Cell. Mol. Life Sci. 64 (2007) 803 – 814
1420-682X/07/070803-12
DOI 10.1007/s00018-007-6414-6

Cellular and Molecular Life Sciences

Review

SV40 association with human malignancies and mechanisms of tumor immunity by large tumor antigen

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Received 19 September 2006; received after revision 8 November 2006; accepted 13 December 2006
Online First 26 January 2007

Abstract. SV40 was discovered as a contaminate of poliovirus vaccine lots distributed to millions of individuals in the United States between 1955 and 1963 while contaminated vaccine batches were later circulated worldwide. After SV40 was observed to cause *in vitro* animal and human cell transformations and *in vivo* tumor formations in animals, the search for a connection between the virus and human malignancies has continued to the present day. Different molecular methods have been used to detect SV40 gene products in a variety of human cancers, though SV40 causality in these tumor types has yet to be established. These data, however, are not without controversial issues related to inconclusive SV40 serological and epidemiological evidence alongside tools and methodologies that may contribute to false-positive results in human specimens. This review will also explore how vaccination against SV40 protein products may be used to help prevent and treat individuals with SV40-expressing cancers.

Keywords. Simian virus 40, Simian virus 40 large tumor antigen, cancer; immunotherapy, brain neoplasm, osteosarcoma; non-Hodgkin lymphoma, malignant pleural mesothelioma.

1. Early history of SV40

Beginning in the early 1950s, viral infected rhesus and cynomolgus monkey kidney cell cultures were unknowingly used to prepare formulations of the poliovirus vaccine. In 1960, this contaminant, designated simian virus 40 (SV40), was discovered in rhesus monkey kidney cells when cytopathic effects were observed once the virus was transferred to an African green monkey kidney cell culture [1]. Interestingly, SV40 was unique from other simian viruses known at the time since these cytopathic effects, marked by cytoplasmic vacuolization of infected cells, were not evident in its naturally derived host, ultimately leading to its elusive presence. In 1961, the US federal government required all vaccines to be free of SV40. Unfortunately, existing inactivated polio vaccine contaminated lots were not recalled, and millions of individuals (i.e. estimated between 10–30 million children and adults [2]) were exposed to live SV40 between 1955 and 1963. Though the main source of SV40 exposure to humans has been attributed to the failure to properly inactivate the virus in the poliovirus vaccine, a few individuals were also inoculated with experimental vaccines targeting respiratory syncytial virus or adenovirus that contained SV40 contaminants [2]. Additionally, an experimental live polio vaccine

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was distributed to a small number of volunteers that was contaminated with high titers of SV40 [1], but since the live attenuated vaccine did not become licensed until after 1963, it was presumed to be free of SV40 when it was administered on a much larger scale. In retrospect, this was far from the case, as batches of the live polio vaccine produced by one company until 1978 and dispensed worldwide were recently found to harbor infectious SV40 [3].

After its discovery, concerns progressively mounted with the occurrence of and properties of SV40. The virus was found to be in low concentration in inactivated lots of the polio vaccine [2]. Subsequent experimentation determined that formaldehyde treatment, as was performed to inactivate the poliovirus in the vaccine, did not completely inactivate all SV40 virions [4]. Studies confirmed the ability of SV40 to induce tumors *in vivo* in neonatal hamsters [5-8] and to transform human cell lines *in vitro* [9,10]. Subcutaneous tumor nodules could also be demonstrated in terminally ill human patients injected with SV40 transformed cells [11]. The primary focus therefore was to determine whether a correlation existed between rises in human cancers due to the administration of SV40 contaminated vaccines. However, SV40's potentially damaging effects were mixed. Epidemiological studies involving children and adults appeared to indicate no statistically significant increases in malignancies with those individuals receiving contaminated versus SV40-free polio vaccines [2, 12, 13]. The human response to direct SV40 exposure was also documented in healthy adults and children. With intranasal and oral injections, the antibody response to SV40 and virus recovery was minimal [14, 15]. In contrast, anti-SV40 antibody remained prevalent at least 3 years after individuals were subcutaneously inoculated with the inactivated SV40-contaminated poliovirus vaccine, suggesting a prolonged viral infection [16].

In the 1970s, one convincing report, in particular, presented data from over 50,000 pregnant women vaccinated with the inactivated poliovirus vaccine between 1959 and 1965 [17]. The study concluded that children born to mothers who had received SV40 contaminated lots of the vaccine during pregnancy were at a twofold higher risk of developing malignancies most likely of neural origin and occurring within the first 4 years of birth. Other studies also began to surface during this time detailing the isolation of SV40-related virions and gene products from individuals with melanoma [18] and progressive multifocal leukoencephalopathy (PML) [19, 20], although PML eventually became associated with infection of the human polyomavirus, JC virus (JCV) [21]. SV40-like DNA sequences or antigen expression were also demonstrated in several types of adult brain tumors [22–25] through hybridization and immunofluorescence techniques. Again, reports during this time period downplayed the association of SV40 with human tumors. In one study, SV40-related antigens were undetected in a series of adult brain neoplasms [26]. These SV40 Tag-negative cerebral cultures were derived from a total of 80 brain tumors at the Johns Hopkins School of Medicine, and SV40 Tag expression was probed using sera from hamsters with transplanted SV40 tumors. Additionally, Fraumeni and colleagues performed an 8-year follow-up of infants vaccinated with the SV40-contaminated oral polio vaccine and observed no significant increases in cancer mortalities [27].

The trend of SV40 detection in human samples has extended into the modern day and now includes additional sets of cancers. However, these data are not without controversy, the least of which involves issues such as false-positive results or whether a direct causal relationship between SV40 and certain forms of human cancer indeed exists. It is noteworthy that the aforementioned and other epidemiological studies [28–36] remarking on the association of SV40 with human cancer are plagued with shortfalls, including the inability to clearly distinguish SV40 exposure in individuals studied and latency effects associated with receiving contaminated polio vaccines. Such issues prompted the Institute of Medicine of the National Academy of Sciences in 2002 to conclude that current epidemiological evidence is inadequate to definitively accept or reject a link between human malignancies being the result of SV40-contaminated poliovirus vaccines [37]. Since components of these types of studies are lacking, the awareness and cause for concern surrounding SV40 has been based entirely on the detection of viral remnants in a variety of human cancers.

SV40 biology and oncogenicity

SV40 is composed of a non-enveloped icosahedral capsid with a double-stranded DNA genome that encodes both structural and non-structural protein products. SV40 is also a member of the polyomavirus genus, which includes two other polyomaviruses, JCV and BK virus (BKV), that commonly infect humans. JCV and BKV are recognized as human oncogenic viruses and are primarily associated with PML and hemorrhagic cystitis, respectively [38]. Since all three polyomaviruses are related, they share 70% nucleic acid sequence similarity, though the viruses can easily be distinguished at the genomic level. The viruses are also serologically distinct; however, early and modern

applications are hard pressed to distinguish SV40 from JCV and BKV, as JCV- and BKV-like particle antibodies cross-react with SV40. This technological difficulty has called into question the reliability of serological data supporting or denying the role of SV40 in human tumors [39].

Asian macaques, especially the rhesus species (*Macaca mulatta*), are the known natural host for SV40. Infection of healthy immunocompetent monkeys appears to be non-pathological and benign [40]. On infection of the kidney and SV40 endocytosis into the cell, the virus is transported to the nucleus, where early region messenger RNA (mRNA) is transcribed and early proteins such as large tumor antigen (Tag) are translated. SV40 Tag initiates replication of the viral genome by binding the viral DNA origin of replication and recruiting cellular replication machinery such as DNA polymerase alpha-primase. SV40 Tag also functions to promote the transcription of structural viral genes upon replication by suppressing the production of early gene mRNA. In this scenario, capsid proteins are synthesized and SV40 virions are assembled and released into the cytoplasm upon cell lysis. In order to prevent such a destructive infectious cell cycle, the immune system of a healthy host maintains a low-level viral state, thus achieving an asymptomatic state [41]. In contrast, SV40 administration into immunocompromised monkeys eventually leads to viremia and viruria alongside high and prolonged levels of anti-SV40 antibody, indicating an observable infection and immune response to the virus [40, 42]. Indeed, severe pathological consequences, including widespread SV40 dissemination and tumor formation, can result from infection in monkeys with simian acquired immunodeficiency syndrome [40]. Together with its role in viral replication, SV40 Tag also serves to boost the level of SV40 production within an infected cell by deregulating the cell cycle [41]. This is achieved by binding to and abolishing the functions of the host's retinoblastoma (Rb) protein family, driving the cell into S phase cycle growth. Additionally, SV40 Tag abrogates the function of the tumor suppressor protein, p53, in order to prevent cell apoptosis. In all, these actions by SV40 Tag promote prolonged growth of SV40-infected cells with the potential to induce a malignant host cell phenotype. The specificity and host range of SV40 infection remains largely dependent on the ability of the host polymerase to work concertedly with SV40 gene products such as SV40 Tag [43, 44]. It is in this manner that monkeys and humans support SV40 replication most efficiently. Though SV40 replication is incompatible in hamsters and rodents, many of the first animal studies appeared [5–8], detailing the ability of SV40 to induce specific tumors in newborn hamsters based upon the route of virus inoculation. Ependymomas have been reported to be the result of intracranial injections of SV40 [8], while intravenous injections have caused leukemias, lymphomas and osteosarcomas [45]. Mesotheliomas could also be induced in hamsters through intracardial and intrapleural routes [46]. Thorough *in vitro* analysis has determined that hamster and rodent cells can become briefly transformed with SV40 in an episomal state, though the virus and cell transformation is eventually lost with cell division [44]. However, if SV40 becomes integrated into the host cell genome, cell progeny retain SV40 and a malignant phenotype as Tag continues to be expressed by the cell.

In contrast, human cells, particularly fibroblasts and epithelial cells, allow SV40 replication upon viral entry, but malignant transformation usually does not occur since infected cells are lysed after the assembly of SV40 virions. Interestingly, upon infection with SV40, human cell types such as lung mesothelial cells undergo malignant transformation and immortalization without viral integration into the cellular DNA.

Human cancer relevance to SV40

Beginning in the late 1970s and into the 1980s, occasional reports appeared citing human samples testing positive for SV40-like DNA sequences or gene protein products [18, 22–25, 47, 48]. With the eventual creation and advancement of additional molecular technologies (e.g. polymerase chain reaction [PCR]), it became possible to more accurately ascertain whether SV40 genomic material was indeed present in certain forms of human tumors which are summarized in Table I. To better address the controversies surrounding the results of data implicating the presence of SV40 in many forms of human malignancies, recent meta-analysis on the subject has significantly shown that SV40 is associated with brain tumors, bone cancers, non-Hodgkin lymphoma and malignant mesothelioma [49].

Brain tumors

Interest in SV40 eventually returned with the observation that the virus was present in certain types of brain tumors. In 1992, Bergsagel and colleagues first reported SV40-related Tag sequences in ependymomas and choroid plexus tumors of children by PCR that were distinct from JCV and BKV [50]. Immunohistochemistry analysis also found Tag expression in the nuclei of a portion of these same tumor types, though the Tag protein could not be distinguished from among the three polyomaviruses since the viral proteins evoke an antibody response that commonly

Table 1. Summary of SV40 Detection in Human Cancers

Location	Pathology	Country of origin	Reference no.
Brain			
	ependymoma	Italy, Switzerland, Portugal, USA	50–53
	choroid plexus	Italy, Switzerland, Portugal, USA	50–53
	medulloblastoma	Switzerland, Portugal	52
	other types	Italy, Japan, Portugal, Switzerland, USA	52–56
Bone			
	osteosarcoma	Canada, Germany, Italy, Japan, USA	55, 60, 63–65
Lymphoid tissue			
	non-Hodgkin lymphoma	USA	67–70
Chest and lungs			
	malignant pleural mesothelioma	USA	81

cross-reacts [39]. To expand upon these results, additional work was performed by Lednicky and colleagues [51] on DNAs extracted from the same tumor samples [50] by more comprehensive PCR analysis. In addition to confirming that SV40 viral DNA was present in the majority of tumor samples tested, infectious SV40 from one choroid plexus sample was isolated. Interestingly, viral sequences detected in the studied tumors and isolated infectious virus were distinct from any known laboratory strains and were similar to SV40 sequences obtained from infected monkeys (i.e. archetypal). These sequence data suggested that monkeys and humans are potentially infected with analogous strains of SV40 as a result of a monkey-to-human transmission events. Additional work by others during this time period also amplified SV40 specific sequences through PCR in other childhood brain malignancies such as medulloblastomas [52] and a number of adult brain tumors, including gliomas [52, 53] and meningiomas [53] from Switzerland and Portugal [52] and Italy [53]. At present, further studies have confirmed these early reports of human brain tumors harboring SV40-specific DNA sequences [54–56], but several examples do exist in which SV40 was minimally or not detected in primary brain samples using modern laboratory applications [57–59].

Bone tumors

Osteosarcomas are a rare form of malignant bone cancer that can arise in both children and adults, though the disease normally develops during adolescence. In 1996, Carbone and colleagues were the first to demonstrate SV40-like Tag DNA sequences in a variety of bone tumors from the United States, Canada, Italy and Germany, including osteosarcomas which yielded the majority of SV40-positive samples [60]. Subsequent sequence analysis of DNA amplified in a select few of osteosarcoma tumors were unique to SV40. Additionally, SV40-like positive samples were significantly associated with osteosarcoma patients stricken with Li-Fraumeni [60], a syndrome characterized by increased susceptibility to certain cancers due to one functional p53 allele [61, 62]. Following these initial reports, other accounts of SV40 in bone cancers began to surface. Lednicky and colleagues, again, confirmed the existence of authentic and archetypal SV40 sequences in 50% of osteosarcoma samples derived from patients, though the sequenced SV40 strains differed in the Tag gene from each tumor sample [63], as had previously been observed in human brain malignancies [51]. Mendoza and colleagues were also able to amplify SV40 by PCR in osteosarcoma samples, and through Southern blot hybridization, the authors concluded that SV40 had been integrated into the DNA of 50% of the tumor samples tested [64]. Additionally, a subset of osteosarcoma tissues positive for SV40 DNA by PCR also had disrupted functions in their tumor suppressor proteins, p53 and Rb. More recently, SV40 sequences in osteosarcoma samples from Japan [65] and Italy [55] have been detected and build upon data from the United States associating SV40 with these types of human bone tumors.

Non-Hodgkin lymphoma

HIV-positive patients appear to develop systemic non-Hodgkin lymphoma (NHL) more frequently than the general population [66]. Though the oncogenic herpesvirus Epstein-Barr virus (EBV) has been

shown to be associated with primary central nervous system lymphoma, as a result of HIV infection, EBV has been less defined in AIDS-related systemic NHL. This apparent lack of understanding has led many investigators in the field to pursue the search for other cancer-causing viruses, including SV40, that may be a causative agent for NHL.

Several studies have detected SV40-specific sequences in over 40% of NHL samples tested [67–69], with Burkitt's and diffuse large B cell lymphomas as the most common types affected [67, 68]. A further breakdown of the data has shown that between 22 and 46% of HIV-1-positive patients with NHL also contained SV40 DNA sequences [67, 68, 70]. To further support the link between SV40 and the pathogenesis of NHL, it has been reported that SV40-positive NHL samples commonly have disturbed tumor suppressor genes by promoter methylation [69], and SV40 Tag protein expression was detected in NHL tissues from HIV-1 infected patients [70].

The spectrum of SV40 and NHL studies has not been confined to the detection of SV40 DNA sequences. In fact, many reports downplay those that point to the potential association of SV40 with NHL. Using data from serological experiments, a number of investigators have demonstrated a lack of significant SV40 seroprevalence in patients with NHL [71–74]. Though these noted studies [71–74] relied solely on serological evidence, PCR has been used to show that SV40 DNA sequences were either of low incidence in comparison to controls or unable to be detected in NHL samples from a variety of countries, including the United States [75–79].

Malignant mesothelioma

Malignant pleural mesothelioma (MPM) is an exceptionally lethal cancer that has been causally linked to asbestos exposure [80]. The severity of this disease can range from localized, in which the cancer is confined to the pleura of the chest wall and lungs, to an advanced state where distant organs and tissues become affected. Generally, the onset of MPM occurs in patients decades after exposure to asbestos, and survival upon diagnosis and after standard treatments of chemotherapy, radiation, and surgery is less than 1 year.

The molecular interactions between SV40 and MPM are arguably the best reported of the known SV40-positive tumor types. Among the large volume of supportive data, Carbone and colleagues first described SV40 DNA sequences and SV40 Tag expression in tissue samples taken from MPM patients [81]. Following this discovery, SV40 Tag was found to complex with p53 [82] and the Rb protein family [83], while SV40 infection was discovered to cause a variety of tumor suppressor gene inactivations in mesothelial cells [84]. Additionally, MPM cells were found to be easily susceptible to transformation by SV40 in comparison to other known human cell types [85] and that the malignant phenotype of the cell was maintained with the expression of SV40 Tag [86]. Taken together, a proposed mechanism of infection has resulted from these and other data reporting on interactions between SV40 and MPM that are distinct from human epithelial and fibroblast cells [85]. Upon SV40 entry into the cell, the virus remains episomal rather than integrated into the host DNA. SV40 Tag is expressed but becomes bound to the high levels of p53 common in mesothelial cells, and consequently SV40 replication and the eventual host cell lysis are suppressed. Therefore, a large portion of infected mesothelial cells are not lysed, and these cells become easily transformed due to their prolonged exposure to SV40 Tag. These transformed mesothelial cells also become immortalized from increased telomerase activity which is a result of infection of SV40 and the expression of viral gene products, including SV40 Tag [87].

Currently, it is hypothesized that SV40 works either alone or in conjunction with asbestos to induce a malignant phenotype in mesothelial cells that eventually leads to MPM. This thought arises since SV40 and asbestos have been shown to be cocarcinogens *in vitro* [85], and epidemiological data indicate that only 5–10% of individuals exposed to high levels of asbestos are stricken with the disease, while 10-20% of MPM cases occur in those patients that have had no known exposure to asbestos [88]. Therefore, within this hypothetical cocarcinogen framework, it is possible that upon inhalation of asbestos fibers and over an extended latency period, the immune response is impaired, and SV40-transformed mesothelial cells are afforded the opportunity to grow in an environment shielded from immune detection [44, 88].

A few reports have been unable to observe SV40 sequences in MPM in countries such as Finland, Turkey and Austria [89–92]. These findings seem to fall in line with the geographic distribution of poliovirus vaccines since the inhabitants of these latter countries did not receive the SV40-contaminated version. Proponents of the SV40 connection with mesothelioma have also criticized the methodologies and reporting of other studies yielding negative SV40 results in human MPM tissues that should have had some degree of SV40-positive reactivity based upon the work of other investigators in the field [93]. These issues included concerns based on low PCR sensitivity methods [94–98] and conflicting results published by one group [99, 100]. More recently, Mayall and colleagues [101] and Manfredi and colleagues [102]

were unable to amplify SV40 DNA sequences in human MPM samples from the United States and United Kingdom, two countries that administered SV40-contaminated polio vaccines.

Prevention and treatment

It remains promising that a vaccine could be constructed to treat diseases in which SV40 gene products, such as SV40 Tag, are expressed.

Our laboratory has employed an animal model with a challenge system that both utilizes a tumorigenic cell line, expressing SV40 Tag, and simulates widespread tumor metastasis [103, 104]. This experimental metastasis system accounts for tumor burden in challenged animals by way of the appearance of lung tumor foci and survival. If mice are immunized with recombinant SV40 Tag prior to tumor inoculation, no tumor foci appear on the lungs, and animals achieve complete systemic tumor immunity. However, without priming the immune response to SV40 Tag, mice succumb to tumor challenge and do not normally survive longer than 3 weeks.

Further immune analysis of the protective effect observed in this system has led us to hypothesize that SV40 Tag-specific antibody is important within the course and period following tumor challenge. CD4+ T lymphocytes also appear to be required prior to tumorigenic inoculation of animals [105]. In this sense, it is likely that upon activating T helper-2 type CD4+ T cells, SV40 Tag-specific B lymphocytes become primed and activated to differentiate into plasma cells that secrete SV40-specific antibody. Though this may not be the only role CD4 cells play in this animal model of tumor immunity, the effect of the ensuing *in vivo* antibody response is also less characterized. It remains plausible that tumor cells are scavenged and removed from the system by macrophages and/or natural killer cells that target SV40-specific antibody complexed to the tumor cells in a manner described as antibody-dependent cell-mediated cytotoxicity. Ongoing work within our laboratory is further delineating the overall immunologic response to this tumor challenge system.

We have also further explored the effects of DNA vaccination within this experimental metastasis model in mice in a prophylactic setting [106]. Briefly, systemic tumor immunity is observed only with a vaccine that efficiently evokes both a cell-mediated and humoral-based immune response to SV40 Tag. This hypothesis is a result of data that show the effects of two plasmids with differing promoter strengths that control the expression of SV40 Tag. Mice immunized with the more versatile plasmid elicit an antibody response and remain clear of lung tumor foci. Again, this scenario of plasmid immunization resembles our recombinant Tag protein work in that SV40 Tag antibody appears to be directly linked to tumor immunity.

These mouse studies relate to human scenarios where individuals are stricken with malignancies in which SV40 Tag protein has either been shown to be expressed or SV40 Tag DNA sequences have been amplified. With a clear understanding of the immune mechanisms needed to effectively clear or prevent the tumor presence, an appropriate vaccine could be tailored to activate the necessary immune cell subsets in order to achieve the goal of tumor immunity [107]. For example, within this scenario, individuals at high risk for asbestos exposure and thus MPM could be immunized with plasmid DNA expressing SV40 Tag. SV40-infected cells are kept at bay by the primed immune response, and the patient is less likely to develop a malignant phenotype in the pleura of the chest cavity and lungs upon exposure to asbestos fibers. Should evidence of mesothelioma be detected, the individual could be boosted with a recombinant Tag protein immunization to further augment the immune response to SV40 Tag.

SV40 and uncertainty

Since its discovery, SV40 has been embroiled in the controversial question as to whether the virus demonstrates a credible threat to human health. Though the number of publications supporting the occurrence of SV40 within human malignancies is abundant, doubt still persists surrounding its apparent connection to cancer.

Other oncogenic viruses have demonstrated stronger associations to human malignancies than SV40. For instance, human papillomavirus (HPV) [108] has been definitively linked to cancers of the cervix in women. Specific HPV DNA is readily detected in tumor samples ($>99\%$), and the serological evidence for infection in patients is clear and long-lived. In comparison, SV40 infection in selected human tumors and its potential causal relationship is a more difficult case to make.

Human infection

Once SV40 was found to be a contaminate of rhesus and cynomolgus monkey kidney cell cultures used to prepare early vaccines, studies were initiated to test the human response to the virus. Morris and colleagues first described the symptoms in adult volunteers exposed intranasally to the SV40-contaminated respiratory syncytial virus vaccine [14]. After 1 week, the virus was isolated from throat swabs from only a few patients, and the antibody response to SV40 was

considerably low overall in the majority of patients. The authors concluded from these results that SV40 infection via the respiratory route produced a subclinical response, as most volunteers did not demonstrate an observable infection at the time of and in the months following vaccine inoculation. Melnick and Stinebaugh [15] further demonstrated that SV40 could be isolated for as long as 1 month from the stool samples of children administered the SV40-contaminated oral polio vaccine, though no antibody response was evident from the groups tested. Together, these short-lived SV40 infections by way of the respiratory and oral routes were in contrast to the infection resulting from subcutaneous inoculation of the virus [16]. Children given doses of the inactivated SV40-contaminated poliovirus vaccine through this route developed detectable antibody levels that lasted at least 3 years. However, these early results and their reporting on SV40 antibody must be collectively viewed with knowledge about serological cross-reactivity among the polyomaviruses [39].

With these infectious studies in hand (particularly intranasal and oral SV40 administration and the ensuing temporary infection), it was useful to determine whether SV40 was an infectious agent spreading person-to-person worldwide in the human population. The likely options for this initial virus source included SV40-contaminated vaccines and/or a crossover event to humans from SV40-infected monkeys [42]. Though the virus naturally resides in healthy Asian macaque monkeys without signs of illness, it is hypothesized that SV40 transmission to susceptible animals occurs predominately through virus shedding in the urine and is localized to the kidney [2, 40]. Likewise, it was plausible that SV40 could be isolated in SV40-infected immunocompromised human patients. However, Shah and colleagues were unable to isolate SV40 sequences by PCR in urine specimens from HIV-infected male patients, while BKV and JCV sequences were readily detected in 22 and 31% of those samples, respectively [109]. Further studies on this matter reached similar conclusions by indicating the lack of an SV40 presence in sewage samples from several European countries (France, Spain, Sweden) and South Africa [110]. In contrast, this same group relayed information that human waste in India (where rhesus macaques and humans live and interact closely) tested positive for SV40, suggesting that the virus is being transmitted in the country [111]. To further support the claim that SV40 infection has occurred on a much wider scale with subclinical responses, Butel and colleagues detected SV40-specific sequences in 5% of urine specimens from healthy children ranging in age from 6 to 16 years [112].

Many studies have also ruled out the scenario in which the virus is simply confined to malignancies such as brain, bone and lung cancers. Molecular evidence supports the role of SV40 in contributing to the pathogenesis of kidney disease in both children [112] and adult [113] renal transplant patients who did not receive the SV40-contaminated polio vaccine. SV40 footprints have also been found in two patients suffering from late-onset hemorrhagic cystitis associated with complications of bone marrow transplantation [114]. The virus has additionally been amplified at different levels in otherwise normal tissues from donors [53,55,65,67,75,81].

Seroprevalence

Serological surveys began to be explored in the 1970s and have continued today to assess the extent to which SV40 is prevalent in the human population [40]. As might be expected, individuals in direct contact with non-human primates or SV40 have maintained the highest percentage (up to 55%) of serum samples yielding SV40 antibody [115–118]. Next in prevalence but much lower come those who were potentially exposed to SV40 through the administration of contaminated poliovirus vaccines [117, 119–122], and then samples from people who were not given the vaccine and have had no known interaction with SV40-infected monkeys [117, 119–125]. These latter data are striking in that they suggest that SV40 circulation in the human population existed before and after the wide-scale administration of contaminated vaccines between 1955 and 1963. However, although investigators such as Shah and colleagues [116, 119, 123] initially supported this view; they now contend that past and current serological data do not support SV40 as a widespread infectious agent. The levels of antibody titers to SV40 have been explored in population-based studies [39, 126, 127] and individuals with MPM [94], NHL [71–74], brain [128], or bone cancers [94, 126], and no convincing increases in antibody within particular groups have been found. In most of these cases, the argument put forward is that the low presence of serum reactivity to SV40 is instead an indication of the cross-reactivities of antibodies to BKV and JCV that commonly infect humans [39].

Viral DNA detection

After SV40 was discovered by Sweet and Hilleman [1] and determined to hold great potential to affect public health, the virus became a common item in laboratories for extensive investigation [40]. Interestingly, due to this intent fixation, SV40 was the first eukaryotic viral genome to be sequenced among other molecular advances, and eventually helped lead to the construc-

tion of plasmid DNAs using portions of the SV40 genome [129, 130].

Recently, Lopez-Rios and colleagues [131] have linked amplifiable SV40 DNA to possible plasmid contamination in tumor samples. When these investigators initially analyzed MPM tumor samples using common SV40 primers that target a region of the SV40 Tag gene, over 50% of samples gave positive results. However, when these amplified products were looked at further, it was determined that this region contained sequences also found in many common plasmids utilized in the laboratory. Upon redesigning primer pairs that excluded this common plasmid region, 6% of MPM samples showed faint SV40 detection. Altogether, these data add to the uncertainties involved with SV40 and seem to explain the confusing observation that SV40 gene products are amplified by some and not others in the field. Other concerns have also been raised over the use of PCR in determining the presence of SV40. These include the use of PCR methods and primer sets that might be more conducive to yielding nonspecific DNA products and reproducibility among laboratories studying similar tumor types [41]. Yet investigators stand by their positive PCR data and assert that true SV40 is indeed found in a variety of human tumors and is not simply a result of compounding issues such as contamination with plasmid DNA [132, 133].

Summary and future work

The overall low degree to which SV40 and specific antibody response have been reported in tissue and sera samples from cancer patients and the general population has made it a difficult case to make for its role in human disease. With the activity needed for SV40 to induce a malignant phenotype, it is generally acknowledged that the human response to such an infectious and actively replicating virus would prompt an antibody response to viral products and the ability to isolate high copy numbers of DNA from tissue samples as is observed in HPV infections. However, it cannot be ruled out that SV40 may not follow the above logic to cause human pathogenesis [41]. Not only would this property be distinctive from most viral infections that cause human disease, but it would also be unique in terms of the BKV and JCV infections – two polyomaviruses that are easily isolated and cause observable immune responses in humans. No doubt, SV40's viral characteristics will continue to elude investigators until additional steps are performed to better address the issue [41, 42]. These improvements include development of a highly specific serologic assay to determine the occurrence of SV40 antibody in clinical samples that rules out cross-reactivity to BKV and JCV viral proteins. PCR methodologies must also be modified and standardized in order to reduce the potential for false-positive results (i.e. from plasmid contamination or nonspecific products) while increasing reproducibility among laboratories studying similar tissue types. Finally, new epidemiological studies must be undertaken that address issues such as defining whether individuals were indeed exposed to SV40 by contaminated vaccines or other means (i.e. prolonged exposure to non-human primates), while also taking into account the rarity of SV40-associated cancers and/or the latency effects of SV40 exposure [134].

With these concerns and ideas for future assessment, there still remains an overabundance of data by a large number of laboratories implicating SV40 as a human oncogenic virus. Altogether, *in vitro* analysis has detailed the mechanisms surrounding transformation of animal and human cell lines, while *in vivo* animal models have clearly shown tumor formation upon SV40 inoculation. It is also no coincidence that the same malignancies observed in neonatal hamsters due to certain routes of SV40 administration, for instance, correspond to cancers seen in humans. Alongside the molecular evidence detecting SV40 in tissues by PCR and DNA sequencing, numerous publications have also backed these modern results with older techniques, including viral rescue and hybridization to isolate products akin to SV40 [133].

Vaccine development within this field also remains an intriguing opportunity and viable course to help target SV40 malignancies through either prophylactic or therapeutic means. Since SV40 Tag is an essential protein expressed by the virus for both replication and transformation, it could be utilized as a vaccination tool to help prime the immune response and thwart the outgrowth of SV40-infected cells. These vaccination modalities have the potential to help individuals stricken with cancers of the brain, bone, lymphoid tissues, lungs, and chest cavity.

Acknowledgements. This work was supported in part by a National Institutes of Health grant RR-12317. D.B.L. is a Dean's Scholarship Award recipient.

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