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Failure to Confirm Presence of SV40 Sequences in Human Tumours

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THE MONKEY polyomavirus, simian virus 40 (SV40), is a potent tumour-inducing virus in laboratory animals. Although SV40 has not been associated with human disease [1, 2], DNA sequences similar to those of SV40 have been found recently in ependymomas and choroid plexus tumours of chidren using a polymerase chain reaction (PCR) technique [3]. Our interest in the possibility that these sequences may be involved in the oncogenesis of other malignancies prompted us to reproduce and further investigate these data.

DNA from ependymomas was provided by H. Budka (Institute of Neuropathology) and an SV40-transformed rat fibroblast cell line by Ch. Czerni (Institute for Cancer Research). DNA was isolated from paraffin-embedded tissue and cell homogenates using standard methods. As DNA in paraffin-embedded tissue can be degraded, isolation of intact DNA was proven by amplification of β-globin sequences. To achieve high sensitivity for the amplification of SV40 DNA, we used a nested PCR protocol with outer primers: SVO for 5'TGATGAATGGGAGCAGTGGTGGAA 3'; SVO.rev 5' CCCACCTGGCAAACTTTCCTCAAT 3', amplifying a 490 bp fragment, followed by a second PCR with inner primers SV.for3 and SV.rev, as used by Bergsagel and associates [3]. The products were analysed on agarose gels and stained with ethidium bromide. Figure 1 shows dilution experiments of DNA isolated from the SV40-positive rat cell line, indicating a detection limit of our assay system of <10 copies of SV40.

In contrast to the findings of Bergsagel and associates [3], SV40-like DNA sequences could not be found in any of the 10 ependymomas investigated. The discrepancy of 10 out of 11 ependymomas positive for SV40-like sequences in the study by Bergsagel versus none in our series could have two possible explanations: firstly, the fact that in our system, the outer 3' primer (SVO.rev) was positioned 307 base pairs downstream of SV.rev, so that if only a shorter sequence was present, there would be no amplification. However, the 490 bp fragment we amplified is situated in a highly conserved region, well within the early region of SV40, which encodes for the T-antigen required for the initiation and presumably maintenance of transformation [4].

Secondly, SV40 might not be found with the same incidence in different areas. SV40 does not naturally infect humans, but it did contaminate polio vaccine in the 1950s and 1960s [1, 2]. If this were the ultimate source of the SV40-like DNA, a different prevalence of SV40 sequences can be expected in various countries. However, since all our ependymoma cases were negative, there is a lack of evidence that the described SV40 sequences [3] play a key role in the pathogenesis of this rare disease.

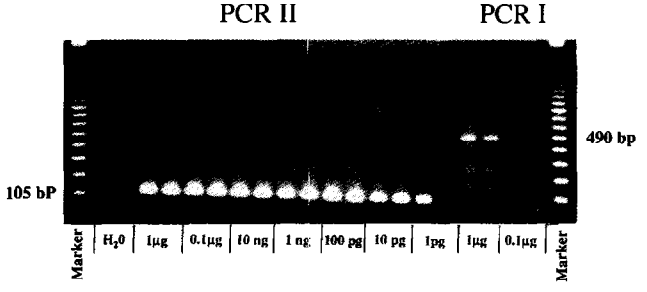

Figure 1. After the first polymerase chain reaction (PCR I) the SV40 DNA (490 bp) was hardly visible when 0.1 µg template was used; after PCR II with the nested primer, the inner product (105 bp) could be adequately detected in 10 pg of total cellular DNA; at 1 pg one of the two reactions was negative.

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Flow Cytometric Gating on Cytokeratin-containing DNA Aneuploid Breast Cancer Cells Improves the Prediction of Recurrence

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INCREASED S-PHASE fraction (SPF), as estimated by flow cytometry, is associated with an increased risk of recurrence for breast cancer patients [1, 2]. However, the analysis with single parameter flow cytometry is influenced by the presence of

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