

Increasing evidence for involvement of SV40 in human cancer

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SV40, a small DNA virus, is known to possess strong oncogenic potential. Millions of people were exposed to SV40 as an unknown contaminant of some early poliovaccines. This article briefly summarizes the increasing evidence of the association of SV40 with certain types of human cancer, including mesotheliomas and brain tumors. Unanswered questions pertaining to the pathogenesis of human infections by SV40 and the functional role of the virus in tumor development are noted. It is concluded that SV40 should be considered a candidate human tumor virus and that vigorous efforts to clarify the role of the virus in human disease should be supported.

1. Introduction

The polyomavirus SV40 is a highly tumorigenic DNA virus, with the large T-antigen functioning as a potent viral transforming protein (Fig. 1) [4,5,8,11, 30]. Although SV40 was inadvertently administered to millions of people as an unrecognized contaminant of many poliovaccines between 1955 and early 1963 [8, 15,41], no short-term adverse effects on human health were recognized and those observations led to the belief that SV40 was harmless in humans. It is time to re-evaluate that conclusion.

Occasional reports of the detection of SV40 in human brain tumors started appearing in the 1970's [8], but limitations of available technology dampened the impact of those observations. The first report of detection of SV40 DNA in human brain tumors using polymerase chain reaction (PCR) and sequence analysis technologies appeared in 1992 [3]. Subsequently,

many independent studies reported similar findings involving several types of tumors [1,8,10,25].

This paper will be a brief synopsis of the evidence linking SV40 to human tumors. Although some have been skeptical of the validity of the general observations [43,49] and the evidence does not yet constitute proof of viral etiology, it is the opinion of this author that the totality of data is persuasive and deserves serious consideration. The potential benefit to the public, if the virus were to be established as a factor in human carcinogenesis, would be significant and warrants that these leads be vigorously pursued with the full support of the NIH.

2. Independent studies and complementary approaches

There have been over 50 reports of the detection of SV40 DNA in human tumors [1,8,10,25]. These reports have focused predominantly on mesotheliomas, brain tumors, and osteosarcomas and have originated from geographically distinct regions of the world. As an example of the reproducibility of detection of SV40 sequences in certain types of tumors, results involving brain tumors are summarized in Table 1.

Estimates of SV40 positivity among different types of human tumors vary widely, but generally are above 50% for mesotheliomas and certain brain tumors and about 35% for bone tumors [1,25].

Complementary technical approaches have indicated the presence of SV40 in human tumors. These approaches have included (i) PCR assays of tumor cell DNA using primer sets directed against one or more regions of the SV40 genome, (ii) sequence or Southern blot analysis of PCR products, (iii) immunohistochemistry (IHC) using antibodies to SV40 T-antigen, (iv) protein extractions followed by Western blot assays to detect SV40 T-antigen, (v) *in situ* hybridization to detect SV40 messenger RNA, (vi) microdissection of mesothelioma samples to separate tumor cells from normal cells, (vii) isolation of infectious virus

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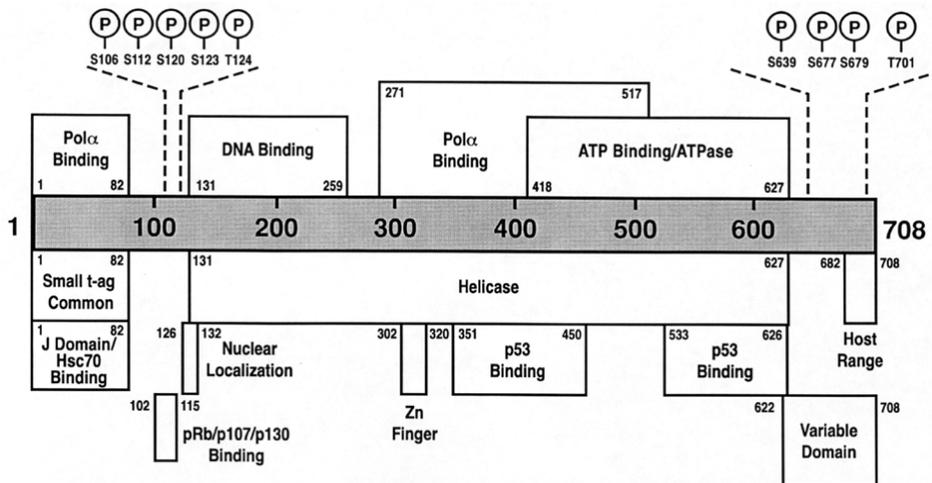


Fig. 1. Functional domains of SV40 large tumor antigen (T-ag). The numbers given are the amino acid residues using the numbering system for SV40-776. Regions are indicated as follows. Small t-ag Common: region of large T-ag encoded in the first exon; the amino acid sequence in this region is common to both large T-ag and small t-ag. Pol α Binding: regions required for binding to polymerase α -primase. J Domain/Hsc70 Binding: region required for binding the heat shock protein hsc70. pRb/p107/p130 Binding: region required for binding of the Rb tumor suppressor protein, and the Rb-related proteins p107 and p130. Nuclear Localization: contains the nuclear localization signal. DNA Binding: minimal region required for binding to SV40 Ori DNA. Helicase: region required for full helicase activity. Zn Finger: region which binds zinc ions. p53 Binding: regions required for binding the p53 tumor suppressor protein. ATP Binding/ATPase: region containing the ATP binding site and ATPase catalytic activity. Host Range: region defined as containing the host range and Ad helper functions. Variable Domain: region containing amino acid differences among viral strains. The circles containing a P indicate sites of phosphorylation found on large T-ag expressed in mammalian cells. S indicates a serine and T indicates a threonine residue. Reproduced from Butel and Lednický [8] as modified there from Stewart et al. [47].

from tumor samples, and (viii) inhibition of SV40-positive tumor cell proliferation by an SV40 early region antisense construct. A recent multi-institutional study in which several participating groups detected SV40 DNA in coded mesothelioma specimens provided strong evidence for inter-laboratory reproducibility of results [52].

3. Common arguments have been ruled out

An argument raised against the observation that SV40 is present in human tumors is that the tumor-associated virus has been misidentified, i.e., that it is not SV40 but is either one of the known human polyomaviruses (JCV or BKV) or a new, previously unrecognized virus. It is certainly possible that JCV or BKV are present in some human tumors and may be etiologically important [2,23,28]. In some studies in which SV40-specific primers were used, confirmatory tests were not carried out to prove that amplified sequences were actually SV40 specific, but in many studies, the PCR products were proven by sequence analysis or Southern blot hybridization to be of SV40 origin [1, 25]. Up to four separate sections of the viral genome

have been amplified from some tumors [31,32], making it highly likely that authentic SV40 was present in those tumors. SV40 genomic DNA was cloned directly from a meningioma [27] and infectious SV40 was rescued from a choroid plexus carcinoma [31]; these human brain tumor isolates have been sequenced in entirety and shown to be authentic SV40 [48]. Thus, SV40 sequences detected in some human tumors have been unambiguously discriminated from those of other polyomaviruses.

Another frequently posed argument is that the positive tumor results are due to laboratory contamination of tumor specimens. Laboratory contamination is a valid concern, given the sensitivity of the PCR assay, the presence of SV40-based plasmids in many laboratories, and the possibility of cross-contamination of PCR reactions with positive control plasmids. It is not possible to know whether contamination may have occurred in any of the studies reporting positive results, but this potential problem as the explanation for all virus-positive tumors has been ruled out (Table 2). Reports often describe extensive precautions that were taken to avoid contamination of samples during specimen processing or analysis, such as the use of dedicated rooms from which plasmids and viruses were

Table 1
Detection of SV40 in human brain tumors

Tumor type	No. positive/No. tested (%)		Ref.
	SV40 DNA	SV40 T-antigen	
Brain tumors	11/32 (34)		22
Brain tumors	14/53 (26)	18/68 (26)	18
Astrocytoma	1/8 (12)		26
Astrocytoma	8/17 (47)		34
Astrocytoma		11/15 (73)	54
Choroid plexus	10/20* (50)	4/5 (80)	3
Choroid plexus	5/6 (83)		34
Choroid plexus	6/16 (38)		21
Choroid plexus	0/7 (0)**		38
Ependymoma	10/11 (91)	3/6 (50)	3
Ependymoma	8/11 (73)		34
Ependymoma	4/13 (31)		51
Ependymoma		8/8 (100)	54
Ependymoma	1/25 (<1)		53
Ependymoma	9/16 (56)		21
Ependymoma	0/10 (0)**		38
Glioblastoma	10/30 (33)		34
Glioblastoma	7/28 (25)		21
Glioblastoma	0/13 (0)**		38
Glioblastoma		4/8 (50)	54

*Infectious SV40 recovered from one tumor.

**Tumors from Finland.

Table 2

Evidence against laboratory contamination of SV40-positive human tumor specimens

– Use of rigorous laboratory precautions during sample processing and analysis
– Use of diagnostic control plasmids
– Sequence differences between tumor-associated DNAs and laboratory strains of SV40
– Detection of SV40 T-antigen in tumor cells by immunohistochemistry
– Extraction of T-antigen protein from tumor samples
– Detection of SV40 mRNA in tumor cells
– Presence of SV40 DNA in microdissected tumor cells and not in normal cells
– Finding of similar tumor types positive in independent studies
– Isolation of infectious SV40 (non-laboratory strain) from tumors

excluded. Positive control plasmids containing diagnostic engineered restriction sites [29] have been used to allow differentiation of sequences amplified from tumors from the positive controls.

The most definitive evidence against contamination comes from sequence analyses that revealed that tumor-associated sequences could be distinguished from known laboratory strains of SV40 [8,31,32,48]. Viral DNA detected in tumors often has an archetypal regulatory region arrangement, similar to the arrangement of natural isolates, without the duplications in the enhancer that are typical of laboratory strains. A variable domain at the C-terminus of the T-antigen gene can be used to differentiate SV40 strains [47] and, in

the majority of cases, tumor-associated sequences have differed from all known laboratory strains.

IHC assays have detected the expression of T-antigen in tumor cells [3,9,12,33,52], T-antigen protein has been extracted from some tissue specimens and shown to be complexed with p53 [9,54], and SV40 mRNA expression has been demonstrated in tumors [9]. None of these results would be possible if samples were accidentally contaminated with SV40 virus or plasmid DNA during sample preparation.

Microdissection of mesothelioma samples followed by PCR assays detected SV40 DNA in the tumor cells and not in adjacent, nonmalignant cells [45,46]. If laboratory contamination had occurred, viral sequences presumably would have been distributed randomly. Independent studies have consistently found ependymomas and choroid plexus tumors to have higher SV40 positivity rates than glioblastomas or medulloblastomas, observations that are not compatible with laboratory contamination, which would be expected to be distributed randomly among samples, regardless of tumor type.

Other concerns routinely raised about the etiologic significance of virus-cancer associations do not apply to this system [5]. There is agreement that SV40 is tumorigenic and that SV40 can transform human cells [5, 8, 11]. Strong evidence exists that SV40 can infect humans, based on serological surveys [7,8,17,20,24,42, 44,55], detection of SV40 DNA in pediatric renal transplants [6], and excretion of virus by infants who were recipients of SV40-contaminated poliovaccines [35], in addition to the detection of SV40 DNA in human tumors [1,8,25]. The fact that SV40 DNA has been found associated with several histological types of tumors is not evidence against viral specificity; although some human tumor viruses (such as hepatitis B virus) produce a single type of cancer, others (such as Epstein-Barr virus) are associated with multiple tumor types [5]. SV40 is able to induce tumors in several different tissues in rodents, and the human tumors found to contain SV40 DNA are the same tumor types as those produced in rodents following virus inoculation [8].

4. The conundrum of negative studies and unexpected findings

Several studies have reported failures at detection or infrequent detection of SV40-related sequences in mesotheliomas or brain tumors [13,19,37,38,49,53] (Table 1). Assuming that the positive reports are accurate, explanations need to be considered for the negative

studies. Negative results could reflect unrecognized variables, perhaps including (i) the patient population studied, (ii) the geographic origin of the tumors, (iii) the age of the patients, (iv) the numbers and types of tumors examined, (v) sample collection or processing (with deparaffinization and DNA extraction methods significant technical variables) [29], or (vi) the sensitivity of analytic methods employed (such as amounts of DNA tested or the numbers of PCR cycles used) [29]. Geographic differences may be significant and may reflect variations in exposure to the virus and/or environmental cofactors. For example, mesotheliomas from Finland and Turkey and brain tumors from Finland have been found to be SV40 negative [13,19,38], whereas the same investigators detected SV40 DNA in similar tumors from other countries [13,21,52] (Table 1). It has been noted that SV40-contaminated poliovaccines were not used in Finland and Turkey [13,19].

Several epidemiology studies have failed to detect an elevated risk of cancer among recipients of potentially contaminated poliovaccines [16,36,39,50]. However, these analyses have not ruled out SV40 as a human pathogen. Cohort-based epidemiological studies have certain limitations; as poliovaccines were not uniformly contaminated with SV40, it cannot be known which individuals in the "exposed" cohort actually were exposed to SV40 or to what level of infectious virus. These studies have assumed that no human exposure to SV40 has occurred except through the vaccines used from 1955 to 1963. As there is strong evidence that humans continue to be exposed to SV40, perhaps by horizontal transmission, the cohort analyses are further complicated. A recent survey by Fisher et al. [14], in contrast, concluded that there may be an increased incidence of brain and bone tumors among recipients of the contaminated poliovaccines.

Discrepant results and unexpected observations illustrate that there is much we do not know about SV40 and human infections. Until pathogenesis parameters are defined and technical variables are identified, it is to be expected that there will be conflicting reports and differing interpretations of virus-tumor associations [5].

5. Conclusions

At this time, SV40 should be considered a candidate human cancer virus. A compelling case can be made that SV40 infects humans today and may be found in association with certain types of human tumors. However, the biological role the virus may play

in the etiopathogenesis of those tumors remains to be determined. Unanswered questions include: (i) the source(s) of virus causing human infections, (ii) routes of viral transmission, (iii) tissue distribution of viral infection in humans, (iv) geographic differences in the prevalence of virus infections, (v) predisposing host factors that influence susceptibility to infection and disease, (vi) the biological effects of different virus variants, (vii) the state of viral DNA in tumor cells, (viii) expression of viral genes during tumorigenesis, and (ix) possible co-factors involved in cancer causation.

During the early stages of unraveling new virus-disease associations, discordant reports are to be expected [5]. However, such discrepancies should not paralyze scientific inquiry and prevent exploration of potentially important human disease leads. Considering the wealth of information accumulated about SV40 as an oncogenic virus and the mounting evidence of SV40 infections in humans, the possible involvement of the virus in human tumor development is a credible hypothesis that deserves to be addressed without delay.

The significance of establishing an SV40 role in the etiology of human cancer would be profound. Viral markers could be used for the diagnosis of tumors having a viral component and might provide useful prognostic information; preliminary data, in fact, suggest that SV40 is associated with poorer survival in patients with mesothelioma [40]. The encyclopedic knowledge of the functions of T-antigen could lead to cancer treatments targeted toward the viral transforming protein. Finally, new means of cancer prevention could be developed aimed at blocking virus infection, including viral vaccine approaches.

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