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Is There a Role for SV40 in Human Cancer?

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

The question of whether Simian Virus 40 (SV40) can cause human tumors has been one of the most highly controversial topics in cancer research during the last 50 years. The longstanding debate began with the discovery of SV40 as a contaminant in poliovirus vaccine stocks that were used to inoculate approximately 100 million children and adults in the United States between 1955 and 1963, and countless more throughout the world. Concerns regarding the potential health risk of SV40 exposure were reinforced by studies demonstrating SV40's potential to transform human cells and promote tumor growth in animal models. Many studies have attempted to assess the relationship between the potential exposure of humans to SV40 and cancer incidence. Reports of the detection of SV40 DNA in a variety of cancers have raised serious concerns as to whether the inadvertent inoculation with SV40 has led to the development of cancer in humans. However, inconsistent reports linking SV40 with various tumor types has led to conflicting views regarding the potential of SV40 as a human cancer virus. Several recent studies suggest that older detection methodologies were flawed, and the limitations of these methods could account for most, if not all, of the positive correlations of SV40 in human tumors to date. Although many people may have been exposed to SV40 by polio vaccination, there is inadequate evidence to support widespread SV40 infection in the population, increased tumor incidence in those individuals who received contaminated vaccine, or a direct role for SV40 in human cancer.

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INTRODUCTION

The debate over whether or not Simian Virus 40 (SV40) can cause human cancer has been one of the most contentious topics in oncology in the last 50 years. The inadvertent exposure of nearly 100 million people in the United States, and many more throughout the world, to SV40 through the administration of contaminated poliovirus vaccines, and the subsequent finding of the oncogenic activity of the SV40 viral large and small T antigens, has led to concern over the potential of SV40 to cause cancer in humans. Since its discovery in 1960, thousands of studies investigating the biology of SV40 have been reported. Although much progress has been made elucidating the mechanisms of transformation by SV40, the issue surrounding the role of SV40 in human cancer has remained unresolved.

The aim of this review is to give a broad overview of the evidence implicating SV40 in human cancer, and to relate these studies to the biologic mechanism of SV40-mediated transformation. We seek to evaluate the biologic plausibility of the association of SV40 with human cancer. In particular, we highlight several recent advances in SV40 detection methodology that have helped to clarify the controversy surrounding SV40 and human cancer.

DISCOVERY OF SV40

The discovery of SV40 was reported in 1960 as a contaminant present in polio vaccine stocks.[1] At this time, polio vaccine was being produced in primary kidney cells and tissue harvested from rhesus and cynomolgus macaques. Safety testing of vaccine preparations on cultures of African green monkey kidney cells uncovered the presence of SV40, a "vacuolating agent," that induced a cytopathic effect in some cultures.[1] SV40 was detected in preparations of both the inactivated, or Salk, poliovirus vaccine and the live attenuated, or Sabin, poliovirus vaccine. In the United States, the Salk inactivated poliovirus vaccine (IPV) was used to inoculate 98 million children and adults between 1955 and 1963, whereas about 10,000 people received the Sabin oral polio vaccine (OPV) through clinical trials between 1959 and 1961.[2,3] Because OPV was not licensed in the United States until 1963, after the establishment of government regulations requiring all poliovirus vaccine to be free of SV40, vaccination with IPV from 1955 to 1963 is thought to be the primary source of human exposure to SV40 in the United States. An adenovirus vaccine developed by the United States military and used to vaccinate Army recruits during this period was also found to be highly contaminated with SV40.[4] However, because of its limited distribution, this vaccine is not

thought to have posed a significant risk for exposure of SV40 into the general population.

Both the Salk and Sabin vaccines, including poliovirus stocks, cell cultures, and the methodologies necessary to generate the vaccine, were distributed to many countries throughout the world. In at least some instances, vaccines produced in other countries were also contaminated with SV40 virus. A recent report provides evidence that some polio vaccines prepared by an Eastern European vaccine manufacturer were contaminated with SV40 and used in the former Union of Soviet Socialist Republics as late as 1978.[5]

The extent of the contamination of poliovirus vaccine stocks with viable SV40 virus is unclear. The formalin inactivation process used in the production of IPV was not as effective in inactivating SV40, and thus it is possible that live SV40 remained present in IPV stocks.[6] Safety testing of early IPV lots was not performed systematically, and it is unclear whether any testing was done before 1961. Furthermore, not all batches of the polio vaccine were tested for SV40, and SV40 did not contaminate all of those vaccine stocks tested. Among vaccine lots found to be contaminated, the titer of infectious SV40 present varied greatly.[7] In 1961, the US government passed regulations requiring all polio vaccine produced to be free of SV40.[2] However, stocks of vaccine produced before 1961 were not recalled, and thus contaminated vaccine may have continued to be used for immunizations until 1963. This combination of factors has made it difficult to know precisely who was inoculated with SV40-containing polio vaccine, but given the fact that most people received several vaccinations, it is likely that all those vaccinated during this period received some exposure to SV40.[2]

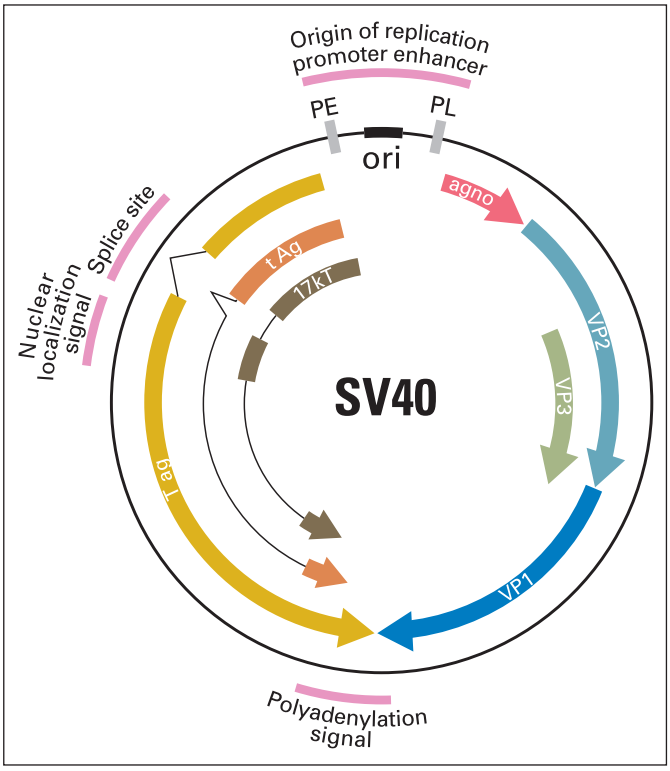

Fig 1. Simian Virus 40 (SV40) genome organization. Regulatory elements of the SV40 genome used in the construction of laboratory plasmids are highlighted in red, including the small t antigen (t Ag) intron splice site, the large T antigen (T ag) polyadenylation signal, promoter, enhancer, and origin of replication. The early (PE) and late (PL) promoters, origin of replication (ori), major SV40 viral proteins are also represented. agno, agnoprotein.

SV40

SV40 is the most well characterized member of the *Polyomaviridae* family of small DNA tumor viruses. The genome of SV40 consists of 5,243 base pairs of double-stranded DNA and encodes seven viral proteins in overlapping reading frames.[8,9] The viral genome encodes 3 structural proteins of the virus (VP1, VP2, and VP3), two proteins important for the viral life cycle (large T antigen and small t antigen) and two small proteins of unknown function (agnoprotein and 17kT).[10,11] In addition, the SV40 genome contains two strong transcriptional promoters, an origin of replication, several splicing sites, and a polyadenylation signal (Fig 1). Many of these viral DNA elements have proven to be invaluable tools for molecular biology research and have been inserted into nearly every mammalian expression plasmid vector used to this day.[12,13]

SV40 virus naturally infects certain species of Asian macaques, in particular the rhesus monkey. SV40 establishes a low-level infection in its host, where it persists in the kidneys without apparent effect. It is thought that SV40 may be excreted through the urine of the infected host and transmitted through the oral or respiratory route.[2] SV40 is closely related to two human polyomaviruses, JC virus (JCV) and BK virus (BKV). Infection with JCV and BKV is prevalent in the human population, although the mode of transmission is not clear. BKV and JCV exist as inapparent infections in immunocompetent hosts, but can produce pathologic effects in immunocompromised individuals through the destruction of infected cells.[14] BKV has been associated with renal nephropathy, affecting as many as 5% of renal-transplant recipients.[15,16] JCV can cause progressive multifocal leukoencephalopathy, a particular problem for HIV-infected individuals, and has recently been reported in patients with multiple sclerosis or Crohn's disease treated with natalizumab.[17,18]

SV40 AND CELLULAR TRANSFORMATION

Soon after SV40 was discovered, several groups reported that the virus could form tumors in rodents as well as induce transformation of primary cultures of human cells.[19-23] Concern over the potential public health risk for cancer posed by the inadvertent inoculation of polio vaccine recipients with SV40 led to an intense investigation into the biology of SV40. As a result of these studies, many important advances in the field of molecular biology have come to fruition. Some of these findings include mechanisms of DNA replication, RNA splicing, transcriptional regulation, identification of the first protein nuclear localization signal, identification of the tumor suppressor p53, and the discovery of cell cycle regulation by the retinoblastoma protein (pRb).[24-29]

Since the discovery of SV40, much progress has been made in understanding its oncogenic potential. The process of cellular transformation induced by SV40 typically depends on the integration of the viral DNA into the host genome in a manner that allows a high level of expression of the major viral oncogenic proteins, large T antigen and small t antigen. SV40 large T antigen can cooperate with small t antigen and additional cellular oncogenes to fully transform a variety of primary human cells.[30-32] Expression of large T antigen alone, in the

absence of other SV40 viral proteins, is sufficient to transform a variety of primary rodent cells.[33-35]

Large T antigen's transforming properties depend on its ability to bind to and perturb the cellular p53 and pRb tumor suppressor proteins. p53 and pRb normally function to prevent tumorigenesis by controlling cellular proliferation and apoptosis. Cellular distress signals such as DNA strand breaks, absence of growth factors, oncogene activation, and hypoxia can serve to activate the p53 and pRb tumor suppressor pathways. In many human tumors, the p53 and pRb proteins may be absent or mutated due to genetic alterations, thus resulting in uninhibited cellular proliferation and tumorigenesis. The functional inactivation of these tumor suppressor proteins is a common molecular mechanism of cancer occurrence with nearly all human cancers having disruptions in these two pathways.[36-38] Similarly, direct inactivation of p53 and pRb by binding to SV40 large T antigen causes unregulated cell growth leading to cellular immortalization and transformation in certain cell types.

In addition to its role in promoting growth, large T antigen provides essential functions necessary for the replication of the virus. Large T antigen binds specifically to the origin of replication to initiate viral DNA replication. Large T antigen has intrinsic adenosine triphosphatase and DNA helicase activity necessary to unwind the viral DNA and promote replication. The helicase domain of large T antigen is contained within and overlaps with the p53-binding domain. Therefore large T antigen provides essential functions to promote cellular proliferation and viral replication.

It is important to note that SV40 has shown oncogenic activity in cellular and animal models in a manner similar to human papillomavirus (HPV). Oncogenic strains of HPV encode the E6 and E7 viral oncoproteins that cause cellular transformation by targeting the p53 and pRb proteins, respectively, for inactivation.[39] In nearly every case of cervical cancer, the HPV genome is integrated into the chromosomes of affected cervical cells in such a way to permit high level and constitutive expression of the E6 and E7 viral oncoproteins.[40,41] In addition, specific antibody responses to HPV viral capsid proteins can be detected in cervical cancer patients. It should be noted that SV40 viral DNA has rarely, if ever, been found to be incorporated into the chromosomes of cells derived from a human cancer. Therefore, if SV40 is present in human tumors, it is likely to exist as a viral episome, and thereby require large T antigen expression for persistence.

ASSESSMENT OF THE ROLE OF SV40 IN HUMAN CANCER

Studies demonstrating the oncogenic potential of SV40 in animal and laboratory models prompted concerns regarding the public health risk of individuals exposed to SV40-contaminated polio vaccine and increased incidence of cancer. Several lines of evidence have been used to assess the relationship between exposure of humans to SV40 and cancer incidence and mortality: population-based and case control studies of people likely exposed to SV40-contaminated polio vaccines, serologic studies assessing potential exposure to SV40, detection of viral DNA in tumor tissue using polymerase chain reaction (PCR), and immunohistochemistry for large T antigen. Using these methods of assessment, SV40 has been implicated in a wide range of human cancers including mesothelioma, osteosarcoma, and non-Hodgkin's lymphoma, as well as a variety of childhood brain tumors such as ependymoma and choroid plexus tumors. However, many other studies have failed to demonstrate an association of SV40 with these same tumor types. Inconsistent reports of the association of SV40 with human tumors have led to conflicting views regarding the potential of SV40 as human cancer virus.

Concerns regarding the possible health threat posed by SV40 and the accumulating evidence that SV40 may be present in human tumors led the Institute of Medicine (IOM) Immunization Safety Review Committee to assess the potential that SV40 from contaminated polio vaccine, as well as other possible sources, is present in humans and contributes to the development of cancer. In 2003, the IOM published their findings confirming that there was substantial evidence that SV40 contaminated many of the early lots of polio vaccine and that SV40 had the potential to cause human cancers.[3] However, the committee was troubled by the inconsistencies encountered in the detection of SV40 in human tumors and thus was unable to come to an authoritative resolution. Instead, the IOM recommended that more specific and sensitive tests be developed to resolve these issues.

EPIDEMIOLOGIC EVIDENCE

In the United States, the major source of human SV40 exposure was the mass immunization program for poliovirus in the late 1950s and early 1960s. However, inadequate safety testing done on vaccine stocks during this period has made it difficult to determine who was inoculated with SV40-containing vaccine and how much viable SV40 was present in the contaminated vaccine. Furthermore, little is known about the potential of SV40 to infect humans. Because the mode of transmission of SV40 is not known, it is difficult to determine whether person-to-person transmission has occurred within the population or if other sources of SV40 exposure exist in the population independently from the poliovirus vaccine.[42-47] This ambiguity in defining exposed and control individuals has made it difficult to assess the risk of SV40 exposure and cancer. Despite these limitations, numerous epidemiologic studies have analyzed cancer incidence data in relation to probability of SV40 exposure. These studies have been extensively reviewed elsewhere and only a few pertinent examples will be briefly discussed here.[48]

Cancer incidence has been assessed for people with high risk of exposure to SV40-contaminated poliovirus vaccine, such as those born between 1955 and 1963, compared with people with low risk of exposure, those born in 1964 and later. No significant increase in overall cancer incidence between exposed and unexposed birth cohorts has been demonstrated for medulloblastoma, osteosarcoma, ependymoma, mesothelioma, choroid plexus tumor and non-Hodgkin's lymphoma.[49-55]

A series of long-term follow-up studies have attempted to assess cancer incidence of SV40-exposed individuals over their lifetimes. One particular cohort of 1,073 newborn infants with documented exposure to SV40 through poliovirus vaccination has provided insight on the effects of SV40 exposure in the neonatal period. Studies of neonates inoculated with SV40-containing poliovirus vaccine and followed up after 6 to 8 years, 17 to 19 years, and 35 years all found no excess risk of cancer attributable to SV40 exposure.[56-58] These studies are of particular interest because laboratory experimentation in hamsters has demonstrated that age at the time of exposure is an important factor in susceptibility to the oncogenic effects of SV40. Animals

injected with SV40 virus as newborns developed tumors with a frequency of almost 100% while older animals were more resistant to tumor formation after injection with SV40.[59]

Another study of US Army veterans assessed the likelihood of exposure to SV40-contaminated adenovirus vaccine (those individuals recruited between 1959 and 1961) in relation to the development of certain types of SV40-associated cancers. The study found no increased risk for brain tumors, mesothelioma, or non-Hodgkin's lymphoma associated with exposure to adenovirus vaccine despite the presence of potentially high titers of SV40 in the vaccine.[60]

Despite some limitations, taken together, these epidemiologic studies have been unable to demonstrate an association between SV40 exposure and cancer incidence. There was no trend of increased number of cancer cases relating to vaccine distribution in the years during and immediately after immunization with contaminated vaccine, and there has been no such trend in the population to date.[7,52]

SEROLOGIC EVIDENCE OF PRIOR EXPOSURE TO SV40

Early Serologic Studies

Early serologic studies conducted on individuals who received SV40-contaminated polio vaccine found that those individuals immunized with SV40-contaminated IPV developed neutralizing antibodies to SV40 at various levels. Immune response appeared to reflect the amount of contaminant present in the vaccine; only 30% to 50% of individuals mounted a significant antibody response against formalin-inactivated SV40 after three doses of vaccine. Antibody titers persisted for a period of up to 3 years postinoculation.[61] Seroconversion may have resulted from an antibody response mounted against formalin-inactivated virus proteins or live SV40 contaminating the vaccine. Thus, seroconversion rates are conservative indicators of SV40 exposure, reflecting any immune response to SV40.

Interestingly, Melnick and Stinebaugh found that children who received OPV did not develop neutralizing antibodies even though they may have received large doses of live SV40 compared with the potentially inactivated SV40 in IPV. However, some children did excrete low levels of SV40 in their stool for up to 5 weeks after ingestion of the vaccine, indicating viral clearance. This study suggests that oral SV40 exposure probably did not result in a substantial or sustained infection of SV40 in vaccinated individuals.[43]

Another early study explored the serological responses of adult volunteers inoculated with SV40 intranasally. The authors found that SV40 introduced by the respiratory route elicited a neutralizing antibody response in about two thirds of the volunteers. In general, low levels of SV40-specific antibodies were detected, suggesting that exposure to SV40 by the respiratory route induces only a low-grade infection in humans.[44]

One might expect that if SV40 infection of humans is contributing to cancer development, individuals suffering from related cancers would develop higher titers of antibodies to SV40, and in particular antibodies to the oncogenic SV40 large T antigen. By analogy, cervical cancer patients often have detectable antibodies not only against the HPV L1 viral capsid protein but also to the E7 and E6 viral oncogenes that are necessary for the oncogenic transformation.[41] Although animals with SV40-induced tumors have been shown to mount a specific antibody response to T antigen, this has not been the case for humans.[62] Two early studies showed that cancer patients did not demonstrate an increased prevalence of SV40 antibodies.[63,64] Furthermore, none of these early studies were able to detect antibodies specific to SV40 T antigen.[61,63,64]

Several early studies reported the presence of SV40 neutralizing antibodies at low levels in the population. SV40 seropositivity has been demonstrated in individuals with no history of immunization with contaminated IPV or other possible route of exposure to SV40.[46,63-65] Shah et al[65] detected antibodies to SV40 in children born after 1964, well after government regulations required IPV to be free of SV40, as well as in people born before 1954. These studies suggest that humans may become infected by SV40 independent of poliovirus vaccine exposure.

Serologic studies of SV40 infection in humans have been complicated by the discovery of the two human polyomaviruses JCV and BKV in 1971.[66-68] These two viruses are highly homologous to SV40 and are prevalent among the human population. The large T antigens of JCV and BKV share many functional domains with and have high homology to SV40 T antigen. In addition, the viral capsid proteins, including VP1, of JCV, BKV, and SV40 are highly homologous. Because polyomaviruses tend to be highly species specific, the finding of these two human polyomaviruses was significant in addressing the specificity of human serum antibodies against SV40.[2] It is possible that the early reports of SV40 antibody detection in human sera represented some degree of cross reactivity with antibodies against the highly related BK and JC viruses.

Recent Advances in Serologic Detection

The ability to determine human serum antibodies specific for SV40, JCV, or BKV has been greatly facilitated by the development of recombinant virus-like particle (VLP) -based assays. The VLP assays detect antibodies that are specific to the major capsid protein VP1 of SV40, BKV, and JCV.[69] The development of virus-specific assays allows for the detection of antibodies specific to SV40, BKV, and JCV in human sera, as well as the ability to determine the degree of serologic cross reactivity by competitive absorption studies. Using these methods, recent reports have confirmed high levels of cross-reactivity of human sera between SV40 and JCV or BKV. SV40-reactive antibodies found in human sera are typically present only simultaneously with antibodies to JC or BK.[70,71] Competitive inhibition assays have shown that preincubation of SV40-reactive human sera with BKV or JCV VLPs can reduce the SV40 reactivity of the sera (19% to 61% in one study by de Sanjose et al[73]), suggesting that most of the seropositivity for SV40 is actually caused by cross reactivity to BKV or JCV.[71-74] In addition, many individuals have antibodies to JC or BK viruses but not SV40. Furthermore, antibody titers against JCV or BKV are often much higher than those detected against SV40. It should be noted that the VLP assay can not completely rule out prior exposure to SV40 because antibodies generated against SV40 could cross react with JCV or BKV and thereby become attributed to infection to a human polyomavirus.

Many studies have examined seroprevalence for SV40 in cancer patients compared with controls using VLP assays. Recent serologic studies using the VLP-based technology have been unable to demonstrate an association between SV40 seroprevalence and either immunization with poliovirus vaccine or cancer incidence (Table 1). There was no significant difference in seropositivity for SV40 between cancer patients compared with controls in these studies.[47,70-77]

Table 1. Summary of Serologic Studies Using Highly Specific Virus-Like Particle Assays

Reference	Participants	Participant SV40-Specific Seropositivity (%)	
		Control	Case
de Sanjose et al, [73] 2003	1,107 Spanish adults (520 lymphoma cases, 587 controls)	9.5	5.9
Carter et al,[71] 2003	699 samples (122 childhood osteosarcoma cases, 90 prostate cancer cases, 487 controls)	7.7-8.3	2.46 (osteosarcoma) 5.6 (prostate cancer)
Viscidi et al, [74] 2003	130 healthy US adults	10.2	N/A
Engels et al,[77] 2004	1,342 US adults (724 NHL cases, 622 controls)	9.6-10.5	7.2-9.8
Engels et al, [75] 2004	254 North American zoo workers (109 non-human primate workers, 145 "other" workers)	10 ("other" workers)	24 (non-human primate workers)
Engels et al, 2004[47]	250 US pregnant women that received poliovirus vaccination (50 mothers of cancer cases, 200 mothers of controls)	12 (at beginning of pregnancy) 3 seroconversion	13 (at beginning of pregnancy) 8 seroconversion
Engels et al, [70] 2005	180 US adults (85 NHL cases, 95 controls)	5	6
Rollison et al, [72] 2005	510 US adults (170 NHL cases, 340 controls)	1.6	1.8
Lundstig et al, [76] 2005	288 Swedish children, 282 pregnant Finnish women, 100 Swedish benign skin tumor cases	7.6 (children) 5.7-7.1 (pregnant women)	9 (benign skin tumor)

Abbreviation: NHL, non-Hodgkin's lymphoma.

Two recent studies by Engels et al[70,77] confirmed these findings and compared SV40 seropositivity between humans and animals in parallel. Infected hamsters with SV40-induced tumors had readily measurable antibodies to T antigen resulting from T antigen expression within tumor cells. However, in human non-Hodgkin's lymphoma cases, there was no difference in SV40 seropositivity compared with controls (approximately 5% to 10%) in either study (Table 1). Furthermore, in humans, SV40 reactivity was frequently blocked by JC or BK VLPs but not by SV40 VLPs, while macaques and hamsters readily demonstrated SV40 VLP-specific antibodies.[70,77] Taking into account the competitive inhibition assays, Engels et al estimate the seroprevalence of SV40 among individuals born before 1963 to be 1.0 to 1.6% regardless of cancer incidence.[77] These well-controlled studies highlight the lack of significant antibody response in humans to SV40 and the unlikelihood that the exposure of humans to SV40 is contributing to cancer development.

PCR-BASED DETECTION OF SV40 IN HUMAN TUMORS

There are many reports that associate SV40 with human tumors that have relied on PCR-based viral DNA detection assays. Although many studies have been conducted looking for evidence of SV40 DNA in various tumor types, the data have been largely inconsistent. Several reports have detected SV40 DNA in mesothelioma, ependymoma, non-Hodgkin's lymphoma, and bone cancers.[78-87] However, other studies of these same tumor types have been unable to consistently detect SV40 DNA using similar methods.[54,88-93] The conflicting data generated by these reports has called into question the design of some of these studies as well as the methodology used to detect SV40 DNA sequences.

Among the studies that have reported the detection of SV40 DNA in human tumor tissue, SV40 DNA was absent from many tumors and was often present at low levels when it was detected.[87,88] Studies reporting the detection of SV40 DNA in tumor tissue often did not mask the case-control status of their specimens or did not test normal tissues in parallel as controls. Even when negative controls were included, they occasionally were positive for the presence of SV40 DNA. In one report, SV40 DNA was detected in normal (nontumorigenic) tissues of osteosarcoma patients at about the same frequency as in tumor tissues.[80] Other studies have detected SV40 DNA in normal tissues from healthy individuals.[94-97]

It is interesting to note that among the studies that have detected SV40 DNA in human cancers, many of these findings were in tumors samples from patients who were not likely to have been exposed to SV40 through poliovirus vaccination because of their young age.[80-82,84] In these cases, SV40 exposure was presumably from another source that was not identified. It has been suggested that infants could have been exposed to SV40 by maternal transmission if their mothers received contaminated poliovirus vaccine while pregnant. However, there has been no evidence of mother-child transmission of SV40 to support this hypothesis.[47]

Some studies could detect SV40 DNA with certain sets of PCR primers but not others.[82,83,88] Reports of "SV40-like" DNA detected in tumor tissue could be the result of PCR detection of highly homologous JC or BK DNA sequences although many studies have verified the presence of SV40 by sequencing the PCR amplification products.[78-80,82,83] These discrepancies have led to questions regarding the sensitivity and specificity of PCR-based detection of SV40 as well as the possibility of false-positive results caused by laboratory contamination of PCR reactions by SV40 sequences.

Two multicenter studies have carried out efforts to resolve the difficulties associated with the detection of SV40 DNA in human mesothelioma tissue samples using PCR.[89,98] The study conducted by Strickler et al[89] used masked specimens and tested each sample twice in a blinded fashion. However, the contamination of a set of control samples with SV40 DNA cast some doubt on the reliability of the data generated in that study. In contrast, Testa et al[98] tested only a small case series, and did not do so in a blinded fashion. Furthermore, water rather than normal tissue was used as the negative control in that study, leaving open the possibility that the sample tissues may have

been contaminated with SV40 DNA during processing. Unfortunately, these studies did not clarify the confusion surrounding SV40 and human cancer.

RECENT ADVANCES IN PCR DETECTION

Although several reports regarding PCR detection of SV40 sequences in human tumors have been published since the IOM report in 2001, most have been unable to settle the debate surrounding inconsistencies in PCR detection of SV40.[99-106] The question of laboratory contamination of PCR reactions has been raised repeatedly in reports of SV40 detection in human cancer, but had never been adequately addressed until recently. A few studies have made significant progress in addressing the concerns raised by the IOM.[106-108] One study in particular has helped to clarify the issue of laboratory contamination that has been of substantial concern in accurately detecting SV40-specific DNA sequences.

Over the years, many regulatory elements from the SV40 genome have been incorporated into molecular biology plasmids to facilitate heterologous gene expression (Fig 1). Because of the inclusion of these sequences, the potential for the presence of SV40 viral DNA fragments, even in laboratories that do not specifically study SV40, is extremely high. In a recent report, Lopez-Rios et al[108] screened 80 human pleural mesothelioma tissue samples for SV40 by PCR and encountered difficulties with reproducibility of SV40 detection in some samples using previously described conditions. To reduce false-positive results, the authors redesigned their PCR primers to differentiate between SV40 sequences present in common laboratory vectors from SV40 DNA sequences essential for the viral life cycle (Fig 2). By using the redesigned primers, they were able to detect SV40 DNA that corresponded to common laboratory plasmids, but they were unable to detect specific SV40 sequences corresponding to regions of the virus critical for its replication and transformation functions.[108] Although the authors had never knowingly worked with SV40 DNA before, their study revealed SV40 sequences present in a plasmid used in their lab for other purposes.

Many studies that have reported SV40 DNA in human tumors have used PCR primers (eg, PYVfor/PYVrev; SV5/SV6). These PCR primers were favored because they amplified a region of SV40 that could be distinguished from BK and JC viral DNA. However, these primers recognize a fragment of the SV40 genome that includes the SV40 small t antigen intron and splice sites that has been used in countless numbers of laboratory plasmids to enhance recombinant gene expression.[12] Nearly every plasmid used in laboratories today contains one or more of the following SV40 viral DNA elements: the small t antigen splice, the large T antigen polyadenylation signal, the early or late promoters, and the origin of replication. Many studies that reported detection of SV40 DNA in human tumor samples used primers that could amplify these DNA regulatory sequences. Following the report by Lopez-Rios et al, it is likely that the selected PCR primers yielded positive results in tumor samples because they could amplify SV40 DNA from contaminating laboratory plasmids. This insightful and carefully controlled study illustrates the high probability of false-positive results obtained using PCR primers directed at regions of SV40 that can be found in widely used plasmids. Furthermore, it underscores the prevalence of SV40 DNA in laboratories and should invoke extreme caution regarding the possibility of contamination by SV40 DNA. A recent report by Manfredi et al[107] has further confirmed the work of Lopez-Rios et al with similar findings (Table 2).

The results of these reports call into question all previous studies that have used PCR methods to detect SV40 in human tumors because they were conducted with primers that can amplify DNA present in common laboratory plasmids. All future studies on the association of SV40 with human cancer will need to address the concerns regarding PCR contamination raised by these reports. At a minimum, any study reporting the identification of SV40 in a human tumor should include additional PCR primer sets that amplify regions of SV40 viral DNA that are not typically present in laboratory plasmids as well as primers that can selectively amplify contaminating plasmid DNA.

MECHANISM OF SV40 ONCOGENESIS

If SV40 is playing a significant role in human oncogenesis, its gene products should be detected in tumor cells. Other oncogenic viral proteins such as HPV E6, Epstein-Barr virus EBNA2, and human herpesvirus 8 LANA1, are routinely detectable in target cells of human

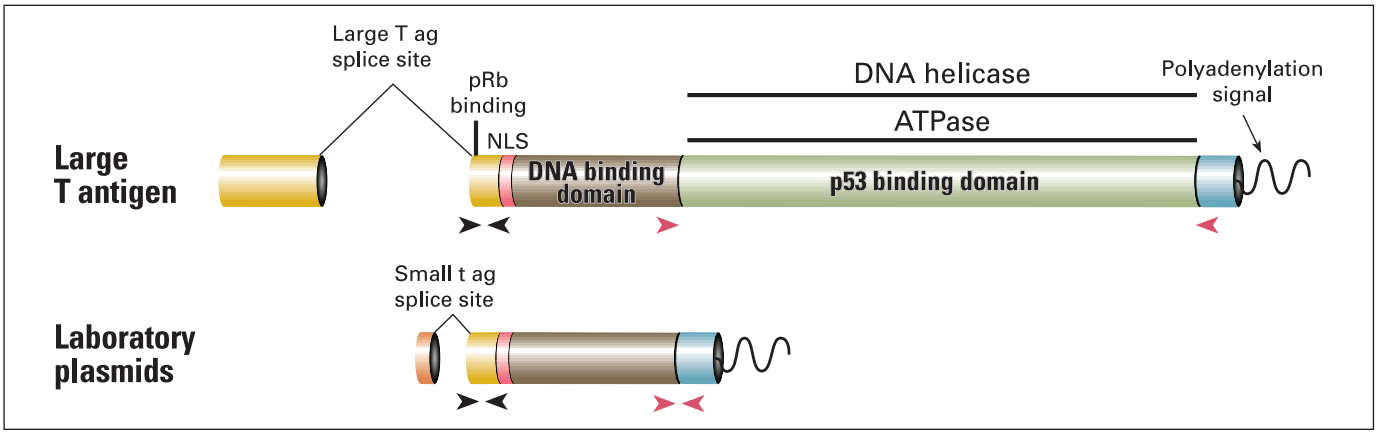

Fig 2. Simian Virus 40 (SV40) DNA frequently amplified by polymerase chain reaction (PCR). The inclusion of T antigen sequences in laboratory plasmids has led to a high risk of contamination of PCR reactions when certain primer sets are utilized. Black arrowheads indicate high-contamination risk primers while red arrowheads indicate primers that distinguish between SV40 viral DNA and SV40 DNA from laboratory plasmids. T ag, large T antigen; t ag, small t antigen; pRb, retinoblastoma protein; ATP, adenosine triphosphate.

Table 2. Summary of Key PCR Studies Published Since the Institute of Medicine Report on the SV40 Contamination of Polio Vaccine and Cancer in 2003 Using Low-Contamination Risk PCR Primers

Reference	Sample Type	SV40-Positive Tumors by PCR Using Specific Primers			Detection of T Antigen (method)
		%	No. Affected	Total No.	
Lopez-Rios et al,[108] 2004	Frozen mesothelioma tissue	6	4	71	Negative (RT-PCR and immunohistochemistry)
Rollison et al,[106] 2005	Adult and pediatric brain tumor tissues; specimens were masked and tested by two independent laboratories	1.8 (no samples tested positive in both labs)	4	225	Not done
Manfredi et al,[107] 2005	Formalin-fixed, paraffin embedded mesothelioma tissue	0	0	69	Negative (immunostaining)

Abbreviation: PCR, polymerase chain reaction; RT-, reverse transcriptase.

malignancies.[109] To resolve the debate over SV40 and human cancer, methods should be focused on the detection of SV40 large T antigen, the major oncogenic protein of the virus.

In most studies, SV40-mediated transformation follows a model analogous to that of HPV. HPV has been proven to cause human cancer, with more than 99% of cervical carcinomas resulting from persistent infection by high-risk HPV.[39] HPV tumorigenesis requires the persistent expression of E6 and E7 in host cells. Both E6 and E7 genes as well as proteins can be consistently detected in HPV-associated cancer tissue.[39] If SV40, like HPV, is capable of causing human cancer, a similar situation should occur, with the expression of biologically active T antigen detectable in all tumor cells. Detection of SV40 DNA fragments by PCR alone in human tumors is not sufficient to imply a causative role for SV40 as a human cancer virus, especially due to the likelihood of PCR contamination. It is necessary to demonstrate the presence of functional protein as well as mRNA specific for large T antigen in human tumors to support a contribution of SV40 to carcinogenesis.

Few studies in the past have provided evidence of T-antigen expression in human tumor samples, and the results that have been obtained have provided weak or negative data in this regard. Within tumor tissues positive for SV40 by PCR, T antigen detection by immunohistochemistry was often undetectable or inconsistent with the PCR results.[83,110] Other recent studies were similarly unable to detect T antigen in tumor tissue by immunohistochemistry or immunostaining using highly specific and sensitive T antigen antibodies to test hundreds of mesothelioma, CNS tumor, and non-Hodgkin's lymphoma samples.[107-109,111,112]

Furthermore, evidence of the tumorigenic function of T antigen in human tumors is required to demonstrate SV40-dependent oncogenesis. Because T antigen's transforming properties depend on its ability to bind to and disrupt the cellular p53 and pRb tumor suppressor proteins, T antigen binding to these two proteins should be evident in tumor cells where SV40 is responsible for transformation. Few studies have attempted to demonstrate T antigen association with either p53 or pRb in human tumor tissue, with only rare examples of tumor tissue containing detectable complexes of T antigen and p53 or pRb.[113-115] These experiments are critical to the implication of SV40 in human cancers because the inactivation the of p53 and pRb tumor suppressor pathways is required not only to establish, but also to maintain T antigen–mediated transformation.

Data from animal models of SV40-induced cancers suggest that T antigen is required to induce and maintain SV40-mediated transformation and should therefore be present in all tumor cells where SV40 is contributing to tumorigenesis.[116-118] However, it has also been suggested that SV40 could transform cells through a hit-and-run mechanism in which T antigen would be required to induce, but not maintain, the transformed phenotype, and therefore may not be detectable in all tumor cells. To test this model, one study conditionally expressed SV40 T antigen in the salivary glands of mice. Transformation was induced in cells expressing T antigen. When T-antigen expression was experimentally reduced in these cells after 7 months of continuous exposure, the transformed phenotype persisted even in the absence of T-antigen expression.[119] In this experiment, it is likely that these cells acquired additional genetic mutations, allowing the maintenance of the transformed phenotype in the absence of T-antigen expression; it is important to point out that a high level of T-antigen expression driven by chromosomally integrated viral DNA was required for an extended period of time to initiate cellular transformation. Although a hit-and-run mechanism for SV40-mediated human carcinogenesis seems unlikely, high levels of biologically functional T-antigen expression that appear to be dependent on clonal integration of SV40 viral DNA that should be readily detected in tumor cells are undoubtedly required for the initial hit. To date, there has been no report that has demonstrated high level of persistent expression of large T antigen in a human tumor.

CONCLUSIONS

Although many people may have been exposed to SV40 through their inoculation with the polio vaccine, there is inadequate evidence at this time to suggest widespread SV40 infection in the population or increased tumor incidence among those individuals who received contaminated vaccine. Recent studies suggest that flawed detection methodology may account for most, if not all, positive correlations of SV40 in human tumors to date. Advances in serologic methods through the use of VLP assays specific to SV40, BKV, and JCV, as well as improved PCR primer design to reduce false-positive results attributed to laboratory contamination of SV40 DNA, have provided significant insights into the question of SV40 and human cancer. Taken together, the studies conducted to date have failed to provide convincing evidence implicating SV40 as a human pathogen.

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Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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GLOSSARY

SV40 (simian virus 40): A polyomavirus isolated from rhesus monkey kidney cell cultures used to produce polio virus vaccine in 1960. SV40 infects several species of monkeys but typically does not cause symtoms or disease. SV40 has been shown to induce transformation in human and mouse cells in vitro and thus has served as an important laboratory tool for studying oncogenesis as well as other biological processes. The major oncogenicprotein of SV40, large T antigen, binds to and inactivates the tumor suppressors p53 and pRb.

pRB (RB phosphorylation): Phosphorylated form of RB, the retinoblastoma susceptibility gene product. Phosphorylated RB has important ramifications for cell cycle progression. In the phosphorylated state, RB is unable to bind to the transcriptional factor E2F (also important for cell cycle regulation). This results in an excess of free E2F, which can then induce transcription of genes involved in cell cycle progression. Hence, phosphorylation of RB allows cells to progress through the G1 checkpoint into S phase of the cell cycle.

JCV (JC virus): A human polyomavirus isolated in 1971 from the brain of a patient with progressive multifocal leukoencephalopathy and named after the initials of that patient, JC. The virus has a greater than 70% nucleotide sequence homology to BKV and SV40. JCV exists as an unapparent infection in immunocompetent hosts but can produce pathologic effects in immunocompromised individuals. JCV can cause PML, a particular problem for individuals with HIV, and has recently been reported in patients with multiple sclerosis or Crohn's disease treated with natalizumab.

BKV (BK virus): A human polyomavirus isolated in 1971 from urine of a renal transplantation patient and named after the patient's initials, BK. The virus has greater than 70% nucleotide sequence homology to JCV and SV40. BKV exists as an unapparent infection in immunocompetent hosts, but can produce pathological effects in immunocompromised individuals through the destruction of infected cells. BKV has been associated with renal nephropathy, affecting as many as 5% of renal transplant recipients.

OPV (Sabin oral polio vaccine): A live attenuated oral polio vaccine developed by Albert Sabin in 1961.

IPV (Salk inactivated poliovirus vaccine): An inactivated polio vaccine developed in 1955 by Jonas Salk. The IPV is delivered by injection.

Episome: A genetic particle of certain cells (ie, bacteria) that can exist on their own in the cytoplasm or as part of a chromosome. A plasmid is an example of an episome.

Helicase: A protein that unwinds DNA at replication forks.

VLP assay (virus-like particle assay): A test such as an enzyme immunoassay based on the recognition of virus capsid proteins in a biologically relevant conformation. VLP assays are often used to test the specificity of antibodies against viruses.

ERRATA

The September 10, 2006, article by Rojo et al entitled, "Pharmacodynamic Studies of Gefitinib in Tumor Biopsy Specimens From Patients With Advanced Gastric Carcinoma" (J Clin Oncol 24:4309-4316, 2006) contained an error in the spelling of S. Ramon y Cajal. It was originally published as S. Ramon Cajal and should have been S. Ramon y Cajal.

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The September 10, 2006, Biology of Neoplasia article by Poulin and DeCaprio, entitled "Is There a Role for SV40 in Human Cancer?" (J Clin Oncol 24:4356-4365, 2006) contained an error in the Authors' Disclosure of Potential Conflicts of Interest section. In addition to "Novartis Pharmaceuticals (B)," "Venable LLP (A)" should have been disclosed for James A. DeCaprio in the Consultant category.

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The September 20, 2006, article by Ng et al entitled, "Prospective Study of [^{18}F]Fluorodeoxyglucose Positron Emission Tomography and Computed Tomography and Magnetic Resonance Imaging in Oral Cavity Squamous Cell Carcinoma With Palpably Negative Neck" (J Clin Oncol 24:4371-4376, 2006) contained an error. In Figure 1, the x-axis was labeled "Specificity," while it should have been "1–Specificity."

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