Asbestos, Simian virus 40 and malignant mesothelioma

S C Stenton

Department of Respiratory Medicine and Regional Unit for Occupational Lung Disease, Royal Victoria Infirmary and University of Newcastle upon Tyne, Newcastle upon Tyne, UK

Introductory article

Simian virus 40 large T antigen (SV40LTAg) primer specific DNA amplification in human pleural mesothelioma tissue

C Pepper, B Jasani, H Navabi, D Wynford-Thomas, AR Gibbs

Background. DNA sequences and immunoreactivity associated with Simian virus 40 transforming factors, large T and small t antigens (SV40LTAg), suggestive of an aetipathogenetic link have been identified in fresh frozen tissue of a high proportion of recent cases of pleural mesotheliomas from the United States, Italy and Germany. SV40 is not normally infective in man though it can transform human cells in tissue culture. A large cohort of people in the western world was accidentally parenterally inoculated with live SV40 through contaminated polio vaccines given between 1959 and 1961, and this might be a factor in the current continuing rise in the incidence of mesothelioma in the United States, Britain and Europe. The present study investigated the presence of SV40LTAg DNA in recently diagnosed cases of mesothelioma in Britain and the feasibility of detecting the SV40 DNA in archival tissue for retrospective analysis of cases in the peri-vaccination period.

Methods. DNA was extracted from fresh frozen and/or rehydrated formalin fixed, paraffin embedded tissue sections from nine recently diagnosed cases of mesothelioma, nine cases of pulmonary adenocarcinoma, and three reactive pleurae, and amplified by the polymerase chain reaction (PCR) using the primer pairs used previously on fresh frozen tissues — namely, the SV primer set directed at the LTAg gene sequence unique to SV40 and the PYV primer set directed at a sequence shared by SV40 and papovavirus strains BK and JC, respectively.

Results. PCR positivity with the SV primer set was restricted to four of the nine cases of mesothelioma. In contrast, six of the nine mesotheliomas, two of the nine adenocarcinomas, and one of the three reactive pleurae showed positivity with the PYV primers. The fresh frozen and corresponding formalin fixed, paraffin embedded tissue results concorded well with each other.

Conclusions. Our data provide evidence for the association of SV40LTAg primer specific DNA with human pulmonary mesothelioma in the British population. (Thorax 1996;51:1074–6)
SV40 and human cancer

The introductory article has added to a growing body of knowledge suggesting that Simian virus 40 (SV40) DNA can be identified in several types of human malignancy. The techniques used in the study by Pepper and colleagues were chosen to replicate previous work, and although they are not described in detail in their rapid communication, they can be largely inferred. The region of the viral genome under investigation codes for its large T antigen (TAg; Fig 2). It is well characterised and appropriate primer sequences for polymerase chain reactions (PCR) are available. PCR involves adding oligonucleotide “primer” sequences from two areas of a gene to the material under investigation together with a DNA polymerase. The region of the gene between the two primer sequences is amplified, provided it is present in the parent sample. In this study the primers were expected to amplify a 105 base-pair sequence and the reaction produced DNA fragments of approximately the correct length in four of nine mesotheliomas.

SV40 is a small double stranded DNA virus which causes asymptomatic infection in the kidneys of rhesus monkeys. It belongs to the group of papovaviruses which include mouse polyomavirus, human BK and JC viruses, and papillomaviruses. BK and JC viruses (named after their natural hosts) are associated with progressive multifocal leukoencephalopathy. These papovaviruses share with adenoviruses (another DNA virus of vertebrates) a potent ability to induce tumours in species which are not their natural hosts. Papillomaviruses are associated with cervical cancer but there is, to date, no convincing evidence that other papovaviruses cause malignancy in humans.

SV40-like DNA was identified in human tumours in 1992 by Bergsagel and colleagues who were looking for JC- or BK-like DNA in childhood choroid plexus tumours and ependymomas. When PCR was performed with polyomavirus (PVY) primers under conditions of low stringency—that is, allowing DNA amplification despite some base-pair mismatching—amplification products of approximately the correct size were detected. However, when a portion of the amplified DNA was sequenced it was found to be identical to that of a region of SV40 TAg and different from the DNA of the JC and BK viruses in several respects. When the authors repeated the PCR under conditions of high stringency using SV40 primers they detected DNA which hybridised with a further SV40 oligonucleotide sequence in 20 of 31 of the tumours.

Immunohistochemical staining revealed nuclear SV40 TAg in seven of 11 cases. Seventeen tumour samples were restudied blindly in a different laboratory using primers for three different parts of the SV40 genome. DNA from 14, including all eight which had previously been considered positive. The tumour DNA was transfected into monkey kidney cells and, in one case, produced what were regarded as typical viral cytopathic effects.

At around the same time SV40 was noted to induce mesotheliomas when inoculated into the pleural cavities of hamsters and so Carbone and coworkers extended Bergsagel’s observations with a study of human mesotheliomas. SV40-like DNA was identified in 29 of 48 tumours but in only one of 28 lung tissue samples from the same subjects, and in none of 48 other solid tumours. A 132 base-pair DNA segment which was sequenced showed only a single nucleotide difference from SV40 TAg DNA. Nuclear TAg was detected immunohistochemically in 11 of 14 tumours, and all of 26 serum samples contained anti-TAg antibodies. One further study identified SV40-like DNA in eight of 11 malignant mesotheliomas and in none of seven control samples.
On the other hand, Strickler and colleagues failed to repeat the amplification of SV40 DNA from mesotheliomas. They failed to identify any amplified DNA, and the potential exists for misidentification and for false positive and false negative results. 

Amplifying DNA sequences from tissue samples is often fixed in formaldehyde and embedded in paraffin is not a simple matter and the potential exists for misidentification and for false positive and false negative results. The introduction of PCR has shown only 1961 is not known but, as 60% of rhesus monkeys used for laboratory experiments, it is possible that the PCR primers have failed to identify SV40 DNA in brain tumours (table 1) and the contradictory observations need to be resolved.

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Vesicles. SV40 genes have been used to transform or properties and binding of p110, suggesting a common inhibition and anchorage dependence, reduced re-quired for SV40 binding to p110 are also essential for
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SV40 produced mesotheliomas in all 12 injected ham-
sters in one study,21 suggesting that SV40 is, if anything, a more potent cause of mesotheliomas in the hamster than asbestos. It is noteworthy that the tumours which are most readily induced in hamsters are also those from which viral DNA has been isolated in humans. It might also also be relevant that the mesothelium and renal tubular
cells (the Simian host tissue of the virus) are unusual in being mesodermally derived tissues with epithelial characteristics. T this might suggest either a viral tro-
phism for these particular tissues or the expression of gene products which the virus might interact to produce malignant changes.

In vitro studies also support the possibility of a role for SV40 in inducing magergically. Most cells cultured in vitro grow as a monolayer on culture plates for only a limited number of divisions before the cells become senescent. Some cells infected with viruses undergo “transformation” and display loss of contact inhibition and anchorage dependence, reduced re-
quirements for serum growth factors, and the ability to undergo ªtransformationº and display loss of contact and each virus has developed proteins which serve
this function. T hey have structural similarities and are termed “early” antigens because of the timing of their expression during the infective cycle. They are dis-
tinguished from “late” antigens which code for viral coat proteins. In the case of SV40, the large tumour antigen (large T antigen or TAg) is the principal early protein. It is multifunctional and in “permissive” cells – that is, those which allow viral replication – it binds to viral DNA and in the presence of other cellular proteins unwinds it and allows replication. TAg also binds and inactivates a number of tumour suppressor gene products which normally have inhibitory influences on the enzymes necessary for DNA replication, it up-
regulates the expression of insulin growth factor 1 which stimulates cell division, and has self-regulating prop-
erties. A small t antigen is produced by differential splicing of RNA of the same gene and has an important role in facilitating TAg effects.

The tumorigenic properties of DNA viruses represent the outcome of their replication strategy in cells which, for unknown reasons, do not allow viral replication. Under these circumstances the viral DNA can become randomly incorporated into the host genome and, if its products are expressed, they can inactivate suppressor gene products and so release a stimulus to unregulated cell growth.20 Human cells are semi-permissive for SV40 and allow both viral replication and DNA incorporation into the host genome.

Several nuclear proteins are known to be bound and inactivated by viral early antigens such as SV40 large T antigen. The best characterised of these are p110, the protein product of the retinoblastoma gene, and p53. The retinoblastoma gene sited on the long arm of chromosome 13 is the prototypic tumour suppressor gene and is associated with retinoblastomas, osteo-
sarcomas, and various other tumours, though not mesotheliomas.20 It is under-phosphorylated in the early phases of cell division and becomes progressively more phosphorylated as division progresses. It is thought that a reduction in the levels of hypophosphorylated p110 by SV40 TAg binding neutralizes its growth arresting
properties. T he amino acid residues 105–115 which are required for SV40 binding to p110 are also essential for human cells to transform properties.26 T hey are homologous with sequences on the adenovirus early region IA pro-
tein which are essential for that virus’s transforming properties and binding of p110, suggesting a common mechanism for viral cell transformation (fig 3).26 T he sequences of SV40 DNA which have been amplified from human tumours code for the p110 binding region. P53 is the most important tumour suppressor gene involved in human cancer with more than 50% of tumours exhibiting loss of function.20 T its levels are in-
creased by breaks in DNA strands and this leads either to arrest of cell replication for a sufficient period to allow DNA to be repaired, or to cell death (apoptosis). P53 has thus been named the “guardian of the gen-
ome”.1 SV40 and other viral proteins bind to p53 and inactivate it, reducing its DNA repair, apoptotic, and growth inhibitory functions, and might in this way contribute to the development of malignancy.

Structure of SV40 and mechanism of oncogenesis

Viruses such as SV40 carry only a limited amount of genetic material and depend heavily on host cell func-
tions for their replication. T his requires them to subvert normal cellular functions towards virus replication, and each virus has developed proteins which serve
A variety of malignancies. Mutations of proto-oncogenes and intracellular signalling proteins are recognised. Colleagues have recently reported a correlation between mesotheliomas and chromosomal abnormalities being present in a virus in a tumour is not the same as the generation of hydroxy radicals from intracellular known to be potently tumorigenic in animals and has mechanisms exist which could explain an exposure with SV40 and asbestos is not sufficient to alterations to the genetic material. In vitro, cultured If the observation is confirmed then immuno-histochemical staining for p53 has been reported in 35–70% of mesotheliomas, even in the absence of abnormalities of the gene, and this p53 antigen appears to be of wild type rather than mutant. Pap and colleagues have recently reported a correlation between SV40 large T antigen and p53 levels in mesotheliomas, offering further support for the hypothesis that SV40 contributes to malignancy by binding and inactivating p53 and other nuclear proteins.

**Conclusions**

The reports of SV40-like DNA in human mesotheliomas require confirmation, and the type and location of the viral genome need to be further characterised. However, the current information raises the possibility that SV40 is an important cofactor in the development of mesotheliomas. If evidence of seropositivity or viral DNA is present in about 5% of the general population and in 60% of those with mesotheliomas, then that suggests an approximately 30-fold increased risk of malignancy for those carrying the virus. Only limited reassurance can be taken from the cohort studies of populations exposed to SV40 as they all had limited power to detect even a large effect on a rare tumour. Demonstrating the presence of a virus in a tumour is not the same as demonstrating an aetiological role but plausible molecular mechanisms exist which could explain an oncogenic role for SV40. The virus belongs to a class of heterogeneous is observed both between tumours and between different parts of the same tumour. Some are observed more frequently than might be expected by chance and might be of aetiological significance, but overall there is little evidence of mutations of known oncoproteins in malignant mesotheliomas. Abnormalities of the retinoblastoma gene or the p53 gene do not appear to be important. On the other hand, immuno-histochemical staining for p53 has been reported in 35–70% of mesotheliomas, even in the absence of abnormalities of the gene, and this p53 antigen appears to be of wild type rather than mutant. Pap and colleagues have recently reported a correlation between SV40 large T antigen and p53 levels in mesotheliomas, offering further support for the hypothesis that SV40 contributes to malignancy by binding and inactivating p53 and other nuclear proteins. Most of the chromosomal abnormalities induced by asbestos appear to be random and a considerable degree of heterogeneity is observed both between tumours and between different parts of the same tumour. Some are observed more frequently than might be expected by chance and might be of aetiological significance, but overall there is little evidence of mutations of known oncoproteins in malignant mesotheliomas. Abnormalities of the retinoblastoma gene or the p53 gene do not appear to be important. On the other hand, immuno-histochemical staining for p53 has been reported in 35–70% of mesotheliomas, even in the absence of abnormalities of the gene, and this p53 antigen appears to be of wild type rather than mutant. Pap and colleagues have recently reported a correlation between SV40 large T antigen and p53 levels in mesotheliomas, offering further support for the hypothesis that SV40 contributes to malignancy by binding and inactivating p53 and other nuclear proteins.
agents targeted at this molecule might have a useful therapeutic effect. It would be paradoxical if a series of fortuitous findings which linked an unforseen effect of polio vaccination to an unforseen effect of asbestos exposure was to provide the clue which led to an effective treatment for malignant mesotheliomas.

1. Peto J, Hodgson JT. Malignant mesotheliomas currently cause approximately 1000 deaths per year in the UK.
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