlling T-Ag expression are the sole determinants of the ability of T-Ag to transform particular cell types (Saenz-Robles et al., 2001). Stochastic effects are probably also a factor in the development of tumors in transgenic mice as was shown for T-Ag-induced pancreatic tumors (Christofori et al., 1994; Efrat and Hanahan, 1989).

Although rodent cells do not support SV40 replication (and thus infection causes cell transformation) and monkey cells are permissive for SV40 replication (and thus infection causes cell lysis), human cells are unique in that they support transformation and low-level SV40 replication. In other words, human cells are semipermissive for SV40 replication. The transformation of human cells by SV40 was first reported in 1962 (Shein and Enders, 1962). SV40 can transform many types of human cells in culture. SV40-transformed human fibroblasts exhibit many of the features of the transformed phenotypes that were enumerated for rodent cells (see above). However, unlike rodent cells, human cells are not immortalized by SV40. Rather, they exhibit an extended life span: primary human fibroblasts can undergo ~50–60 population doublings before they begin to undergo senescence whereas SV40-transformed fibroblasts can undergo 70–80 doublings (Arrington and Butel, 2001; Bryan and Reddel, 1994).

After 70–80 population doublings, SV40-transformed fibroblasts enter a “crisis” period when most of the cells die by apoptosis (Macera-Bloch et al., 2002; Ponten, 1971). The few cells that survive crisis are capable of continuous cell growth and are termed immortal. The interaction of SV40 T-Ag with p53 and pRB enables the transformed phenotype and growth in the extended life span phase. Immortalization requires additional event(s) caused by the mutagenic effects of T-Ag (described above) and only occurs at a frequency of $10^{-8}$–$10^{-5}$. One such event is the inactivation of the tumor suppressor gene SEN6 (Banga et al., 1997; Jha et al., 1998). Interestingly, ectopic expression of human telomerase (hTERT) can avert crisis in SV40-transformed primary cells (Zhu et al., 1999), and indeed SV40 T-Ag, ras oncogene, and hTERT in combination result in direct tumorigenic conversion of normal human epithelial and fibroblast cells (Hahn et al., 1999).

In an early series of interesting studies, Jensen et al. (1964) demonstrated that SV40 is tumorigenic in humans. Cells from human volunteer donors were transformed ex vivo by SV40 and then autologously reimplanted subcutaneously. These SV40-transformed cells grew as subcutaneous nodules for 2 weeks but then regressed presumably because of an immune reaction against viral antigens. Histologically, the nodules were sarcomas. Normal control cells did not form nodules. This was a one-time study that would seem unlikely to be approved by contemporary IRBs.

The role of SV40 in the etiology of human cancers is perhaps one of the most contentious issues in cancer research today. The first report of an association of SV40 with human cancer was made by Soriano et al. (1974).
SV40 and SV40 T-Ag were detected in malignant melanoma metastases from a patient who had antibodies to viral capsid and T-Ag (Soriano et al., 1974). Since then, there have been a large number of reports of the association of SV40 and human tumors and these have been reviewed recently (Arrington and Butel, 2001). However, no further studies have been reported on the association of SV40 with melanoma specifically and it would be interesting to examine melanomas using modern techniques and find out the extent of correlation of SV40 with melanoma.

Many reports have described the association of SV40 with mesothelioma, a rare but aggressive cancer of mesothelial cells. A major factor in the development of mesothelioma is thought to be exposure to asbestos. The frequent association of SV40 with mesothelioma has led to the hypothesis that the virus is a cofactor in the development of this malignancy. In cell culture studies, human mesothelial cells have been found to be nonpermissive for SV40 replication and consequently to undergo cell transformation in response to SV40 infection. Asbestos was found to be a synergistic factor for transformation in these experiments (Bocchetta et al., 2000). It has also been reported that human mesothelial cells are permissive for BKV replication but refractory to infection by JCV. This may provide an explanation as to why SV40 is associated with mesothelioma rather than the more ubiquitous human polyomaviruses, BKV and JCV (Carbone et al., 2003a, 2003b).

Despite the many reports of SV40 in mesothelioma samples, several studies have failed to detect SV40 sequences as reviewed recently (Arrington and Butel, 2001). To address this technical issue, Testa et al. (1998) conducted a multicenter study. SV40 DNA was detected by PCR using primers directed at two conserved regions, and positive clones were verified by Southern blot and sequencing. Immunostaining of selected samples demonstrated SV40 T-Ag expression. However, another multicenter study reached the opposite conclusion (Strickler, 2001). The problematic technical issues that may underlie this contradiction have been reviewed recently (Garcea and Imperiale, 2003). The two major concerns are the number of PCR cycles used in DNA amplification and the specificity of the antibodies used in immunohistochemistry. A large number of PCR cycles (40–60) are necessary to detect SV40 DNA, raising the possibility that there may be fewer than one viral genome per cell. Furthermore, the antibodies that are used to detect SV40 T-Ag are cross-reactive with the T-Ags of JCV and BKV, raising obvious issues of specificity.

SV40 has also been reported to be associated with some brain tumors (reviewed in Arrington and Butel, 2001) and with non-Hodgkin lymphoma (Shivapurkar et al., 2002; Vilchez et al., 2002). The requirement for 45 cycles of PCR for reproducible detection suggests that the virus was present in low copy number in non-Hodgkin lymphomas. Interestingly, SV40 has been found in association with pediatric tumors of the choroid plexus and ependymomas using PCR and T-Ag immunohistochemistry (Bergsagel et al., 1992). This is the type of tumor that is seen in transgenic mice containing the SV40 early region (Table 3). SV40 has also been detected in some medulloblastomas by PCR and immunohistochemistry (Huang et al., 1999; Krynska et al., 1999a, 1999b).

What is the nature of the SV40 DNA that is associated with human tumors? DNA sequences of SV40 from human tumors have been isolated and compared to those of laboratory strains and monkey isolates (Lednicky et al., 1995; Stewart et al., 1996, 1988). These data yielded the following conclusions: (1) The SV40 DNA found in human tumors is authentic. (2) The SV40 DNA sequences found in human tumors differ from the sequences of the reference strain SV40-776 and the other laboratory strains (SV40-B2 and VA45-54). This argues against contamination of samples in the laboratory as a source of the SV40 DNA. The promoter region of SV40 isolated from monkeys or from human tumors has a single copy of a 72-bp sequence that is duplicated in laboratory strains. (This duplication may represent an adaption to growth in tissue culture.) (3) There was no compelling evidence for human-specific strains of SV40 or for tumor type-specific associations.

Recently, more convincing evidence for the association of SV40 with human cancer has emerged that is free from the technical caveats of earlier studies that were discussed above. Mendoza et al. (1998) reported a study of 35 osteosarcoma tumors of which 9 were positive by PCR. Analysis of total cell DNA from tumors by Southern blot hybridization found that about half of the PCR-positive tumors had detectable SV40 DNA that was integrated into chromosomal DNA (1–4 copies per cell). In another study, microdissection of mesothelioma frozen tumor revealed the presence of SV40 DNA sequences in the tumor cells but not in adjacent nontumorous tissues (Shivapurkar et al., 1999). Malkin et al. (2001) studied two unrelated patients with Li Fraumeni syndrome who have one germ-line allele of p53 that is mutated. These patients had multiple tumors and some were due to loss of the single wild-type p53 allele. However, in choroid plexus of tumors of these patients, normal p53 was maintained but SV40 DNA sequences and T-Ag were detected. It has been suggested that p53 haploinsufficiency of individuals with Li Fraumeni syndrome predisposes them to develop SV40-associated in susceptible tissues such as the choroid plexus (Garcea and Imperiale, 2003). Indirect evidence for a link between SV40 and cancers comes from the observations that mesotheliomas from Finland and Turkey (where polio vaccine was not administered) are negative for SV40 (Hirvonen et al., 1999; Emri et al., 2000). Direct evidence for SV40 was provided by Lednicky et al. (1995) who reported the isolation of infectious SV40 from a sample of choroid plexus tumor tissue after lipofection of tumor DNA into monkey kidney cells. Waheed et al. (1999) have demonstrated that an adenoviral vector expressing antisense transcript to SV40 early region induced growth arrest and apoptosis in SV40-positive human mesothelioma cells growing in culture.
Recently, Arrington et al. (2004) reported a meningioma in a scientist who had a risk of laboratory exposure to SV40. SV40 DNA was isolated from the tumor and cerebrospinal fluid of the patient and was found to have had an identical sequence to the laboratory source. It would be of interest to examine cancer samples from other individuals with prior occupational exposure to SV40. Thus, the evidence for the association of SV40 with human cancer continues to strengthen and should no longer be dismissed on the grounds of the technical issues regarding PCR and antibody specificity.

Is the association of SV40 with human tumors causal or incidental? Does SV40 initiate tumorigenic events or do tumors offer a microenvironment that favors viral replication in humans with latent SV40 infection? SV40 has a very potent oncogene, T-Ag, and is highly tumorigenic in cell cultures and animals so it would be surprising if SV40 were associated with tumors merely by chance. However, there still remains a lack of clear epidemiological evidence. The Institute of Medicine (IOM) recently reviewed the evidence associating polio vaccines and SV40 with human tumors and concluded that the epidemiological studies are sufficiently flawed that the evidence was inadequate to conclude whether or not contaminated polio vaccine caused cancer (IOM report, 2002). The IOM report also acknowledged that the biological effects of SV40 were consistent with its being a tumor virus.

Conclusions

Three polyomaviruses have been discussed that are thought to have a possible role in the etiology of human malignancies. Studies on the molecular biology of these viruses and their effects on cultured cells and in animal models form a coherent picture with what is know about these viruses and their associations in a clinical setting. However, it remains to be stringently proven that they have a causal role in human neoplasia. Problems include the following: The viruses are ubiquitous in nature but the associated cancer is rare. The incubation period between infection and appearance of cancer is long. The initial viral infection is usually subclinical making it difficult to establish when it occurred. The human viruses JCV and BKV do not productively infect animal models. Environmental cofactors (e.g., cocarcinogens) or host factors (e.g., immune status) modulate pathogenesis. These considerations make it difficult to apply Koch’s postulates to the polyomaviruses. Zur Hausen has proposed alternative criteria for defining a causal role for an infection in cancer (zur Hausen, 1999): (1) epidemiological plausibility and evidence that a virus infection represents a risk factor for the development of a specific tumor; (2) regular presence and persistence of the nucleic acid of the respective agent in cells of the specific tumor; (3) stimulation of cell proliferation upon transfection of the respective genome or parts therefrom in corresponding tissue culture cells; (4) demonstration that the induction of proliferation and the malignant phenotype of specific tumor cells depend on functions exerted by the persisting nucleic acid of the respective agent.

Evidence has been presented here that the JCV, BKV, and SV40 polyomaviruses fulfill at least the last three criteria. These viruses transform cells in culture and produce tumors in inoculated animals or transgenic mice with patterns consistent with their putative role in human tumorigenesis, as we have described. The presence of viral DNA and viral gene expression in a subset of human tumors has been established.

The differences between the polyomaviruses can be ascribed, at least in part, to divergences in viral tropisms with respect to cells in culture, laboratory animals, and human cancers. As we have described, the important determinant of tropism is the noncoding regulatory region. Whether differences in functions between the transforming proteins of the polyomaviruses are important is still not fully resolved. However, in the cases where functions have been compared between polyomaviruses, conservation of function was always found (Table 1). Taken together with the high degree of sequence conservation of the polyomavirus transforming proteins (Table 2), it seems likely that they all operate by the same mechanisms.

Future studies of polyomaviruses will be aided by recent technical advances. Oligonucleotide-based microarray approaches should permit the evaluation of the expression of many thousands of genes at once to generate transcriptional profiles that can be used to examine the effects of polyomaviruses in cell culture systems and in tumors. Radhakrishnan et al. (2003) correlated changes in gene expression in primary human astrocytes after JCV infection with gene expression alterations that were observed in astrocytes within PML lesions of brain tissue from patients with neuro-AIDS. Proteomics-based approaches could be used to assess the status of T-Ag in tumors. For example, two-dimensional electrophoresis could be used to resolve T-Ag/p53 followed by mass spectrometry to evaluate the identity of the T-Ag protein (JCV, BKV, or SV40) and the status of the p53 protein (wild-type or mutated).

Rather than to focus on a debate over whether polyomaviruses “cause cancer”, it might be better to view the viral oncoproteins as putative targets for therapeutic intervention. Treatment of a particular cancer, whether by gene therapy or by small molecule inhibitors, is predicated upon a knowledge of the signal transduction pathways that have become dysregulated within the tumor. Usually, cancer is thought of as a disease that progresses through the gradual accumulation of multiple successive genetic “hits” leading from normal cells to fully malignant metastatic tumors (Fearon and Vogelstein, 1990). These hits include activation of oncogenes, knockout of tumor suppressors, and events that increase mutation rates or destabilize the genome. Each hit represents a target for strategies designed to reverse malignancy. Activation of expression of latent polyomavirus may represent such a hit. The highly multifunctional polyoma-
viral transforming proteins impinge on many cellular signal transduction pathways (Table 1) and thus molecular strategies for the disruption of their interactions with cellular proteins may represent a fruitful avenue for the development of new types of therapeutic interventions. For example, ELISA-based assays for T-Ag/p53 interaction have been used to screen chemical libraries for agents that inhibit T-Ag binding to p53 (Carbone et al., 2003a, 2003b). Delivery of antisense transcripts to the early region of SV40 using an adeno viral gene therapy vector achieved significant growth inhibition and apoptosis of T-antigen-expressing human mesothelioma cells in culture (Waheed et al., 1999).

Agents that target the polyomavirus proteins have the potential of providing new therapies for certain cancers and of answering questions about the importance of polyoma virus protein function in neoplasia.

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