Asbestos, Simian virus 40 and malignant mesothelioma

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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 aetiopathogenetic 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 which had previously been considered positive. The reaction produced DNA fragments of approximately the correct size in four of nine mesotheliomas. 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 TAg DNA. Nuclear TAg was detected immunohistochemically in 11 of 14 tumours and all of 26 serum samples. Hence the correct length in four of nine mesotheliomas and in none of seven control samples. SV40-like DNA was detected 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. 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 TAg DNA. Nuclear TAg was detected immunohistochemically in 11 of 14 tumours and all of 26 serum samples. Hence the correct length in four of nine mesotheliomas and in none of seven control samples.

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. SV40-like DNA was identified in 29 of 48 adults being seropositive. They are structurally very similar to SV40 with 69% homology of their DNA. BK causes cystitis and nephritis in immunosuppressed patients and JC is 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.
On the other hand, Strickler and colleagues failed to replicate the original PCR on tissue from 50 mesotheliomas using the same primers as Bergsagel and others, and failed to identify any amplified DNA capable of hybridising with SV40 sequences. Their results could not be explained by any obvious artefact: 48 of the 50 specimens amplified DNA using primers for B-globin genes, indicating that amplifiable DNA was present, and positive results were obtained from Simian and human cell lines known to have SV40 DNA incorporated into their genome. Serum SV40 antibodies assayed using a viral culture plaque inhibition assay were detected in only three of 34 samples. Other workers have failed to identify SV40 DNA in brain tumours (table 1) and the contradictory observations need to be resolved.

Amplifying DNA sequences from tissue samples which are 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 introductory article showed only that the SV40 primers amplified DNA sequences of approximately the correct length and did not demonstrate that they contained SV40-like sequences. However, the DNA sequence has been confirmed in other laboratories and it seems unlikely that the PCR primers are amplifying either JC or BK viruses or parts of the normal human genome. However, and despite the structural similarities with SV40, it is possible that the DNA originates from a previously unrecognised human virus. Contamination by laboratory SV40 strains is also possible as PCR is extremely sensitive to small amounts of extraneous DNA, and SV40 is a commonly used laboratory virus. The SV40-like DNA from some bone tumours was found to have an extra copy of a 72 base-pair enhancer region which is found in laboratory strains but not in wild SV40. On the other hand, sequences identified in brain tumours matched more than one strain of wild virus and were not homologous with laboratory strains, and were thus not due to contamination. Laboratory contamination also does not readily explain the tissue specificity of the findings.

The amplification of extracted SV40 DNA appears to be highly reproducible between laboratories with discordant results having been reported in only 3% of samples, but the method of extraction of DNA from tissue samples does affect the identification rate and might explain interlaboratory differences. The laboratories which identified viral DNA in mesothelial tissue also found immunohistochemical evidence of viral protein production whereas other laboratories found neither, raising the possibility of geographical differences in viral presence. However, serological and immunohistochemical tests are not well characterised and the 100% identification of antibodies in one laboratory and the almost 0% identification in another suggests that the differences are technical.

Doubt also remains about the extent of tissue infection with viral DNA. The mesothelioma data suggest that it is localised to the site of origin of the tumour, whereas the presence of viral DNA in blood from patients with osteosarcomas suggests that it is more widely distributed. It is not known whether the entire viral genome is usually present, though that sometimes seems to be the case, or whether it is truly incorporated into the host genome. Incorporation of SV40 into host DNA occurs commonly in vitro but in human bone tumours the DNA appeared to be present in short sequences and might have been episomal. At this stage it seems clear that the observations of SV40 DNA in human tumours can neither be accepted without reservation nor dismissed, and that further work is necessary to refine and standardise the identification techniques and to explain the discrepancies. In the meantime, it is worth considering how Simian viral DNA might have come to be present in tumours and whether there are other clues suggesting that it might have an aetologically significant role.

### Human exposure to SV40 and oncogenic properties

SV40 was identified in 1961 in the kidney cells of rhesus monkeys which were used from the early 1950s to culture poliovirus for the newly developed Salk polio vaccine. SV40 produced no cytopathic effects in the Simian kidney cells and by the time its presence was recognised 62% of the US population had been immunised with potentially infected vaccines. The extent of contamination of vaccines administered before 1961 is not known but, as 60% of rhesus monkeys used to culture poliovirus were infected and as SV40 is more resistant than poliovirus to chemical inactivation, it is thought that up to 30% contained live SV40. High levels of infective viral particles (10,000 per ml) were found in some samples, and SV40 antibodies were found in 20% of vaccinated schoolchildren. Oral polio vaccines were not licensed for clinical use until 1962 when SV40 had been largely eliminated from the culture system, and were probably not contaminated to any significant extent. Contamination of some adenovirus and hepatitis vaccines has been reported.

Most patients currently suffering from mesotheliomas are old enough to have received contaminated polio vaccines but that is not the case for the children with cerebral tumours in whom viral DNA was identified, and this suggests that there are other potential sources of infection. Support for this comes from the identification of SV40 antibodies in 2% of blood samples obtained before the introduction of polio vaccines, in 3% of schoolchildren born after vaccines were cleared of SV40, and in 4% of elderly patients with no history of polio vaccination. The significance of these serological studies is not entirely certain because of the possibility of cross reactions with anti-JC and anti-BK antibodies.

Contact with monkeys can lead to infection but the range of natural SV40 host animals is narrow and animal contact cannot explain the background 2-5% seropositivity rate. The virus can replicate in the nose and intestinal tracts of humans and can be shed for several weeks after infection, but nothing is known about whether transmission between humans occurs.

Shortly after its identification, SV40 was found to be highly oncogenic when injected into immature ham-

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>SV40-like DNA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesothelioma</td>
<td>29/48 (60%)</td>
<td>11</td>
</tr>
<tr>
<td>Normal lung</td>
<td>1/28 (4%)</td>
<td>11</td>
</tr>
<tr>
<td>Stenon</td>
<td>1/20 (5%)</td>
<td>11</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>40/126 (32%)</td>
<td>8</td>
</tr>
<tr>
<td>Lung cancer/adenocarcinoma</td>
<td>0/19 (0%)</td>
<td>4, 11</td>
</tr>
<tr>
<td>Normal brain</td>
<td>1/20 (5%)</td>
<td>6, 9</td>
</tr>
<tr>
<td>Normal lung</td>
<td>1/28 (4%)</td>
<td>11</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>4/33 (12%)</td>
<td>5, 6, 12</td>
</tr>
</tbody>
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#### Table 1: Frequency of identification of SV40-like DNA
Virus such as SV40 carry only a limited amount of genetic material and depend heavily on host cell functions for their replication. This requires them to subvert normal cellular control mechanisms and to express viral proteins in order to replicate. After infection, the SV40 virus enters proliferating cells and infects specific tissues in which the virus can replicate. In the presence of host cell factors that support viral replication, the virus can enter the cell nucleus and replicate its genome. The SV40 virus is known to be able to take mesothelial cells one step along a pathway towards full malignant expression but that other steps are often necessary.

Structure of SV40 and mechanism of oncogenesis

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Pathogenesis of malignant mesothelioma

Modern concepts of oncogenesis emphasize the multi-step nature of tumour development. This model suggests that tumours develop as a result of a series of genetic and epigenetic alterations that accumulate over time. The development of malignant mesothelioma is a complex process that involves the interaction of genetic and environmental factors. The most important risk factor for malignant mesothelioma is exposure to asbestos. Other factors that may contribute to the development of malignant mesothelioma include smoking, radiation exposure, and infections with certain viruses. It is thought that exposure to asbestos and other environmental factors may lead to changes in the genetic material of mesothelial cells, leading to the development of malignant mesothelioma.
The reports of SV40-like DNA in human mesotheliomas require confirmation, and the stage 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 not known. Surface iron molecules can catalyse generation of hydroxy radicals from intracellular hydrogen peroxide and other potentially toxic free radicals can be produced by phagocytosis-induced augmentation of the respiratory burst, but these chemical mechanisms do not readily explain the importance of the physical properties of fibres in determining their malignant potential. Asbestos can cause physical disruption of the cellular cytoskeleton and this may lead to chromosomal instability during cell division, and might directly break or damage chromosomes leading to alterations in the genetic material. In vitro, cultured mesothelial cells ingest asbestos fibres, develop chromosomal abnormalities, and have a prolonged lifespan. They do not, however, produce tumours when injected into animals. Although cells with chromosomal abnormalities are more easily transformed by SV40, co-exposure with SV40 and asbestos is not sufficient to produce malignant cells using this model.

Facilitation of entry of foreign DNA is another potential mechanism through which asbestos can lead to malignant change. Free DNA can be detected in blood and bronchoalveolar lavage fluid, and is probably present locally in high concentrations at sites of inflammation. In vitro, asbestos fibres are at least as effective at incorporating viral DNA into cells as standard calcium phosphate coprecipitation methods. It is therefore perhaps relevant that the timing of the infected polio virus epidemic coincided with the peak exposure of the working population to asbestos (fig 1) as the presence of fibres might have led to incorporation of SV40 specifically into airway or mesothelial cells.

M od 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 oncogenes in malignant mesotheliomas. Abnormalities of the retinoblastoma gene or the p53 gene do not appear to be important. On the other hand, immunohistochemical 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.19 Past 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.10

**Figure 3** Representation of interactions between viral early antigens and host tumour suppressor gene products. P110, P3, p53, p107, E7, E8, E1A, TAg. Adapted with permission from Tannock and Hill.
LEARNING POINTS

- Malignant mesotheliomas currently cause approximately 1000 deaths per year in the UK and the numbers are predicted to rise until about the year 2020.

- Contaminated polio vaccines exposed a substantial proportion of the population to Simian virus 40 (SV40).

- Malignant mesotheliomas currently cause approximately 1000 deaths per year in the UK and Simian virus DNA has been detected in subsequently arising human cancers.

- Simian virus 40 may act as a cofactor in the development of malignant mesotheliomas.

agents targeted at this molecule might have a useful therapeutic effect. It would be paradoxical if a series offortuitous findings which linked an unforseen effect of polio vaccination to an unforeseen effect of asbestos exposure was to provide the clue which led to an effective treatment for malignant mesotheliomas.
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