OPINION

SV40 and human tumours: myth, association or causality?

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An increasing number of scientific reports have described evidence for a polyomavirus, simian virus 40, in a highly select group of human tumours. How did a simian virus infect humans and is the virus a passenger in tumours or is it important in their pathogenesis?

Since the discovery nearly 100 years ago that viruses can cause cancer, scientists have searched for the viral origins of human cancers. The International Agency for Research in Cancer (IARC), a branch of the World Health Organization (WHO), has identified six viruses — human papillomavirus (types 16 and 18), Epstein–Barr virus (EBV), hepatitis B virus, hepatitis C virus, human T-cell lymphotropic virus type I and human immunodeficiency virus type-1 (HIV-1) — as group 1 carcinogens (that is, agents that have been shown to be carcinogenic in humans). It is estimated that these viruses cause ~15% of human malignancies[1]. However, if other viruses for which the evidence is indicative but not definitive — group 2 carcinogens — are included, this figure would almost double[1]. In addition to the viruses that have been identified by the IARC, the footprints of simian virus 40 (SV40; REF. 2), and occasionally other polyomaviruses, have been identified in human tumours. What are polyomaviruses and how do they transform cells?

Polyomaviruses and SV40

Three polyomaviruses — SV40, Jamestown Canyon virus (JCV) and BK virus[3] (BKV) — have been found in humans. Polyomaviruses are small (40 nm diameter) icosahedral, non-enveloped viruses that contain circular, double-stranded DNA. The polyomavirus genome consists of early genes, which encode regulatory proteins that are required for viral replication, and late genes, which encode structural proteins that are needed for virus assembly (BOX 1). SV40 strains can be identified by two regions that show genetic variation. These regions, the viral regulatory region and the T antigen (T Ag) variable domain, are useful for genotyping and strain identification[4,5].

JCV and BKV are of human origin and were isolated in 1971 (REFS 6,7); both viruses are found with high frequency in humans. They usually remain latent, but might, under certain circumstances, induce specific diseases. JCV is associated with the neurodegenerative disease progressive multifocal leukoencephalopathy (PML), which is common in patients with AIDS. BKV is associated with haemorrhagic cystitis, especially in renal transplant recipients. SV40, JCV and BKV share about 70% sequence similarity and can be distinguished at the nucleic-acid level.

SV40 is of monkey origin, but has been identified in a select group of human tumours. Co-expression of SV40 early-region genes, telomerase activity and oncogenic HRAS are sufficient to transform human cells[8]. SV40 contributes to transformation by perturbing several intracellular pathways. Large t Ag simultaneously disables both the retinoblastoma (RB) and the p53 tumour-suppressor pathways, whereas small t Ag perturbs protein phosphatase 2A (PP2A; REF. 8). SV40 is the most potent transforming virus and has been frequently found in human tumours. However, the genomic sequences and viral proteins of JCV and, to a lesser extent, BKV have been identified in some human tumours, particularly of neural origin[9]. On occasion, both JCV and SV40 have been found in brain tumours[9,10]. So, accurate identification of the specific polyomaviruses that are present in tumours is essential to understand their role in human cancer biology.

An extraordinary incident has meant that many humans might now be SV40 carriers. How did a simian virus enter the human population?

How SV40 became a human pathogen

SV40 was discovered in 1960 when distinctive morphological changes (vacuolization) developed in African green monkey kidney cells during safety testing of the poliovirus vaccine[11]. SV40 had previously escaped detection because SV40 grew in rhesus or cynomolgus monkey kidney cells without causing observable cellular changes.

The natural host for SV40 is the Asian macaque — particularly the rhesus (*Macaca mulatta*) — and the virus is presumably spread through contaminated urine. In captivity, other related monkeys can become infected if they come into contact with rhesus. In humans, SV40 establishes low-grade persistent infections in the kidneys of infected hosts and can be detected in peripheral blood[12], but it is possible that other tissues, such as the mesothelium, might also harbour persistent infections[13,14]. The mode of transmission between hosts is not well understood, but viruses excreted in the urine might be transmitted by respiratory or oral routes[15,16].

SV40 was found to be an unrecognized contaminant of both the inactivated poliovirus vaccine (IPV or 'Salk vaccine') and the live attenuated oral poliovirus vaccine[15] (OPV or 'Sabin vaccine') because they were prepared in primary cultures of rhesus monkey kidney cells. Viable SV40 was present in IPV as the procedures used to inactivate the poliovirus were insufficient to fully inactivate SV40 (REFS 15,16). Contaminated IPV and OPV were used in many countries, including the United States, the former Union of Soviet Socialist Republics, Japan, England and Wales, Italy, Mexico and several Central American countries, as well as other locations. Both children and adults of various ages were vaccinated.

The prevalence of SV40 in humans

In the United States, before 1963, ~90% of children and 60% of adults received one or more contaminated poliovirus vaccinations, from which time federal regulations required poliovirus vaccines to be contamination free. In addition to poliovirus vaccines, adenovirus vaccines that were used between 1957 and 1960 also contained SV40, and adenovirus-3 and -7 vaccines, which were used from 1961–1965 for military personnel, carried infectious adenovirus–SV40 hybrids[16]. The poliovirus and adenovirus vaccines that have been produced since those times are presumed to be free of SV40 — because of federal regulations — although there is some evidence that later batches of OPV were contaminated[17].

However, it is not possible to identify precisely those individuals who were exposed to SV40 through vaccination, as very few batches of poliovirus vaccine were tested for contamination, not all tested batches were contaminated, there was an uneven geographical distribution of contaminated batches in the United States and the amount of infectious SV40 varied among batches. It is estimated that up to 30% of the IPV vaccine batches and many pre-licensure OPV batches

Box 1 | The genomics of SV40

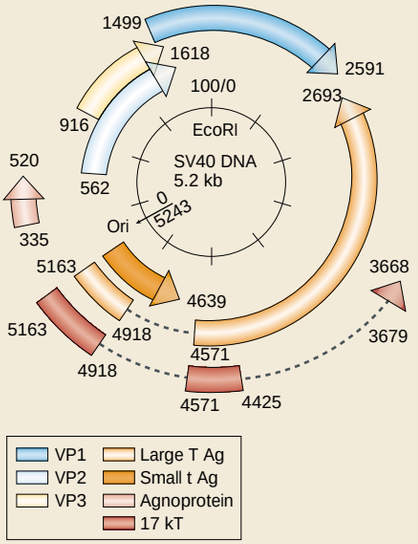
The simian virus 40 (SV40) genome is small (5.2 kb) and contains a limited coding capacity (see accompanying figure). It comprises three parts — a non-translated regulatory region of about 400 bp in size that contains the origin of replication (*ori*) and the promoters and enhancers that control replication; the early region that encodes the replication proteins (T Ag, t Ag and 17 kT protein) is expressed soon after the virus enters a cell; and the late region that encodes the capsid proteins (VP1, 2 and 3) and a maturation protein (agnoprotein), and is expressed efficiently only after viral DNA replication has begun.

The transcriptional promoters and enhancers are located very close to the functional *ori* sequence. The early promoter contains three T Ag-binding sites, some of which overlap the TATA box element that directs the initiation of transcription. Immediately adjacent is a GC-rich cluster (21-bp repeats) that binds the transcription factor SP1. Further upstream is the enhancer region (72-bp element), which contains several transcription-factor-binding sites and acts to increase transcription initiation.

Viral transcription is mediated by cellular RNA polymerase II. Only small amounts of early transcripts are produced (a few hundred molecules per cell), whereas late transcripts are much more abundant (several hundred thousand copies per cell). Early and late transcription proceeds bidirectionally from near the *ori*, with the early and late transcripts being produced from opposite strands of the viral DNA. Alternative splicing of pre-messenger RNAs (pre-mRNAs) produces functional mRNAs.

T Ag is not a transcription factor *per se*, but it can autoregulate the early promoter as the replication cycle proceeds. When T Ag reaches a high enough concentration in the cell, it binds to viral DNA which might block the assembly of functional transcriptional complexes, repressing early transcription. T Ag indirectly contributes to the activation of SV40 late transcription in ways that are not clear, perhaps by stabilizing interactions among transcription factors.

Viral DNA replication requires a functional SV40 *ori*, T Ag protein with intact DNA-binding and helicase activities, and several cellular proteins involved in DNA synthesis. T Ag binds to specific sites in the SV40 *ori*, catalyses local unwinding of the viral DNA, and recruits cellular DNA replication proteins to the complex, including topoisomerase I, replication protein A (RPA) and DNA polymerases. The nature of cellular DNA polymerase primase from different species limits the host range of polyomaviruses. Both monkey and human proteins can replicate SV40 DNA. Topoisomerase II separates the newly joined replicated circular DNA daughter molecules.



were contaminated[16,18]. The amount of SV40 present in the few batches tested ranged from ~10^4–10^5 infectious units/dose for OPV to ~10^2–10^3 infectious units/dose for IPV[16,18,19]. Finally, it is unknown how many individuals actually became infected after receiving contaminated vaccine. About 15–19% of infants who were administered contaminated OPV excreted SV40 in their stools for at least 5 weeks following exposure, providing evidence that vaccinated humans became infected with SV40 (REF. 19).

The prevalence of SV40 infections in humans today is not known. Serological surveys, based on serum neutralization tests, have revealed infection rates of 3–20%, although higher rates are observed among some laboratory workers and monkey handlers (for review, see REF. 15). When compared with infected monkeys, antibody titres in humans are usually low. This indicates that the replication of SV40 is relatively inefficient in humans — perhaps indicative of a poorly adapted virus in a new host species. It was previously reported that individuals who received contaminated OPV did not have a detectable antibody response to SV40 (REFS 16,18), with antibody responses also undetected 2 months after infection in monkeys[20]. Therefore, there is the possibility that the neutralization test is not able to identify all those who are infected with SV40.

Although the prevalence of infection is unclear, indirect evidence indicates that SV40 must now be widely distributed in the human population[2,12,15,16]. SV40-positive tumours have been detected in geographically dispersed areas, including the United States, Canada, the United Kingdom, Europe, China, Japan and New Zealand. An intriguing exception is that SV40-positive tumours have not been found in Finland, Turkey and Austria — countries that reportedly did not use SV40-contaminated poliovirus vaccine[21–23]. So, detection of SV40 seems to be related to the use of contaminated vaccines in different countries.

Which human tumour types are associated with SV40, and are they similar to SV40-induced animal tumours?

The spectra of SV40 tumours

SV40 can infect several cell types of rodent and primate species. However, the susceptibility of cells to support viral replication, lysis or transformation varies greatly according to the cell type and species of origin (BOX 2). The hamster, because of its exquisite sensitivity, is the animal model of preference to study SV40 oncogenesis. SV40 can induce several types of tumour in hamsters; however, the tumour spectrum is highly selective and is dependent on the route of inoculation. Systemic inoculation results in the development of mesotheliomas, osteosarcomas and lymphomas, whereas subcutaneous inoculation results in soft-tissue sarcomas at the site of injection (for review, see REF. 24). Intracranial inoculation results in brain tumours, especially ependymomas. The incidence of mesotheliomas is 100% after intrapleural (mesothelial) inoculation and 60% after intracardiac inoculation.

This selective hamster spectrum stimulated the search for SV40 sequences in the same types of human tumours, and the spectra of tumours in hamsters and humans have been found to be almost identical — the exception being soft-tissue sarcomas, which have not, so far, been comprehensively examined in humans. Apart from lymphomas, the human tumour types that are associated with SV40 are relatively rare, accounting for only ~6% of human tumours. The common human tumours (prostate, lung, colorectal and breast carcinomas) — which constitute nearly half of all malignancies — have low frequencies of association with SV40 (REF. 25). The finding of virtually identical tumour spectra that are associated with SV40 in humans and hamsters is powerful evidence to support the specific association of SV40 with a highly selective group of human tumours.

Overview: SV40 and tumours

Since 1974, an ever-increasing number of publications have reported evidence of SV40 DNA sequences or gene products in human tumours (see TIMELINE). More than 60 studies have used many methodologies to identify SV40 DNA, RNA and proteins — mostly in mesotheliomas, lymphomas, and brain and bone tumours[2]. However, as discussed below, not all studies have found evidence for the virus in human tumours. We briefly highlight some reports about SV40 and the four human tumours with which it is most frequently associated.

Brain tumours. Brain tumours were among the first human tumours to be associated with SV40. After a polyoma-like virus was found in brain tumours, SV40 T Ag was detected in meningiomas in 1975. Subsequently, SV40 has been detected in adult and paediatric human brain tumours, including the rare ependymomas, choroid plexus tumours and medulloblastomas[26–28] — the tumour types that are most frequently induced in hamsters and transgenic mice. Infectious virus was then rescued from brain tumours[5,28,29]. A recent report identified T Ag sequences in 36% of 199 tumours, but not in adjacent brain tissues[27], with BKV and JCV sequences being rarely detected[27].

Bone tumours. Few reports on SV40 and bone tumours have been published. In the largest series, SV40 sequences were detected in 32% of 126 osteosarcomas and 41% of 34 other bone tumour types[30]. In addition to T Ag, other parts of the viral genome have been identified in bone tumours[15].

Lymphomas. Two recent reports convincingly showed the presence of SV40 sequences in non-Hodgkin's lymphomas[25,31] (NHL; for previous reports, see REFS 31,32). Both found nearly identical incidence of SV40 sequences (42% and 43%, respectively) in NHL. Both the diffuse and follicular types of B-cell lymphomas — the most frequent forms of NHL — were positive. SV40 sequences were present in relatively few (9%) cases of Hodgkin lymphomas[25]. JCV and BKV sequences were not detected, and there seemed to be an inverse relationship between the presence of SV40 and EBV[31]. Of interest, the frequency of SV40 in AIDS-associated lymphomas was similar or lower than in tumours arising from patients with HIV-negative or indeterminate status, indicating that the presence of SV40 was not related to immunosuppression. Analysis of five tumours showed SV40 strain variation, with three being identical[31] to a unique strain that was detected only in a vial of contaminated poliovirus vaccine in 1955 (REF. 33). These findings, plus analyses of brain tumours, exclude the possibility of laboratory contamination, and strongly indicate that at least some SV40 in humans originated from contaminated poliovirus vaccines.

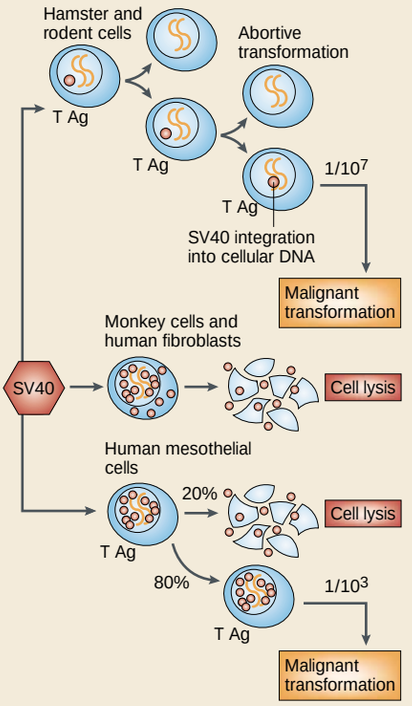
Malignant mesotheliomas. The association of SV40 and human mesothelioma has been well characterized, and appropriate *in vitro* and animal models have been established[13,34]. More than 30 reports, including a multi-laboratory study, have described the presence of SV40 in mesotheliomas, usually at frequencies of ~50%[2,14,32,35–40], although significant geographical variation occurs. We know more about the role of SV40 in the pathogenesis of mesotheliomas than any other human tumour. Therefore, in BOX 3, we use mesothelioma as a model to evaluate the mechanisms by which SV40 contributes to human tumour development.

Viral detection techniques. Several techniques have been used to detect and confirm that SV40 was present in mesotheliomas and other human tumours (see TIMELINE; REF. 40). These include Southern blot hybridization of genomic DNA; T Ag immunofluorescence and immunostaining; polymerase chain reaction (PCR); real-time PCR; DNA

Box 2 | Cellular susceptibility to SV40 lysis and transformation

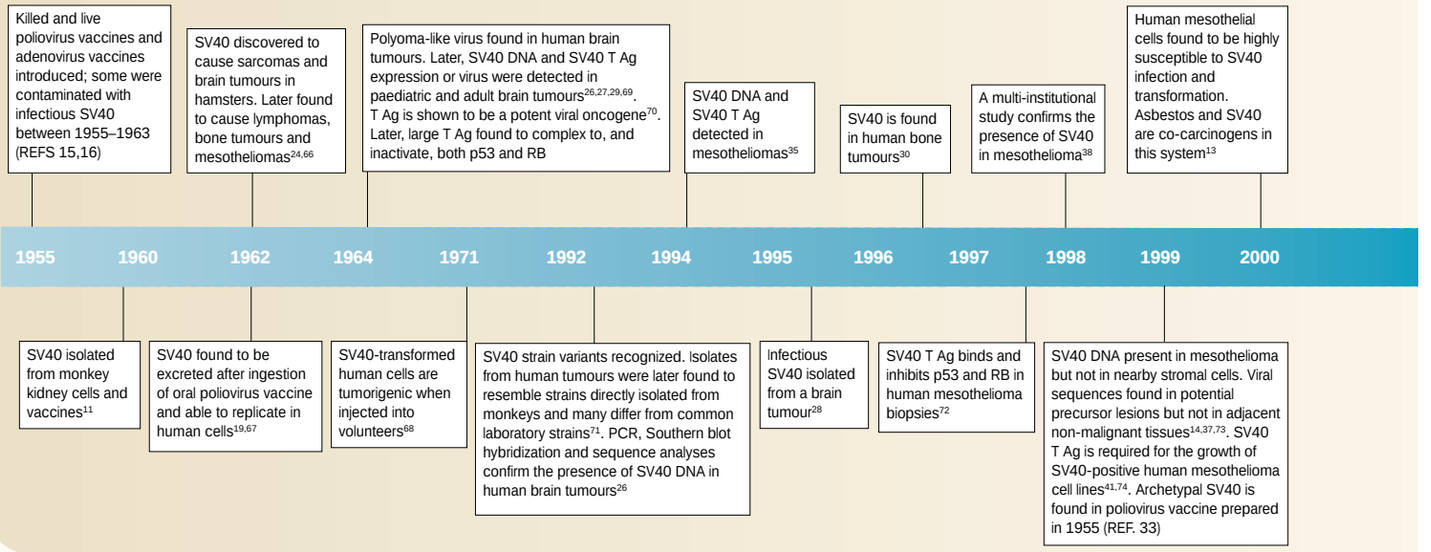
Simian virus 40 (SV40) induces cell-type-specific cytopathic and transforming effects. The virus is unable to replicate in infected, non-permissive rodent and hamster cells; however, T Ag is expressed and causes morphological transformation and cell division. SV40 is maintained in an episomal (non-integrated) state in these cells, so the progeny cells lack SV40 and revert to the normal phenotype within a few passages in cell culture (abortive transformation). Malignant transformation occurs only if SV40 becomes integrated into the host genome. This rare event assures that the SV40 genome is replicated during cell division, and the progeny cells maintain the malignant phenotype (top panel of accompanying figure).

Monkey cells, human fibroblasts and epithelial cells are permissive for SV40 infection, so produce numerous viral particles, which result in cell lysis (middle panel). SV40 infects only a fraction of human fibroblasts (~20%) compared with 100% of monkey cells ('semipermissive' replication), but once human fibroblasts are infected, SV40 replication is as efficient as observed in monkey fibroblasts[13,16,40]. The resulting cell lysis prevents human fibroblasts from undergoing malignant transformation, except, occasionally, when cells are infected at a low multiplicity of infection (about one virus/cell). In this case, rarely, SV40 becomes integrated into the host cell genome and the late-gene-coding sequences are disrupted, which prevents viral assembly. Integration occurs in less than $1/10^7$ infected human cells. Therefore, it was thought that SV40 was unlikely to cause human cancer because it was believed that SV40 had to be integrated into the human genome in order to prevent cell lysis.



SV40 infection of human mesothelial cells resulted in a very different behaviour compared with human fibroblasts or epithelial cells, which explained why the former might undergo transformation. Most mesothelial cells were infected, compared with ~20% of fibroblasts, and, remarkably, most SV40-infected mesothelial cells (~80%) survived infection[13,62] (bottom panel). Whereas SV40 replicates in infected mesothelial cells, relatively few viral particles are produced[13] and cell lysis is infrequent. Because there is no pressure for viral integration, SV40 DNA remains episomal[13,63]. Expression of the SV40 T Ag in 100% of the infected cells — with minimal cell lysis — causes a very high rate of malignant transformation (~$1/10^3$ cells). SV40-transformed mesothelial cells are immortal from the very early passages because SV40 directly induces telomerase activity[42].

Timeline | Pivotal events in the history of human infection by SV40 and its association with human cancers



sequencing; mRNA *in situ* hybridization; T Ag immunoprecipitation and western blot; antisense T Ag; *in situ* PCR; microdissection followed by PCR; co-precipitation of T Ag with cellular p53 and RB; electron microscopic demonstration of SV40 in human mesotheliomas; and detection of the specific induction of cellular oncogenes in SV40-positive tumours and the inactivation of tumour-suppressor genes. Abrogation of SV40 T Ag expression by antisense techniques induced growth arrest and apoptosis, in part, by restoring p53 function and enhanced chemosensitivity in SV40-positive mesothelioma cells[41]. SV40 sequences were not detected in microdissected samples of adjacent non-malignant lung, but were present in presumed preneoplastic lesions in patients with and without mesothelioma, arguing the importance of the SV40 virus in tumour pathogenesis[14,37].

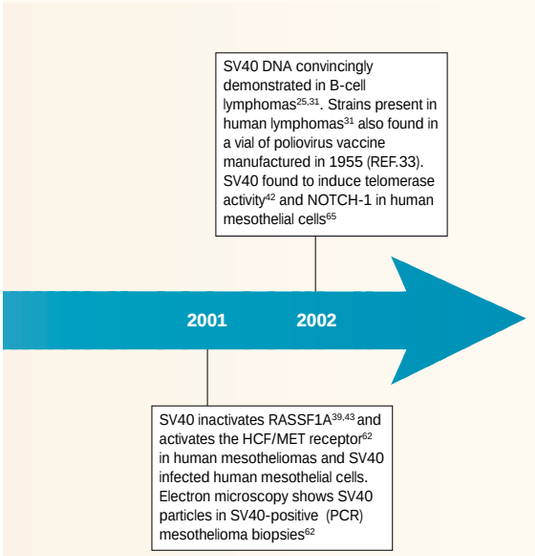
Mesothelial cell susceptibility. An *in vitro* model has been developed to study the pathogenesis of human mesothelioma[13], although the lessons learnt might also be applicable to the pathogenesis of other human tumours. *In vitro* infection of human mesothelial cells by SV40 demonstrated a hitherto unknown virus–cell interaction. Mesothelial cells are uniformly infected by SV40, but are not lysed — they undergo cell transformation at an extremely high rate. The unusual susceptibility of mesothelial cells to transformation might reflect the high levels of endogenous p53 in these cells. Telomerase is activated early after SV40 infection of normal mesothelial cells[42]; however, the development of the malignant phenotype requires many passages and the development of secondary cytogenetic and molecular changes[13,42,43]. T Ag effects on telomerase facilitate the development of these changes[44]. Exposure of mesothelial cells to asbestos enhanced SV40 transformation, indicating that asbestos and SV40 might function as co-carcinogens[40].

Negative interpretations

Several patterns regarding virus–cell interactions emerge from studies with SV40. In transformed hamster cells, the virus is integrated into the host genome. In human tumours, however, SV40 DNA often seems to be present in an episomal (unintegrated) form, although integrated forms might also be present. These have, in fact, been demonstrated in a few studies — the viral copy number and T Ag expression are relatively low, although precise quantitation has not been reported. Differences that are emerging between human and rodent tumours, with respect to the status of the virus, do not constitute negative evidence. The presence of viral DNA in an episomal form is not inconsistent with it having a role in transformation. Weak or absent T Ag expression has been noted in transgenic models of SV40 tumorigenesis in which a 'hit and run' mechanism has been demonstrated[45–47]. Downregulation of T Ag, or even the loss of the entire viral genome, might facilitate immune evasion and cancer growth[46]. These studies have shown that SV40 might initiate carcinogenesis, and then become redundant and lost, allowing the cells to survive and grow. Similarly, EBV — a group 1 carcinogen — is frequently present in human tumour cells in an episomal form, and viral antigen expression might vary with the immune status of the patient[48]. It should be noted that the dose of virus is crucial in non-permissive animals — hamsters and rodents — in which only the input virus is involved in transformation. Dose is less important in semi-permissive human cells because virus replication in some cells produces millions of new viral particles.

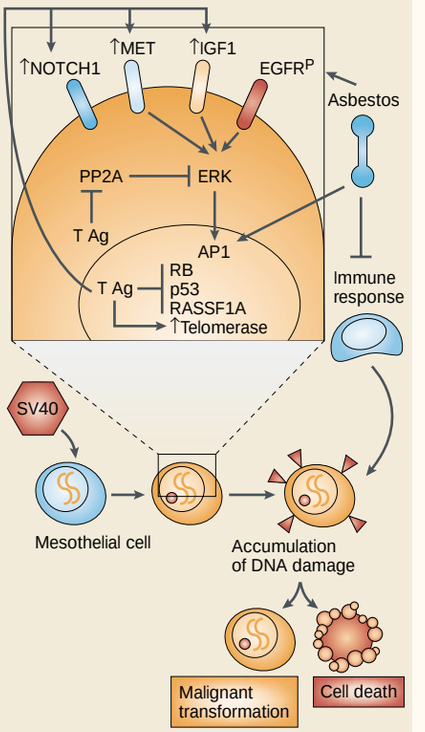
Whereas most of the published studies have found SV40 sequences in the tumour types discussed, occasional studies have yielded negative or inconsistent results (for review, see REF. 2). In addition, the virus–human cell interactions that have been previously discussed might result in false-negative reports[2]. Other reasons for false-negative findings include poor sample quality, inefficient DNA extraction procedures, loss of low-molecular-weight episomal DNA during tissue processing, low-input DNA in PCR reactions, an inadequate number of PCR cycles and the failure to repeat the tests or to adjust testing conditions appropriately. A further consideration for negative findings is the geographical variation in the distribution of SV40-contaminated poliovirus vaccines.

Inter-laboratory studies with coded specimens are one approach to documenting the reproducibility of experimental findings. One such multi-institutional study confirmed the presence and expression of SV40 in mesotheliomas[38], although a subsequent



Box 3 | SV40 and the pathogenesis of mesothelioma

How does simian virus 40 (SV40) induce malignant transformation of human cells? An *in vitro* transformation model exists for mesothelioma and provides an understanding of how two potent carcinogens (asbestos and SV40) interact (see figure). The only known molecular effect of crocidolite asbestos is the activation of the epidermal growth factor receptor (EGFR), which results in the induction of activity of the transcription factor AP1 (REF. 64). Asbestos exposure alone seems to be insufficient for mesothelioma induction[40]. Mesothelial cells are highly susceptible to SV40 transformation, although transformation requires repeated passage[13]. SV40 infection results in the early onset of telomerase activity, which causes immortalization[42]. Expression of T Ag results in binding to and the inhibition of the cellular p53 and retinoblastoma (RB) family proteins. Asbestos seems to increase SV40-mediated transformation of human mesothelial cells *in vitro*, indicating that asbestos and SV40 might be co-carcinogens[40]. In addition, asbestos impairs the local and systemic immune responses, providing a mechanism for avoidance of immune surveillance and for the survival of cells expressing the highly antigenic T Ag protein[40]. SV40 infection induces other changes, including inhibition of protein phosphatase 2A (PP2A) and the tumour suppressor gene *RASSF1A*, and upregulation of NOTCH-1, the MET oncogene and insulin-like growth-factor 1 (IGF1; REFS 36,40,43,62,65). Some changes, such as *RASSF1A* inactivation are late events, occur only after several cell passages[43]. *RASSF1A* is often preferentially inactivated by promoter-region methylation in mesotheliomas that have SV40 DNA sequences[39].



study yielded inconsistent results[49]. The latter study was flawed because of questionable sample processing and contamination by a contracting commercial laboratory and by the lack of inclusion of suitable positive control tumour samples.

Is there solid epidemiological evidence that SV40 has a crucial role in human tumorigenesis?

Epidemiological studies

A missing element in the SV40–human-tumour connection is the lack of solid epidemiological evidence. The inability to identify, with certainty, individuals who are infected with SV40 is a serious impediment to meaningful epidemiology studies that are designed to assess the risk of disease that is associated with SV40 (REF. 16). However, children born from mothers who received potentially contaminated SV40 vaccines had a significantly increased risk of developing brain tumours[50–52]. Studies on children who received potentially contaminated vaccines resulted in ambiguous data because results were not available for the age groups in which most brain tumours and mesotheliomas develop[53,54].

Although the incidence of certain brain tumours, lymphomas and mesotheliomas has greatly increased during the past two decades, conclusive epidemiological evidence that these rises are related to SV40 is lacking. However, the increased incidence of mesotheliomas from almost none (in 1950) to the present 2,000–3,000 cases per year in the United States followed the administration of contaminated poliovirus vaccines. Most mesotheliomas occur in patients who are older than 50 years of age. Therefore, most of the mesothelioma cases that have occurred during the past 40 years developed in patients who were vaccinated as adults at the time when contaminated vaccines were administered — that is 1955–1963. Both children and an estimated 35 million adults received contaminated vaccines; the latter constitutes the cohort in which most mesotheliomas have developed[16,40]. This same group might, however, have been exposed to higher levels of asbestos during the past half century — an accepted cause of mesotheliomas[40]. As SV40 and asbestos are co-carcinogens in the *in vitro* transformation of mesothelial cells[13], both factors might have contributed to mesothelioma development during the second half of the past century.

The routes for transmission of SV40 among humans are not known. Horizontal, and perhaps vertical, transmission might occur, as individuals who are too young to have received contaminated poliovirus vaccine have developed SV40-positive tumours[25,31], or have carried SV40-positive kidney transplants[12,55]. Several strains of SV40 have been isolated from the urine and blood of patients with renal diseases — especially focal segmental glomerulosclerosis — and occasionally from healthy adults[12]. The virus is harboured in renal tubular cells. Rosina Girones (as quoted in REF. 56) recently found high concentrations of SV40 in sewage in India, both in rhesus habitats and in Calcutta, which is outside the monkeys' range. These findings indicate that both monkey-to-human and human-to-human spread of SV40 occur, and this further complicates epidemiological studies.

When SV40 is present in certain human tumours, is it functioning as a passenger (that is, an association) or is it important in tumour pathogenesis (that is, causation)?

Table 1 | SV40 and human mesothelioma — association or causation?

Bradford–Hill criterion	HPV and cervical cancer	SV40 and mesothelioma
Strength of association	The strength of association between HPV and cervical cancer is considered one of the strongest for a human cancer. Recent studies have shown that HPV (all types combined) is present in >90% of cervical cancers	Several studies have found evidence of SV40 in ~50% of human mesotheliomas, both in the United States and in some European countries
Consistency	The presence of HPV in cervical cancer is consistent among a large number of studies, regardless of the HPV testing system used. There are no published studies with negative observations that challenge the association of HPV and cervical cancer	At least 30 studies have reported SV40 in human mesotheliomas using a variety of techniques, whereas four have failed to find an association
Specificity	Specific cancers are related to the presence of HPV. HPV type is also important in the development of specific cancers. HPV is present in the tumour cells. Viral oncogene expression (E6 and E7) occurs in tumour material, but not in stromal cells	SV40 is present in a highly specific group of human tumours. Furthermore, injection of the virus into hamsters produces the same tumours. In human mesotheliomas, SV40 is found only in tumour cells and not in the surrounding non-malignant tissue
Temporality	HPV infections precede pre-cancerous cervical lesions and cervical cancer by years to decades	Little is known, although the virus has been found in preneoplastic lesions. The tremendous increase in the incidence of mesotheliomas over the past several decades was preceded by the administration of SV40-contaminated poliovirus vaccines, as well as by an increased exposure to asbestos
Biological gradient (dose–response)	Unclear, but early studies show that cervical cancer is associated with high viral loads	Unknown. Because humans are permissive to SV40, millions of SV40 particles are produced from few infected cells
Biological plausibility	HPV is a powerful carcinogen that immortalizes human keratinocytes *in vitro*. There are no animal models in which a sexually transmitted PV produces cervical cancer. HPV is present in cervical cancer, where it expresses the oncogenic proteins E6 and E7 that inactivate the host regulatory proteins p53 and RB, respectively. Epidemiological studies support a role for HPV in cervical cancer	SV40 is a powerful carcinogen that, *in vitro*, transforms human mesothelial cells. It induces mesothelioma development in hamsters. SV40 also expresses the oncogenic protein T Ag in human mesotheliomas, which inactivates p53 and RB. Definitive epidemiological studies are lacking
Biological coherence	The association does not conflict with what is known about the natural history of cervical cancer development	The association does not conflict with what is known about the natural history of mesothelioma, and it might explain why those with no history of asbestos exposure can develop the disease
Experimental evidence	*In vitro* and *in vivo* evidence supports a causal role for HPV in the development of cervical cancer	*In vitro* and *in vivo* evidence indicates a causal role for SV40 in mesothelioma development
Analogy	Other DNA tumour viruses can induce cancers in humans, and species-specific papillomaviruses can induce cancers in animals	Other DNA tumour viruses can induce cancers in humans, and SV40 induces mesotheliomas in animals

For suspected carcinogens, there is a distinction between association and causation. The Bradford–Hill criteria[60] can be used to assess the association between simian virus 40 (SV40) and mesothelioma. For comparison, the criteria are applied to the association between human papillomavirus (HPV) and cervical cancer — a relationship that is universally accepted[61].

Passenger or pathogen?

There are several principles of viral carcinogenesis[57]: viruses can cause various tumours in animals and humans; tumour viruses establish persistent infections in their natural hosts; virus infections are more common than tumours in susceptible hosts; tumour induction requires a long latent period; viruses are seldom complete carcinogens (they modulate cellular growth regulatory pathways and secondary molecular events are required for tumorigenesis); and viral markers are usually present in tumour cells. All of these tenets apply to the association of SV40 and human tumours. The evidence presented herein demonstrates a specific association between SV40 and a select group of human tumours and supports a causal role[58,59].

In 1965, Sir Austin Bradford–Hill established nine widely used criteria to determine the strength of an association between a disease and its putative causative agent[60]. In TABLE 1, we apply these criteria to SV40 and human tumours. For comparison, we also apply the criteria to the association between another small DNA oncogenic virus, human papillomavirus (HPV), and cervical cancer, a relationship that is well established[61]. Whereas there is considerable evidence that SV40 has a causative role in the pathogenesis of mesothelioma and brain tumours, for lymphoma and bone tumours the evidence is still insufficient to distinguish between association and causation.

Conclusions

The Institute of Medicine, Washington DC, recently assessed the association between SV40 contamination of the poliovirus vaccine and human cancer (see National Academies web site in online links box). The panels' conclusions about the relationship between poliovirus vaccines and cancer were that because the epidemiological evidence is unsatisfactory, the evidence is inadequate to either accept or reject a causal relationship; the biological evidence is of moderate strength such that SV40 from poliovirus vaccine is related to SV40 infection in humans. The panels' conclusions about the relationship between SV40 and human cancer, regardless of the source of infection, were that: the biological evidence is strong that SV40 is a transforming virus; and the biological evidence is of moderate strength that SV40 exposure could lead to cancer in humans under natural conditions. Similar conclusions

about mesothelioma were reached by a panel of independent experts after a review of data at an international mesothelioma consensus meeting at the University of Chicago[58]. Both panels included scientists who were not involved with SV40 research. The IARC has not evaluated SV40 as a causative agent for human tumours, but on the basis of the large body of evidence summarized here, it is our opinion that SV40 should be included in the list of group 2A carcinogens — that is, those that are probably carcinogenic to humans.

How can these findings impact on clinical medicine? In fact, a Phase I clinical trial is about to be initiated based on the assumption that asbestos and SV40 cooperate in the pathogenesis of mesotheliomas. The National Cancer Institute has funded the production of a human SV40 virus that will be tested in subjects who are at high risk for the development of mesothelioma on the basis of previous high asbestos exposure. It is hoped that by boosting the immune response against SV40, virus-infected mesothelial cells will be eliminated before they become transformed. Accurate identification of SV40-exposed subjects will aid this and other chemoprevention studies. Targeting SV40 — by antisense or other approaches — might form the basis of new therapeutic approaches. The downstream effects of SV40 (such as MET and NOTCH-1 activation, and *RASSF1A* methylation) also offer attractive therapeutic targets. Final proof that SV40 is involved in the pathogenesis of certain human cancers will impact on several aspects of clinical management, including diagnosis, prevention and therapy.

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1. zur Hausen, H. Viral Oncogenesis. in *Microbes and Malignancy* (ed. Parsonnet, J.) 107–130 (Oxford Press, New York, 1999).
2. Jasani, B. *et al.* Association of SV40 with human tumours. *Semin. Cancer Biol.* **11**, 49–61 (2001).
3. Imperiale, M. J. The human polyomaviruses, BKV and JCV: molecular pathogenesis of acute disease and potential role in cancer. *Virology* **267**, 1–7 (2000).
4. Lednicky, J. A. & Butel, J. S. Simian virus 40 regulatory region structural diversity and the association of viral archetypal regulatory regions with human brain tumors. *Semin. Cancer Biol.* **11**, 39–47 (2001).
5. Stewart, A. R., Lednicky, J. A. & Butel, J. S. Sequence analyses of human tumor-associated SV40 DNAs and SV40 viral isolates from monkeys and humans. *J. Neurovirol.* **4**, 182–193 (1998).
6. Gardner, S. D., Field, A. M., Coleman, D. V. & Hulme, B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* **1**, 1253–1257 (1971).
7. Padgett, B. L., Walker, D. L., ZuRhein, G. M., Eckroade, R. J. & Dessel, B. H. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* **1**, 1257–1260 (1971).
8. Hahn, W. C. *et al.* Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Mol. Cell Biol.* **22**, 2111–2123 (2002).
9. Krynska, B. *et al.* Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. *Proc. Natl Acad. Sci. USA* **96**, 11519–11524 (1999).
10. Del Valle, L. *et al.* Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. *Cancer Res.* **61**, 4287–4293 (2001).
11. Sweet, B. H. & Hilleman, M. R. The vacuolating virus, SV40. *Proc. Soc. Exp. Biol. Med.* **105**, 420–427 (1960).
12. Li, R. M. *et al.* Molecular identification of SV40 infection in human subjects and possible association with kidney disease. *J. Am. Soc. Nephrol.* **13**, 2320–2330 (2002).
13. Bocchetta, M. *et al.* Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. *Proc. Natl Acad. Sci. USA* **97**, 10214–10219 (2000).
14. Shivapurkar, N. *et al.* Presence of simian virus 40 sequences in malignant mesotheliomas and mesothelial cell proliferations. *J. Cell. Biochem.* **76**, 181–188 (1999).
15. Butel, J. S. & Lednicky, J. A. Cell and molecular biology of simian virus 40: implications for human infections and disease. *J. Natl Cancer Inst.* **91**, 119–134 (1999).
16. Carbone, M., Rizzo, P. & Pass, H. I. Simian virus 40, polio vaccines and human tumors: a review of recent developments. *Oncogene* **15**, 1877–1888 (1997).
17. Kops, S. P. Oral polio vaccine and human cancer: a reassessment of SV40 as a contaminant based upon legal documents. *Anticancer Res.* **20**, 4745–4749 (2000).
18. Shah, K. V. SV40 infections in simians and humans. *Dev. Biol. Stand.* **94**, 9–12 (1998).
19. Melnick, J. L. & Stinebaugh, S. Excretion of vacuolating SV-40 virus (papova virus group) after ingestion as a contaminant of oral polio vaccine. *Proc. Soc. Exp. Biol. Med.* **109**, 965–968 (1962).
20. Ashekenazi, A. & Melnick, J. L. Induced latent infection of monkeys with vacuolating SV-40 papova virus: virus in kidneys and urine. *Proc. Soc. Exp. Biol. Med.* **111**, 367–372 (1962).
21. Leithner, A. *et al.* Absence of SV40 in Austrian tumors correlates with low incidence of mesotheliomas. *Cancer Biol. Ther.* **1**, 373–379 (2002).
22. Hirvonen, A. *et al.* Simian virus 40 (SV40)-like DNA sequences not detectable in Finnish mesothelioma patients not exposed to SV40-contaminated polio vaccines. *Mol. Carcinog.* **26**, 93–99 (1999).
23. De Rienzo, A. *et al.* Detection of SV40 DNA sequences in malignant mesothelioma specimens from the United States, but not from Turkey. *J. Cell Biochem.* **84**, 455–459 (2002).
24. Carbone, M., Stach, R., Di Resta, I., Pass, H. I. & Rizzo, P. Simian virus 40 oncogenesis in hamsters. *Dev. Biol. Stand* **94**, 273–279 (1998).
25. Shivapurkar, N. *et al.* Presence of simian virus 40 DNA sequences in human lymphomas. *Lancet* **359**, 851–852 (2002).
26. Bergsagel, D. J., Finegold, M. J., Butel, J. S., Kupsky, W. J. & Garcea, R. L. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumors of childhood. *N. Engl. J. Med.* **326**, 988–993 (1992).
27. Huang, H. *et al.* Identification in human brain tumors of DNA sequences specific for SV40 large T antigen. *Brain Pathol.* **9**, 33–42 (1999).
28. Lednicky, J. A., Garcea, R. L., Bergsagel, D. J. & Butel, J. S. Natural simian virus 40 strains are present in human choroid plexus and ependymoma tumors. *Virology* **212**, 710–717 (1995).
29. Krieg, P. & Scherer, G. Cloning of SV40 genomes from human brain tumors. *Virology* **138**, 336–340 (1984).
30. Carbone, M. *et al.* SV40-like sequences in human bone tumors. *Oncogene* **13**, 527–535 (1996).
31. Vilchez, R. A. *et al.* Association between simian virus 40 and non-Hodgkin lymphoma. *Lancet* **359**, 817–823 (2002).
32. Rizzo, P. *et al.* Simian virus 40 is present in most United States human mesotheliomas, but it is rarely present in non-Hodgkin's lymphoma. *Chest* **116**, 470S–473S (1999).
33. Rizzo, P., Di Resta, I., Powers, A., Ratner, H. & Carbone, M. Unique strains of SV40 in commercial polio vaccines from 1955 not readily identifiable with current testing for SV40 infection. *Cancer Res.* **59**, 6103–6108 (1999).
34. Cicala, C., Pompetti, F. & Carbone, M. SV40 induces mesotheliomas in hamsters. *Am. J. Pathol.* **142**, 1524–1533 (1993).
35. Carbone, M. *et al.* Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* **9**, 1781–1790 (1994).
36. Rizzo, P. *et al.* SV40 and the pathogenesis of mesothelioma. *Semin. Cancer Biol.* **11**, 63–71 (2001).
37. Shivapurkar, N. *et al.* Presence of simian virus 40 sequences in malignant pleural, peritoneal and noninvasive mesotheliomas. *Int. J. Cancer* **85**, 743–745 (2000).
38. Testa, J. R. *et al.* A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. *Cancer Res.* **58**, 4505–4509 (1998).
39. Toyooka, S. *et al.* Aberrant methylation and simian virus 40 Tag sequences in malignant mesothelioma. *Cancer Res.* **61**, 5727–5730 (2001).
40. Carbone, M., Kratzke, R. A. & Testa, J. R. The pathogenesis of mesothelioma. *Semin. Oncol.* **29**, 2–17 (2002).
41. Schrump, D. S. & Waheed, I. Strategies to circumvent SV40 oncoprotein expression in malignant pleural mesotheliomas. *Semin. Cancer Biol.* **11**, 73–80 (2001).
42. Foddis, R. *et al.* SV40 infection induces telomerase activity in human mesothelial cells. *Oncogene* **21**, 1434–1442 (2002).
43. Toyooka, S. *et al.* Progressive aberrant methylation of the *RASSF1A* gene in simian virus 40 infected human mesothelial cells. *Oncogene* **21**, 4340–4344 (2002).
44. Wong, J. M., Kusdra, L. & Collins, K. Subnuclear shuttling of human telomerase induced by transformation and DNA damage. *Nature Cell Biol.* **4**, 731–736 (2002).
45. Ewald, D. *et al.* Time-sensitive reversal of hyperplasia in transgenic mice expressing SV40 T antigen. *Science* **273**, 1384–1386 (1996).
46. Salewski, H. *et al.* Increased oncogenicity of subclones of SV40 large T-induced neuroectodermal tumor cell lines after loss of large T expression and concomitant mutation in p53. *Cancer Res.* **59**, 1980–1986 (1999).
47. Tzeng, Y. J. *et al.* SV40 T/t-antigen induces premature mammary gland involution by apoptosis and selects for p53 missense mutation in mammary tumors. *Oncogene* **16**, 2103–2114 (1998).
48. Niedobitek, G., Meru, N. & Delecluse, H. J. Epstein–Barr virus infection and human malignancies. *Int. J. Exp. Pathol.* **82**, 149–170 (2001).
49. Strickler, H. D. A multicenter evaluation of assays for detection of SV40 DNA and results in masked mesothelioma specimens. *Cancer Epidemiol. Biomarkers Prev.* **10**, 523–532 (2001).
50. Innis, M. D. Oncogenesis and poliomyelitis vaccine. *Nature* **219**, 972–973 (1968).
51. Heinonen, O. P. *et al.* Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. *Int. J. Epidemiol.* **2**, 229–235 (1973).
52. Farwell, J. R., Dohrmann, G. J. & Flannery, J. T. Medulloblastoma in childhood: an epidemiological study. *J. Neurosurg.* **61**, 657–664 (1984).
53. Carbone, M., Fisher, S., Powers, A., Pass, H. I. & Rizzo, P. New molecular and epidemiological issues in mesothelioma: role of SV40. *J. Cell. Physiol.* **180**, 167–172 (1999).
54. Strickler, H. D. *et al.* Contamination of poliovirus vaccines with simian virus 40 (1955–1963) and subsequent cancer rates. *JAMA* **279**, 292–295 (1998).
55. Butel, J. S. *et al.* Evidence of SV40 infections in hospitalized children. *Hum. Pathol.* **30**, 1496–1502 (1999).
56. Vastag, B. Sewage yields clues to SV40 transmission. *JAMA* **288**, 1337–1338 (2002).
57. Butel, J. S. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis* **21**, 405–426 (2000).
58. Klein, G., Powers, A. & Croce, C. Association of SV40 with human tumors. *Oncogene* **21**, 1141–1149 (2002).
59. Malkin, D. Simian virus 40 and non-Hodgkin lymphoma. *Lancet* **359**, 812–813 (2002).
60. Bradford-Hill, A. The environment and disease: association or causation? *Proc. R. Soc. Med.* **9**, 295–300 (1965).
61. zur Hausen, H. Papillomaviruses and cancer: from basic studies to clinical application. *Nature Rev. Cancer* **2**, 342–350 (2002).
62. Cacciotti, P. *et al.* SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. *Proc. Natl Acad. Sci. USA* **98**, 12032–12037 (2001).
63. Cacciotti, P. *et al.* The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. *Am. J. Respir. Cell. Mol. Biol.* **26**, 189–193 (2002).
64. Manning, C. B. *et al.* A mutant epidermal growth factor receptor targeted to lung epithelium inhibits asbestos-induced proliferation and proto-oncogene expression. *Cancer Res.* **62**, 4169–4175 (2002).
65. Bocchetta, M., Miele, L., Pass, H. I. & Carbone, M. Notch-1 induction, a novel activity of SV40 required for growth of SV40–transformed human mesothelial cells. (in the press) (2002).

66. Eddy, B. E., Borman, G. S., Grubbs, G. E. & Young, R. D. Identification of the oncogenic substance in rhesus monkey cell cultures as simian virus 40. *Virology* **17**, 65–75 (1962).
67. Shein, H. M. & Enders, J. F. Multiplication and cytopathogenicity of simian vacuolating virus 40 in cultures of human tissues. *Proc. Soc. Exp. Biol. Med.* **109**, 495–500 (1962).
68. Jensen, F., Koprowski H., Pagano, J. S., Pontén, J. & Ravdin, R. G. Autologous and homologous implantation of human cells transformed *in vitro* by simian virus 40. *J. Natl Cancer Inst.* **32**, 917–937 (1964).
69. Bastian, F. O. Papova-like virus particles in a human brain tumor. *Lab. Invest.* **25**, 169–175 (1971).
70. Brugge, J. S. & Butel, J. S. Role of simian virus 40 gene *A* function in maintenance of transformation. *J. Virol.* **15**, 619–635 (1975).
71. Ilyinskii, P. O., Daniel, M. D., Horvath, C. J. & Desrosiers, R. C. Genetic analysis of simian virus 40 from brains and kidneys of macaque monkeys. *J. Virol.* **66**, 6353–6360 (1992).
72. Carbone, M. *et al.* Simian virus-40 large-T antigen binds p53 in human mesotheliomas. *Nature Med.* **3**, 908–912 (1997).
73. Ramael, M. *et al.* Detection of SV40 like viral DNA and viral antigens in malignant pleural mesothelioma. *Eur. Respir. J.* **14**, 1381–1386 (1999).
74. Waheed, I. *et al.* Antisense to SV40 early gene region induces growth arrest and apoptosis in T-antigen–positive human pleural mesothelioma cells. *Cancer Res.* **59**, 6068–6073 (1999).

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Online links

DATABASES

The following terms in this article are linked online to:

GenBank: http://www.ncbi.nih.gov/Genbank/
SV40

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
IGF | MET | NOTCH-1 | p53 | PP2A | *RASSF1A* | RB

FURTHER INFORMATION

AIDS: http://www.aids.org/index.html
Encyclopedia of Life Sciences: http://www.els.net/
EBV | hepatitis B | hepatitis C | HIV-1 | papillomavirus
National Academies: http://National-Academies.org
The International Agency for Research in Cancer: http://www.iarc.fr/
World Health Organization: http://www.who.int/en/

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