Emergent Human Pathogen Simian Virus 40 and Its Role in Cancer

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INTRODUCTION

The polyomavirus simian virus 40 (SV40) is a potent DNA tumor virus, and mounting evidence suggests that it is an emergent human pathogen (1, 10, 12, 13, 39, 49, 50, 66, 111, 123). Recently, the Institute of Medicine of the National Academies concluded that "the biological evidence is strong that SV40 is a transforming virus" and that "the biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions" (111). In addition, two other independent scientific panels have made similar conclusions (53, 131). A recent analysis suggested that SV40 should be included in the list of group 2A carcinogens (i.e., agents for which evidence is indicative but not definitive for carcinogenesis in humans) by the International Agency for Research on Cancer (39). Therefore, as SV40 is recognized as a potent oncogenic agent, it is important to evaluate the increasing data that implicate the virus in some human malignancies. This review examines the biological, pathological and clinical evidence of SV40 pathogenesis and discusses future directions needed to define an etiologic role for the virus in some of these devastating diseases.

History of SV40 Contamination of Polio Vaccines

The discovery of the polyomavirus SV40, as well as its introduction as a pathogen into the human population, was tied to the development and worldwide distribution of early forms of the polio vaccine (13, 95, 111, 123). Inactivated (Salk) and early live attenuated (Sabin) forms of polio vaccines were inadvertently contaminated with SV40 (95, 97, 111). In addition, different adenovirus vaccines distributed to some U.S. military personnel from 1961 to 1965 also contained SV40 (64).

The viral contamination occurred because these early vaccines were prepared in primary cultures of kidney cells derived from rhesus monkeys, which are often naturally infected with SV40 (13, 95, 111). Infectious SV40 survived the vaccine inactivation treatments, and conservative estimates indicate that up to 30 million people (children and adults) in the United States may have been exposed to live SV40 from 1955 through 1963 when administered potentially contaminated polio vaccines (95, 111). Millions of people worldwide were also potentially exposed to SV40 because contaminated polio vaccines were distributed and used in many countries (85, 123). These data led the Institute of Medicine to conclude that "the biological evidence is of moderate strength that SV40 exposure from the polio vaccine is related to SV40 infection in humans" (111).

Shortly after its discovery, SV40 was shown to be a potent oncogenic DNA virus (13). In animal models, the neoplasias induced by SV40 included primary brain cancers, malignant mesotheliomas, bone tumors, and systemic lymphomas (13). Subsequently, many in vitro studies established that the oncogenic capacity of SV40 reflects the disruption of critical cell cycle control pathways (9, 96, 116). During the last decade, numerous published studies from independent laboratories, using different molecular biology techniques, have demonstrated SV40 large tumor antigen (T-ag) or DNA in primary human brain and bone cancers and malignant mesothelioma (1, 13, 39, 50, 123). More recently, studies have demonstrated that SV40 T-ag sequences are significantly associated with non-Hodgkin's lymphoma (NHL) (102, 124, 125). Therefore, the major types of tumors induced by SV40 in laboratory animals are the same as those human malignancies found to contain SV40 markers. A recent meta-analysis (122) of the molecular evidence conclusively established a significant excess risk of SV40 with those selected human cancers.

It is noteworthy that SV40 has been detected in malignancies from children and young adults not exposed to contaminated polio vaccines, as well as in older adults (5, 18, 71, 73, 76, 117, 124, 125, 129, 132, 133). The detection of viral markers in

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young persons, by using molecular techniques, coupled with the isolation of infectious SV40 from tumors (62) and from nonneoplastic specimens (66, 67), suggests that SV40 continues to cause infections in the human population today. In contrast, some retrospective epidemiological studies have failed to demonstrate an increased cancer risk in populations which had a high likelihood of having received potentially contaminated polio vaccine (20, 82, 95, 112, 114). However, the epidemiological data available are recognized to be inconclusive and limited (95, 111, 123), and the Institute of Medicine found that the epidemiological data for cancer rates in people potentially exposed to SV40-contaminated vaccines are inadequate to evaluate a causal relationship (111). This conclusion is based on the lack of data on which individuals actually received contaminated vaccines, the unknown dosage of infectious SV40 present in particular lots of vaccine, the failure to know who among the exposed were successfully infected with SV40, the inability to know if the vaccine "unexposed" cohorts may have been exposed to SV40 from other sources, and the difficulty of monitoring a large population for cancer development for years after virus exposure. These important limiting factors led the Institute of Medicine to "not recommend additional epidemiological studies of people potentially exposed to contaminated polio vaccine."

VIROLOGY AND PATHOGENESIS OF INFECTIONS

Properties of the Virus

SV40 is in the family *Polyomaviridae*, which includes JC virus (JCV) and BK virus (BKV). Polyomaviruses are small, nonenveloped, icosahedral DNA viruses. Their genomes consist of a single copy of double-stranded, circular, supercoiled DNA about 5 kb in length. BKV and JCV share 72% DNA sequence homology, and each shares approximately 70% homology with SV40. Although these viruses are related, they can be distinguished easily at the DNA and protein levels. Genetic differences, particularly in the noncoding, regulatory regions of the viral genomes, may determine important differences in host range. Furthermore, the three viruses can be differentiated serologically by neutralization and hemagglutination assays (52, 56, 98).

The SV40 genome is divided into early and late regions, with the early region coding for the large and small T-ags and the late region encoding the capsid proteins VP1, VP2, and VP3 (Fig. 1). Large T-ag of SV40 strain 776 contains 708 amino acids and is a very multifunctional protein (Fig. 2). The large T-ag is an essential replication protein that is required for initiation of viral DNA synthesis and that also stimulates host cells to enter S phase and undergo DNA synthesis. Because of this ability to subvert cell cycle control, T-ag represents the major transforming protein of SV40. T-ag forms complexes with several cellular proteins; fundamental to T-ag effects on host cells is binding to cellular tumor suppressor proteins (9, 13, 96, 116). These properties help explain SV40's potential as a tumor virus. However, it is important to point out that the oncogenic capacity of SV40 is an accidental side effect of the viral replication strategy; viral proteins (large and small T-ags) in lytically infected cells stimulate host cells into a state capable of supporting viral replication.
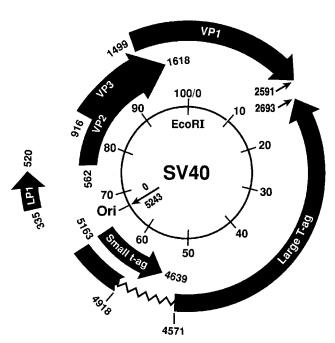

FIG. 1. Genetic map of SV40. The circular SV40 DNA genome is represented, with the unique EcoRI site shown at map unit 100/0. Nucleotide numbers based on reference strain SV40-776 begin and end at the origin (Ori) of viral DNA replication (map unit 0/5243). The open reading frames that encode viral proteins are indicated. The early T-ag proteins are shown on the right, and the late structural (VP) proteins are shown on the left. The beginning and end of each open reading frame are indicated by nucleotide numbers. From reference 13; used with permission.

There is only one known serotype of SV40, but genetic strains exist and can be distinguished by nucleotide differences in the regulatory region (60) and in the variable domain at the extreme C terminus of T-ag, which is defined as the last 86 amino acids of the molecule (residues 622 to 708) (47, 58, 62, 63, 81, 109, 110). Nucleotide differences in the T-ag C-terminal region, including polynucleotide insertions and deletions as well as single nucleotide changes, would change some encoded amino acids. These distinctions at the nucleotide and protein levels have conclusively established that SV40 sequences in human malignancies and other clinical samples are not the result of accidental laboratory contamination (Fig. 3, 4, and 5). However, future studies need to determine whether SV40 strains differ in pathogenic and/or oncogenic capacity. The classic example of DNA virus strains differing in oncogenic capacity is the human papillomavirus group; of the more than 75 types described, of which about 30 cause genital infections, only a few types are associated with the development of cervical carcinoma (68, 135). This identification of high-risk strains has led to the development of preventive interventions, such as the vaccine against human papillomavirus type 16 (54).

Laboratory-adapted monkey strains of SV40 typically contain two 72-bp enhancer elements (Fig. 4 and 5); these are designated "nonarchetypal" or complex regulatory structures (60). In contrast, SV40 isolates from human nonmalignant (Fig. 4) and malignant (Fig. 5) specimens usually (but not always) contain no duplications in the enhancer ("archetypal" structure).
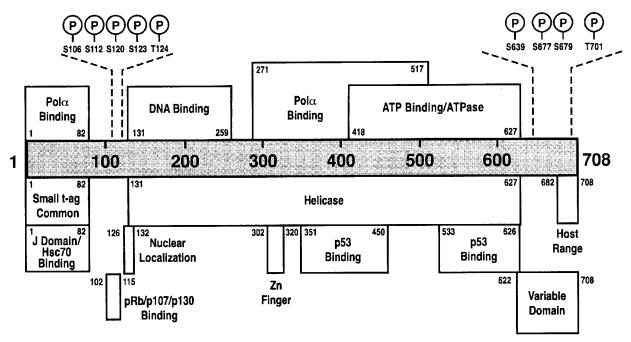
FIG. 2. Functional domains of SV40 large T-ag. Known T-ag functions are identified in boxes above and below the shaded bar, which represents the T-ag protein. The numbers given are amino acid residues based on reference strain SV40-776. The variable domain at the extreme C terminus contains amino acid differences among viral strains and is used for strain identification. From reference 109; used with permission.

Although the function of the SV40 T-ag variable domain is not known, experimental data have suggested that it may be important in some aspect of the virus-host interaction. Embedded within the variable domain of large T-ag is a functional domain, which encompasses amino acids 682 to 708, defined as the host range/adenovirus helper function (hr/hf) domain (Fig. 2). A C-terminal fragment of T-ag can relieve the adenovirus replication block in monkey cells (23, 41, 51, 90, 106) by an unknown mechanism. The hr function was identified because T-ag C-terminal deletion mutants exhibited different growth properties in monkey cell lines; the deletion mutants grew very poorly in CV-1 cells but grew well in BSC and Vero cells (24, 87, 118, 119). Viral DNA was replicated to near-wild-type levels in all three cell types (87, 108). Virions produced by the hr/hf mutants do not assemble properly, seemingly due to an inability to add VP1 to the 75S assembly intermediates (105).

The functional roles for another SV40 early protein, small T-ag, are more elusive. This protein is not essential for virus replication in tissue culture, and there is not a uniform requirement for it in SV40 transformation or tumor induction. However, studies indicate that SV40 small T-ag enhances large T-ag-mediated transformation (96) and is required for complete transformation of human cells in vitro (42). It inhibits cellular protein phosphatase 2A by complexing with the catalytic subunit and regulatory subunit of the enzyme. Small T-ag plays a role in the induction of telomerase in SV40-infected human mesothelial cells (36). Also, recent data indicate that small T-ag is required by large T-ag to upregulate Notch1 expression in SV40-infected and -transformed human mesothelial cells, as well as in SV40-positive human mesotheliomas (7).

Viral Replication Cycle and Effects on Host Cells

An appreciation of the replication cycle of SV40 is fundamental to understanding the oncogenic capacity of SV40 and its potential etiologic role in some human malignancies. The major histocompatibility class I molecules are the specific cell surface receptors for SV40 (4, 8). This initial step in the viral cycle helps explain the broad tropism of the virus and its ability to infect and induce transformation in many types of cells and tissues. In addition, it provides an important distinction between SV40 and the other two polyomaviruses that are able to infect humans, JCV and BKV. JCV uses an N-linked glycoprotein and BKV uses a glycolipid as unique host cell receptors (3). These marked differences are believed to affect the nature of infections by these three viruses in tissues and individuals.

After infection of a cell, SV40 produces large and small T-ags early in the viral replication cycle. These antigens bind and block important tumor suppressor proteins, which include p53, pRb, p107, and p130/Rb2 (1, 13, 59, 96) (Fig. 2). The functions of these intracellular proteins are centered in the control of the cell cycle. The tumor suppressor p53 is believed to sense DNA damage and either pauses the cell in late G_1 for DNA repair or directs the cell to commit suicide through the apoptotic pathway (96, 116). SV40 T-ag binding sequesters p53, abolishing its function and allowing cells with genetic damage to survive and enter S phase, leading to an accumulation of T-ag-expressing cells with genomic mutations that may promote tumorigenic growth. pRb normally binds transcription factor E2F in early G_1 of the cell cycle; T-ag causes unscheduled dissociation of pRb/E2F complexes, releasing E2F to activate expression of growth-stimulatory genes (96, 116). Therefore, SV40 infections in humans may interfere with
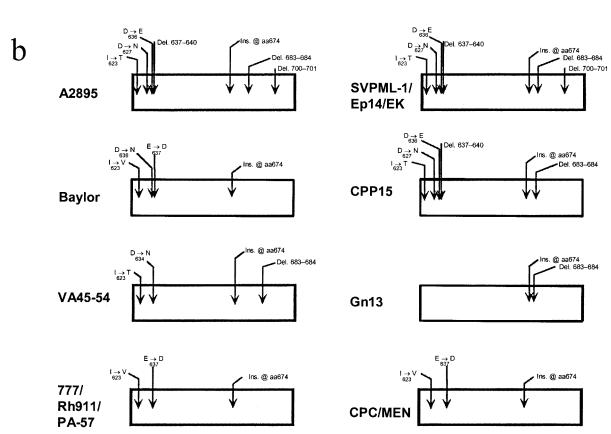
FIG. 3. SV40 large T-ag variable domains. (a) Schematic of large T-ag, showing the location of the variable domain. (b) Amino acid changes in the T-ag C-terminal variable domains of representative SV40 isolates and human primary brain tumor-associated sequences, compared to that of SV40-776. The rectangular boxes represent the T-ag C-terminal region from amino acid (aa) 622 to 708. Virus isolates from monkey kidney cells are shown in the left column. Human brain isolates and primary brain cancer-associated sequences are in the right column. The numbering is according to the system for SV40-776. Del., deletion; Ins., insertion. Arrows indicate the positions and types of amino acid changes. From reference 121; used with permission.

several pathways related to cell cycle control and lead to development of malignancies.

Studies indicate that SV40 can replicate productively in human cells, including fetal tissues (101), newborn kidney cells (101), and different human tumor cell lines (83), although it grows poorly in human fibroblasts (84). Moreover, in vitro assays have shown that human cells can support replication of SV40, establishing that human proteins have the intrinsic ability to cooperate with SV40 T-ag to replicate SV40 DNA (65, 80, 127). Some human cell types undergo visible cell lysis in response to SV40, whereas others fail to exhibit cytopathic changes and produce low levels of virus (84). General conclusions from these early studies are that SV40 can replicate in human cells and that various human cell types display differences in susceptibility to infection by SV40. The basis for the differences is unknown, but T-ag functions are believed to be important (27, 69).

Recent studies have shown that primary human mesothelial cells respond to SV40 very differently from fibroblasts; the mesothelial cells are highly susceptible to SV40 infection and transformation. Most mesothelial cells were infected; few were killed; high levels of p53/T-ag complexes were present; Notch1, the hepatocyte growth factor receptor (Met), and insulin-like growth factor 1 were upregulated; and the tumor suppressor gene *RASSF1A* was inhibited (6, 15, 39, 93). SV40-positive human mesotheliomas show similar changes. The rate of transformation of SV40-infected mesothelial cells was at least 1,000 times higher than that of human fibroblasts (6). These findings emphasize that different human cell types may display dramatically different virus-cell interactions during infection.
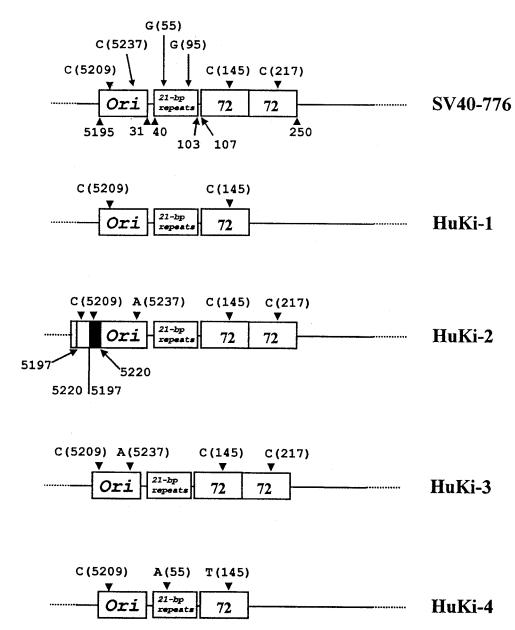
FIG. 4. DNA sequence profiles of SV40 regulatory regions detected in human kidney transplant recipients. *Ori*, viral origin of DNA replication region, which spans nucleotides 5195 to 31; 21-bp repeats, GC-rich region between nucleotides 40 and 103; 72, 72-bp sequence within the enhancer region that is duplicated in some monkey strains (e.g., reference strain SV40-776). Nucleotide numbers are based on SV40-776. Shown are viral sequences associated with transplanted human kidneys (clone designations are on the right). Polymorphisms at the indicated residues are indicated above the boxes. From reference 11; used with permission. For a detailed description of the SV40 regulatory region, see reference 60.

Transmission in Natural Infections

The recognized natural hosts for SV40 are species of Asian macaque monkeys, especially the rhesus macaque (*Macacca mulatta*). SV40, like the polyomaviruses JCV and BKV, establishes persistent infections, often in the kidneys of susceptible hosts (13, 59). An association of primary polyomavirus infection with mild respiratory tract disease, mild pyrexia, and transient cystitis has been reported (32), but the route of infection of these three viruses has not been firmly defined.

SV40 infections may become latent, and the level of virus present may be very low. Both viremia and viruria occur in infected animals, and virus shed in the urine is the probable means of transmission (2, 97). SV40 infections in healthy monkeys appear to be asymptomatic (100), but SV40 causes widespread infections among monkeys that are immunocompromised due to simian immunodeficiency virus infection (47, 58, 81); SV40 sequences and infectious virus were detected in brain, kidney, spleen, and peripheral blood mononuclear cells (PBMCs). These results demonstrate that SV40 can be an opportunistic pathogen in immunosuppressed hosts and that the virus may spread within the host by hematogenous routes.
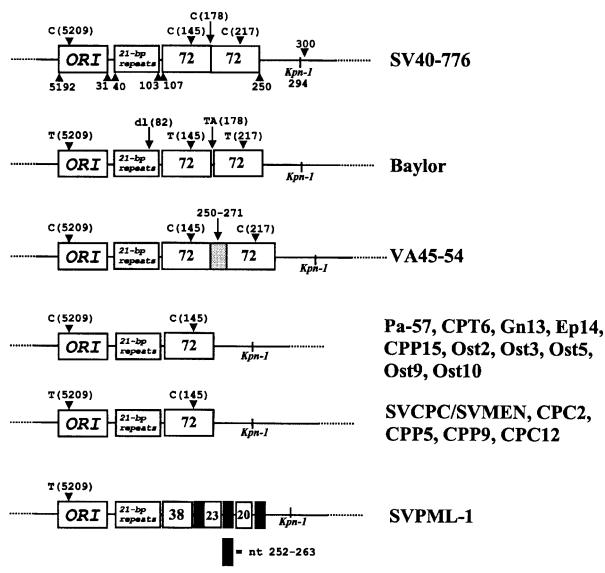
FIG. 5. Regulatory region of SV40. DNA sequence profiles of regulatory regions of SV40 isolates from monkeys and humans and of human tumor-associated DNAs are shown. The diagrams are labeled as described in the legend to Fig. 4. Shown are laboratory-adapted strains (SV40-776, Baylor, and VA45-54), human isolates (SVCPC/SVMEN and SVPML-1), and viral sequences found associated with human brain (CPT, CPP, CPC, and Ep) and bone (Ost) tumors. Tumor-associated sequences usually contain a simple (archetypal) regulatory region without duplications in the enhancer region. From reference 110; used with permission.

Characteristics as a Tumor Virus

The oncogenic capacity of SV40 infections has been well established in laboratory animal models (9, 13, 19, 111, 123). The latent period of tumor development in hamsters infected with SV40 ranges from 3 months to more than a year. The frequency of tumor development is usually over 90% in animals infected as newborns but is reduced in older animals. These data suggest that the age at the time of infection, the route of infection, and the duration of infection may be factors influencing the development of malignancies by SV40.

The neoplasias induced by SV40 in animal models include primary brain cancers, malignant mesotheliomas, bone tumors, and systemic lymphomas (13, 39, 123). Lymphomas are a common malignancy during SV40 infection. In hamsters inoculated intravenously with SV40, systemic lymphomas developed among 72% of the animals, compared to none in the control group (21, 29, 30). The lymphomas were of B-cell origin (22). Following intravenous inoculation, about one-third of the animals developed more than one histologic type of neoplasm, with osteosarcomas being most common after lymphomas. Following intracardiac inoculation, malignant mesotheliomas and osteosarcomas developed in addition to lymphomas (19). An etiologic role of the virus in those cancers was supported because SV40 T-ag was expressed in all malignant cells, animals with tumors developed antibody against SV40 T-ag, and neutralization of SV40 with specific antibody before virus inoculation prevented cancer development (29, 30). Knowledge of these models prompted us, as well as other investigators, to consider the role of polyomavirus SV40 infections in some human malignancies.

TABLE 1. SV40 seropositivity of hospitalized children in Houston, Tex.[a]

Population characteristic	No. SV40 seropositive[b]/ no. of patients (% seropositive)
Age (yr)	
<1–4	1/95 (1.1)
5–9	5/88 (5.7)
10–15	14/154 (9.1)
Sex	
Male	12/181 (6.6)
Female	8/156 (5.1)
Race or ethnicity	
White	11/150 (7.3)
Hispanic	5/85 (5.9)
African-American	3/75 (4.0)
Asian-American	0/5
Other	1/22 (4.5)
Overall seropositivity	20/337 (5.9)[c]

[a] From reference 12, used with permission.

[b] Seropositivity was determined by using an SV40-specific plaque reduction neutralization assay in tissue culture cells.

[c] There was a significant association of SV40 seropositivity with kidney transplantation (6 of 15 [40.0%]) compared to other diagnoses (8 of 238 [3.4%]) ($P < 0.001$).

HUMAN INFECTIONS BY SV40: OVERVIEW OF THE EVIDENCE

Although the prevalence of SV40 infections in humans is not known, studies conducted over the last three decades indicate that SV40 infections are occurring in child and adult populations today. These included individuals who received potentially SV40-contaminated vaccines, as well as in persons born after 1963 who could not have been exposed to those vaccines (5, 11–14, 17, 18, 25, 26, 28, 40, 46, 49, 55, 62, 63, 66, 67, 71–74, 76, 78, 86, 88, 89, 92, 94, 95, 102, 104, 111, 115, 117, 120, 124, 125, 129, 130, 132, 133). In addition, 19% of newborn children and 15% of infants 3 to 6 months old at the time of receiving the oral contaminated polio vaccine were shown to excrete infectious SV40 in their stools for up to 5 weeks after vaccination (75). It is important to point out that the incidence of SV40 infections linked to those vaccines is not known.

SV40 seroprevalence rates in the general populations of the United States and other countries have ranged from 2 to 20% (13, 78, 95). However, differences in the methodology and low sensitivity of the assays used in some studies make it difficult to ascertain the actual prevalence of SV40 infections. A report by Shah et al. (99) found that 18% of adult kidney transplant patients had specific neutralizing antibody to SV40. Another study among adult patients showed the presence of SV40 neutralizing antibodies in 16% of human immunodeficiency virus-infected patients and 11% of individuals not infected with human immunodeficiency virus (49). Among hospitalized children, the overall prevalence of specific SV40 serum neutralizing antibodies was 6% (12); the SV40 seropositivity among children increased with age ($P = 0.01$) and was significantly associated with kidney transplantation ($P < 0.001$) (Table 1). Recently, a study of the prevalence of SV40 infections showed
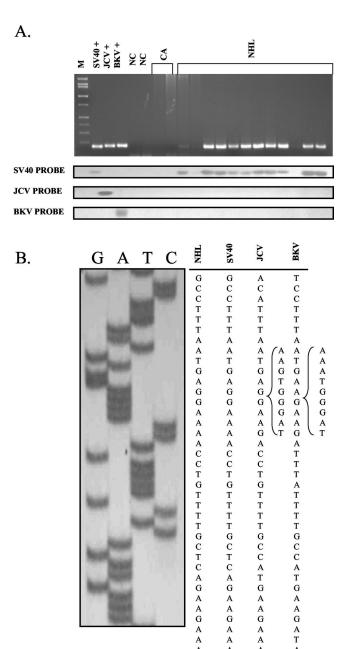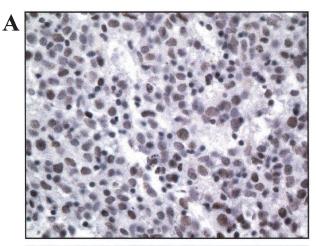

FIG. 6. Detection of SV40 T-ag DNA in NHLs. (A) PCR-amplified polyomavirus sequences after agarose gel electrophoresis and staining with ethidium bromide (upper panel) and after Southern blotting with oligonucleotide probes specific for individual polyomaviruses (lower panels). Lane M, molecular weight marker. Positive control reactions included plasmids specific for SV40 (SV40+), JCV (JCV+), and BKV (BKV+). The negative control (NC) was a reaction without added DNA template. CA, cancer control samples (colon and breast cancers). Hybridization of the PCR products to an oligonucleotide probe specific for SV40 identified the lymphoma-associated viral sequences as SV40 specific; JCV and BKV were not detected. (B) Sequence analysis of the PCR product from the N terminus of the T-ag gene. The sequence from the lymphoma sample (NHL) is identical to that of SV40 and distinguishable from those of JCV and BKV. From reference 125; used with permission.
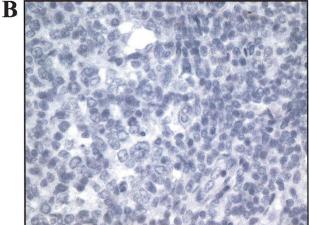
FIG. 7. (A) Immunohistochemical detection of SV40 T-ag in a diffuse large B-cell NHL. (B) Negative control specimen (a reactive lymph node). Specimens were stained by using a monoclonal antibody, PAb416. Note the strong nuclear staining of the majority of lymphoma cells. Magnification, ×400.

TABLE 2. Timeline for discoveries associating SV40 and human malignancies

Yr	Discovery
1970s–1980s	Pre-PCR: SV40 and brain cancers
1992	SV40 DNA (PCR) and expression of large T-ag in brain cancers
1994	SV40 DNA and expression of large T-ag in malignant mesotheliomas
1995	Infectious SV40 isolated from a brain cancer of a 4-year-old child
1996	SV40 DNA in bone cancers
2002	SV40 DNA in lymphomas
2002	Institute of Medicine concluded that "SV40 exposure could lead to cancer in humans under natural conditions"

may represent a pathway for SV40 infections in humans (of unknown frequency), as there are reports of the detection and expression of SV40 T-ag and the presence of viral DNA in cases of primary brain cancers in infants and young children (5, 71, 72, 117, 129, 133). Also, evidence indicates that zoonotic transmission of SV40 should be a consideration in certain populations. Indeed, laboratory workers in contact with SV40-infected monkeys and/or tissues from those animals had a prevalence of antibodies to SV40 in the range of 41 to 55%, suggesting an increased risk for viral infection among this group of workers (43, 134).

Molecular studies of adult patients with renal disease and recipients of kidney transplants found that SV40 cytopathic effects developed in CV-1 cells cocultured with urinary cells or PBMCs from those patients (66, 67). SV40 sequences were detected by PCR in kidney biopsies from 56% of patients with focal segmental glomerulosclerosis. SV40 DNA was localized to renal tubular epithelial cell nuclei in renal biopsies of patients with focal segmental glomerulosclerosis as determined by in situ hybridization. In addition, studies showed that SV40 DNA sequences from the viral regulatory region were detected and identified in the allografts of immunocompromised pediatric renal transplant recipients (Fig. 4) and in the native kidney of a young adult lung transplant patient with polyomavirus nephropathy (11, 12, 77). Different studies have detected SV40 DNA sequences in PBMCs from various patient populations (26, 31, 66, 72, 73, 132). These results demonstrate the nephrotropic and lymphotropic properties of SV40 and indicate that the kidney can serve as a reservoir for the virus in humans. It appears that patients with acquired and/or iatrogenic immunosuppression are a population at risk for SV40. However, the frequency, natural history, and morbidity of the virus in this increasing patient population are unclear.

Large prospective studies using sensitive and specific reagents for SV40 are needed to determine the prevalence of viral infections in the general population and to define groups of individuals at elevated risk for this emerging pathogen. Similarly important is the need for prospective longitudinal studies that address the morbidity and related mortality of these infections. The use of serologic tests alone may not be the most reliable way to conduct these studies. An enzyme immunoassay method for detection of SV40 antibodies in humans recognizes cross-reactivity between SV40, BKV, and

rates of 9% in Hungary and 4% in the Czech Republic (14). Females had a higher rate of SV40 antibodies than males, reaching 16% in Hungary and 8% in the Czech Republic in certain age groups. SV40 infections were found in similar proportions in both countries among persons not exposed to potentially contaminated polio vaccines and in subjects vaccinated in the era of SV40-free vaccines. Minor et al. (78) recently analyzed over 2,000 sera from the United Kingdom and found an SV40 seroprevalence rate of just under 5%. Most of the neutralizing titers were low, and there was no apparent relationship between antibody positivity and polio vaccine usage. These data suggest that SV40 is being transmitted in the human population today, probably at a relatively low prevalence rate. However, conclusions about seroprevalence rates should be viewed with caution, as very little is known about the human immune response to SV40 infections.

Although the mode of transmission of SV40 among humans is unknown, we speculate that different routes may be involved. Studies with laboratory animals indicate that maternal-infant transmission is one possible route of SV40 spread (91). This

TABLE 3. SV40 in primary brain tumors[a]

Reference	Yr	OR	No. with SV40/total no. in: Brain cancer group	No. with SV40/total no. in: Control group	OR and 95% CI[b]
130	1975	5.000	2/7	0/5	
74	1979	2.077	1/7	0/4	
55	1981	4.018	8/35	0/6	
46	1982	13.829	10/30	0/13	
33	1987	1.000	0/29	0/29	
5	1992	401.087	20/31	0/112	
72	1996	1.901	32/83	33/133	
117	1997	5.385	7/33	1/21	
133	1999	32.867	43/65	0/8	
128	2000	3.415	5/267	0/81	
71	2001	25.000	2/2	0/2	
73	2002	1.714	10/25	14/50	
35	2002	1.194	1/47	0/18	
Combined (13 studies)		3.917	141/661	48/482	

[a] Analysis of studies which examined the presence of the polyomavirus SV40 in primary brain tumors compared with control samples. Modified from reference 122; used with permission.

[b] The associations between SV40 and case or control status are reported as ORs for each study (black dots) and 95% CIs (bars), which are displayed on a logarithmic scale. The combined OR is shown as a black square. ORs and CIs are truncated at 100. An OR of >1 indicates that SV40 was more common in brain tumor samples than in control samples.

JCV, complicating interpretation of assay results (126). Similar limitations have been found in serologic methods for identification of human infection with herpes B virus (Cercopithecine herpesvirus 1), which is known also to naturally infect rhesus macaques (*M. mulatta*) (45). Because infection with B virus in humans results in fatal encephalomyelitis or severe neurologic impairment, rapid and conclusive diagnosis is critical in order to control sequelae by this viral pathogen. Serologic assays (including enzyme immunoassay) for B-virus infection in humans are limited by low sensitivity and specificity (45). Currently, cell culture for the three polyomaviruses known to infect humans (JCV, BKV, and SV40) is rarely helpful in establishing diagnosis of infection because of slow viral growth and the requirement for specialized cell lines (52, 56). Serologic assays may be useful for retrospective epidemiological analysis, but they are of minimal use for diagnosis or therapeutic decisions because most overt polyomavirus infections are believed to result from reactivation of latent infections (52, 56). Therefore, the use of modern molecular biology assays is an excellent and preferred alternative for the analysis of SV40 infections in the human population (123). In addition, these sensitive and specific techniques are able to provide insights into the possible infectious etiology of human malignancies (37, 79, 123).

TABLE 4. SV40 in malignant mesotheliomas[a]

Reference	Yr	OR	No. with SV40/total no. in: Mesothelioma group	No. with SV40/total no. in: Control group	OR and 95% CI[b]
17	1994	24.421	29/48	3/51	
25	1995	36.429	8/11	0/7	
86	1996	20.455	4/9	0/12	
88	1998	12.091	7/18	5/100	
40	1998	3,232.998	26/26	0/30	
103	1999	38.333	57/118	0/20	
94	1999	8.727	8/12	11/59	
92	1999	152.565	14/25	0/60	
28	1999	7.800	13/28	1/10	
115	2000	2.036	9/23	6/25	
89	2000	22.612	50/83	0/7	
34	2000	0.525	0/29	0/15	
48	2001	1.000	0/25	0/25	
120	2001	76.304	32/66	0/40	
36	2002	33.000	5/7	0/7	
Combined (15 studies)		16.848	262/528	26/468	

[a] Analysis of studies which examined the presence of the polyomavirus SV40 in malignant mesotheliomas compared with control samples. Modified from reference 122; used with permission.

[b] See Table 3, footnote b. Individual ORs are shown as open circles.

TABLE 5. SV40 in NHLs[a]

Reference	Yr	OR	No. with SV40/total no. in:		OR and 95% CI[b]					
			NHL group	Control group	0.01	0.1	1	10	100	
26	2001	2.1	15/79	19/187						
102	2002	13.3	29/68	8/151						
125	2002	342.8	64/154	0/240						
Combined (3 studies)		5.4	108/301	27/578						

[a] Analysis of studies which examined the presence of the polyomavirus SV40 in NHLs compared with control samples. Modified from reference 122; used with permission.
[b] See Table 3, footnote b. Individual ORs are shown as open squares.

ROLE OF SV40 IN HUMAN CANCER

Experimental Approaches

During the last decade, many studies have shown the presence of SV40 large T-ag DNA or other viral markers in primary human brain and bone cancers, malignant mesotheliomas, and NHL (Fig. 6). Sequence analyses (Fig. 3 and 5) and detection of T-ag protein (Fig. 7) ruled out laboratory contamination of tumor samples. Importantly, infectious SV40 was isolated from a primary brain cancer of a 4-year-old child (62). An important consideration when evaluating the molecular biology data is the sensitivity of methods used to detect SV40 in human tumor samples. Early studies (before 1992) identified SV40-positive neoplasms by using indirect immunofluorescence for viral proteins or DNA hybridization techniques (55, 74, 130), whereas studies after 1992 generally used PCR-based assays.

During the last three decades more than 60 original studies have reported the detection of SV40 in primary brain and bone cancers, malignant mesothelioma, and NHL, whereas a few studies have described an absence of SV40 in those malignancies (16, 33, 34, 44, 48, 70, 113). However, the small numbers of samples tested, the histologic types of malignancies examined, and the laboratory methodologies employed in some cases limit the significance of the results in those studies reported to be negative. Indeed, several steps need to be considered when performing molecular studies of human specimens (1, 50, 61, 107). First, the extraction step of nucleic acids determines whether tissues yield adequate and suitable DNA or RNA for analysis. Unfortunately, with formalin-fixed and paraffin-embedded specimens, degradation of nucleic acids and proteins is a common problem, and the quality of recovered DNA may be poor. If only small amounts of paraffin-embedded tissues are available, the yield of nucleic acids may be inadequate for analysis. Primers directed to a human cellular gene should be used to establish the suitability of a sample for PCR analysis. Because of the sensitivity of PCR-based assays, it is important to rigorously guard against laboratory contamination of samples and controls during processing or testing. Tissue processing and PCR assay setup should be performed in different facilities, from which positive controls (i.e., plasmids) are excluded. Negative tissue controls, extracted and analyzed in parallel, should be included in each experiment to monitor for reagent contamination. The selection of primers and PCR conditions greatly influences the sensitivity and reliability of the assay. Another factor is that tumor specimens usually contain mixtures of normal and malignant cells, in varying proportions. Variations in one or more of these important parameters may explain, at least in part, the ranges in positivity observed among some positive studies and the results obtained in some negative studies.

Summary and Meta-Analysis of Controlled Studies

Table 2 provides a timeline for landmark discoveries associating the polyomavirus SV40 and human malignancies. Although numerous studies have detected SV40 in human primary brain and bone cancers, malignant mesothelioma, and NHL, the small sample sizes and the lack of a control group in some studies made it difficult to make conclusions about the extent to which SV40 may be associated with those human cancers. For this reason, we conducted a meta-analysis of controlled studies (122), an approach which can provide a more balanced and less biased estimate of the evidence than individual studies (57). For inclusion in the meta-analysis, reports had to meet the following criteria: studies were conducted among patients with primary malignancies, the investigation of SV40 was performed on primary cancer specimens and not on cultured cells, the analysis included a control group, and the same laboratory technique was used for both case and control samples. These criteria were established because the use of appropriate controls is crucial in the proper analysis of tissue for viral DNA, especially considering the sensitivity of PCR techniques (38). Thirty-five independent studies met these inclusion criteria. In total, data from 1,793 patients with primary malignancies were evaluated to determine whether SV40 is significantly associated with primary brain cancer, malignant mesothelioma, bone cancer, and NHL.

Thirteen studies fulfilled the criteria for the investigation of primary brain cancers (Table 3). The combined odds ratio (OR) of the studies used in the analysis was 3.9 (95% confidence interval [CI], 2.6 to 5.8). This effect was based on specimens from a total of 1,143 patients, of which 661 were primary brain cancer samples and 482 were control specimens. A modifier detected was the type of sample analyzed (paraffin embedded versus frozen). The adjusted OR was 3.8 (95% CI, 2.6 to 5.7). For malignant mesothelioma, 15 studies fulfilled the criteria; the combined OR of analysis was 16.8 (95% CI, 10.3

to 27.5) and was based on 528 patients with malignant mesothelioma and 468 controls (Table 4). Modifiers detected were the type of control tissue and the method of detection of SV40. The adjusted OR was 15.1 (95% CI, 9.2 to 25.0). The combined OR of the analysis of bone cancers and SV40 was 24.5 (95% CI, 6.8 to 87.9) and was based on 303 patients with bone tumors and 121 controls from four reports (122). The OR for NHL was 5.4 (95% CI, 3.1 to 9.3) and represented 301 cases and 578 controls included in three studies (Table 5). Because there were only three studies that fulfilled the inclusion criteria, further examination of modifying variables was not possible for NHL.

This analysis of published reports found a significant excess risk of SV40 associated with human primary brain cancers, malignant mesotheliomas, bone cancers, and NHL compared to control samples. Therefore, the major types of human malignancies associated with SV40 are the same as those induced by SV40 in animal models. Although the proportion of human cancers containing SV40 varied from study to study, viral prevalence was always greater among primary tumors than among control tissues. Importantly, analysis of data indicated that SV40 may be etiologically meaningful in the development of a specific subset of human cancers. Multiple studies have shown the expression of SV40 mRNA and/or T-ag in cancer cells, the integration of SV40 sequences in some cancers, and SV40 T-ag protein complexed with p53 and pRb in some tumor specimens (1, 10, 13, 39, 50, 76, 122). These findings are compatible with current understanding of how SV40 T-ag mediates oncogenesis. Moreover, microdissection of human malignant mesothelioma samples followed by PCR detected SV40 T-ag DNA only in cancer cells and not in adjacent nonmalignant cells (1, 39, 104). These results from different experimental studies support the conclusion of the Institute of Medicine (111) that "the biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions."

FUTURE DIRECTIONS AND CONCLUSIONS

Mounting evidence indicates that SV40 is a human pathogen, and current molecular biology, pathology, and clinical data, taken together, show that SV40 is significantly associated with and may be functionally important in the development of some human malignancies. Now, prospective studies are needed to determine the prevalence of SV40 infections in different human populations and to assess how the virus is transmitted from person to person. Indeed, the Institute of Medicine recognized that this gap in our understanding of the pathogenesis of SV40 in humans is important and recommended "targeted biological research" of SV40 in humans, including "further study of the transmissibility of SV40 in humans" (111). Considering that molecular biology approaches provide sensitive and specific approaches to analyze infectious diseases and malignancies with a possible infectious etiology, studies using these modern methods should be used to assess the distribution of SV40 infections and morbidity in humans today.

Although in vitro studies have established that SV40 disrupts critical cell cycle control pathways, it remains unknown whether these perturbations are sufficient for the virus to induce the development of malignancies in humans. Therefore, animal models that reproduce key features of SV40 infection and disease in humans are needed. Such models could provide precise evidence of the causal role of a particular pathway in SV40 pathogenesis in target tissues, allow further characterization of the molecular mechanisms of oncogenesis, and provide a preclinical system to test therapeutic interventions for these significant and increasingly common diseases.

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