Rapid communication

Simian virus 40 large T antigen (SV40LTA_g) primer specific DNA amplification in human pleural mesothelioma tissue

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Abstract

Background – DNA sequences and immunoreactivity associated with Simian virus 40 transforming factors, large T and small t antigens (SV40LTA_g), 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 SV40LTA_g 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 LTA_g 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 SV40LTA_g primer

specific DNA with human pulmonary mesothelioma in the British population.

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Keywords: human mesotheliomas, Simian virus 40 (SV40) DNA, polymerase chain reaction (PCR), archival tissue.

Over the past 30 years the incidence of mesothelioma has increased steadily in many industrial countries including Britain, the United States, and some European countries.¹ In Britain it still appears to be increasing although in several other countries these increases have plateaued.² This has been completely or largely attributed to exposure to asbestos and better ascertainment. However, it is possible that the monkey oncogenic virus SV40 which was artificially parenterally introduced into a large cohort of the human population in Britain, some European countries, and the United States through a contaminated polio vaccine given between 1959 and 1961^{3,4} also might have played a part in this process. Support for this hypothesis has come from the work of Carbone *et al*⁵ who detected the presence of SV40-like DNA in 60% of frozen tumour tissues of cases of mesothelioma. Also, inoculation of baby hamsters with the virus has been shown to induce mesothelioma with an incidence of 100%.⁶ However, SV40, unlike its human counterpart papovavirus strains BK and JC, is not normally infective in man though it can transform human cells in tissue culture.^{5,7} One way to investigate the putative carcinogenic role of SV40 in human mesothelioma more directly is to examine the archival mesothelioma tissue from the peri-vaccination period. Since most of the case material has been preserved in a formalin fixed, paraffin embedded state, the present study was set up to confirm the expected association of SV40LTA_g DNA with British cases of mesothelioma and to examine the feasibility of detecting the SV40 DNA in archival formalin fixed, paraffin embedded mesothelioma tissue.

Methods

DNA was extracted from formalin fixed, paraffin embedded tissue block sections of nine recently diagnosed cases of mesothelioma, nine of adenocarcinoma, and three reactive pleurae,

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Figure 1 (A) Demonstration of SV40LTag primer set specific DNA amplification in four out of nine archival mesothelioma specimens. Lanes from right to left: marker (pBR322), specimen 9, positive control SV40 transfected human thyroid cell, specimens 6, 8, 5, 7, 2, 4, 3, and 1 DNA samples, respectively. (B) Reproducibility of DNA amplification. Lanes from right to left: marker (pBR322) DNA, blank block, SV40 transfected human thyroid cell DNA extract, and duplicate DNA extracts from mesothelioma specimens 5, 2, 6, and 1, respectively.

as well as an SV40-transfected human thyroid cell culture preparation.⁸ Four of the mesotheliomas had complementary fresh frozen tissue samples from the same tumour. Crude DNA extracts obtained from frozen sections and rehydrated paraffin sections following proteinase K digestion for 48 hours at 37°C were directly used for PCR amplification. Following this, the DNA samples were amplified using the primers designed by Bergsagel *et al*⁹ including the PYV primers which amplify a 172 bp fragment of SV40LTag common to several papovavirus strains (BK, JC, and SV40), and the SV primers which amplify a 105 bp fragment unique to the SV40LTag gene sequence. The quality of DNA in cases negative for amplification with both the primer sets was assessed using primers KM38 and PC03, specific for the amplification of the ubiquitous 167 bp human β -globin gene sequence.¹⁰

Results

The results for the PCR analyses are summarised in table 1, together with the relevant clinical details. The positive PCR results were found to be reproducible on repeat analysis on separate DNA extracts as illustrated in fig 1A. The SV40LTag related DNA was found to be readily amplifiable in sections taken from frozen and the corresponding formalin fixed, paraffin embedded SV40 transfected cell pellet blocks. In addition, the SV40LTag specific amplification (fig 1B) was restricted to four out of nine mesotheliomas compared with six of nine (67%) showing positivity with the broad spectrum PYV primer set. None of the non-mesothelioma tissue specimens proved positive for SV40 related DNA, but one of the three reactive mesothelial specimens and two of the nine adenocarcinoma specimens showed PYV primer set specific DNA amplifications.

Table 1 Type specific viral DNA detection profiles of individual mesothelial and non-mesothelial tissue specimens

Specimen type	Age; sex; tumour type	β -globin primer specific DNA	SV40LTag primer specific DNA	Papovavirus LTag primer specific DNA
SV40 transfected human thyroid cells (FS)		ND	+	+
SV40 transfected human thyroid cells (PS)		ND	+	+
Mesothelioma 1 (FS)	38; M; epi	ND	+	+
Mesothelioma 1 (PS)		ND	+	+
Mesothelioma 2 (FS)	65; M; biph	ND	+	+
Mesothelioma 2 (PS)		ND	+	+
Mesothelioma 3 (FS)	73; M; sarc	ND	+	+
Mesothelioma 3 (PS)		ND	+	+
Mesothelioma 4 (FS)	63; M; biph	ND	+	+
Mesothelioma 4 (PS)		ND	+	+
Mesothelioma 5 (PS)	54; M; epi	ND	+	+
Mesothelioma 6 (PS)	61; M; sarc	ND	+	+
Mesothelioma 7 (PS)	65; M; sarc	+	—	—
Mesothelioma 8 (PS)	56; F; biph	+	—	—
Mesothelioma 9 (PS)	54; M; epi	+	—	—
Adenocarcinoma 1 (PS)	67; F	ND	—	+
Adenocarcinoma 2 (PS)		ND	—	+
Adenocarcinoma 3 (PS)		+	—	—
Adenocarcinoma 4 (PS)	73; M	+	—	—
Adenocarcinoma 5 (PS)	63; M	+	—	—
Adenocarcinoma 6 (PS)	47; F	+	—	—
Adenocarcinoma 7 (PS)	41; F	+	—	—
Adenocarcinoma 8 (PS)	63; F	+	—	—
Adenocarcinoma 9 (PS)	71; M	+	—	—
Reactive pleura 1 (PS)	52; M; cnsi	ND	—	+
Reactive pleura 2 (PS)	44; M; cnsi	+	—	—
Reactive pleura 3 (PS)	26; M; rep	+	—	—

FS=frozen sections; PS=formalin fixed paraffin embedded sections; epi=epithelial; sarc=sarcomatous; biph=biphasic; cnsi=chronic non-specific inflammation; rep=reactive eosinophilic pleuritis; ND=not done.

Discussion

The results of the present study broadly confirm the findings of Carbone *et al*⁶ in terms of both the pattern of expression and the relative incidence of SV and PYV primer set specific DNA amplification in the mesothelioma specimens from British patients. In addition, the overall findings support the feasibility of analysing the presence of such DNA in archival formalin fixed, paraffin embedded tissue material. This contrasts with the negative findings of Strickler *et al* who failed to detect SV40 DNA in paraffin sections from 50 mesotheliomas, possibly due to methodological differences.¹⁰

Since SV40 is unable to infect the human population naturally, the most likely route of entry is considered to be the accidental parenteral inoculation of large cohorts of American and European populations with SV40 contaminated polio vaccines in the period 1959–61. The fact that SV40LTag related DNA is detectable in archival material should allow examination of stored specimens of mesotheliomas and other types of tumours from the peri-vaccination period. If the tumours from this period are found to be negative for the virus associated DNA, it may become important to

re-evaluate the long held view on the independent aetiological role of asbestos in human mesothelioma and consider an additional putative carcinogenic role of SV40 in this type of human cancer.

- 1 Office of Population Censuses and Surveys/Health & Safety Executive. *Occupational Health Decennial Supplement*. London: HMSO, 1995:127-52.
- 2 Dambuis RAM, Planteydt HT. Mesothelioma. *Lancet* 1995; **345**:1233-4.
- 3 Eddy BE. Identification of the oncogenic substance in rhesus monkey kidney cell cultures as SV40. *Virology* 1962; **17**: 65-75.
- 4 Shah KV, Nathanson N. Human exposure to SV40: review and comment. *Am J Epidemiol* 1976; **103**:1-12.
- 5 Carbone M, Pass H, Rizzo P, Marinette MR, Di Muzio M, Mew DJY, *et al.* Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* 1994; **9**:1781-90.
- 6 Cicala C, Pompetti F, Carbone M. SV40 induces mesotheliomas in hamsters. *Am J Pathol* 1993; **142**:1524-33.
- 7 Bryan TC, Reddel RR. SV40-induced immortalisation of human cells. *Crit Rev Oncogenesis* 1994; **5**:331-57.
- 8 Lemoine NR, Mayall ES, Jones T, Sheer D, Mcdermaid S, Kendall-Taylor P, *et al.* Characterisation of human thyroid epithelial cells immortalised in vitro by simian virus 40 DNA transfection. *Br J Cancer* 1989; **60**:897-903.
- 9 Bergsagel DJ, Finegold MJ, Butel JS, Kupsky WJ, Garcea R. DNA sequences similar to those of simian virus 40 in ependymomas and choroid plexus tumours of childhood. *N Engl J Med* 1992; **36**:988-93.
- 10 Strickler HD, Goedert JJ, Fleming M, *et al.* Simian virus 40 and pleural mesothelioma in humans. *Cancer Epidemiol Biomarkers Prevention* 1996; **5**:473-5.