SV40 Detection in Human Tumor Specimens

To the Editor:

Cristau do et al. (1) provided the first molecular epidemiologic evidence supporting cocarcinogenesis between asbestos and SV40 in human malignant mesothelioma. The authors have also proposed that an inadequate technical procedure to detect SV40 could produce false-positive or false-negative results. Here we provide information for the optimization of the experimental procedure and to prevent laboratory artifacts. It is hoped that this information will help those interested in testing human specimens for SV40.

When testing tumor samples by PCR, it is important to rule out PCR and plasmid contamination. The precautions we recommend to prevent and to detect PCR contamination were described previously (2, 3). To test for laboratory contamination by plasmids, we recommend to run PCR reactions using the primers 5'-GCTCACGCTGTAGATCTC-3' and 5'-TCTAGTGTAGCCGTAGT-3' that amplify a 241-bp portion of the pUC origin of replication present in pBR313 (4) and in virtually all plasmids that are propagated in E. coli (BLAST analysis; http://www.ncbi.nlm.nih.gov/BLAST/).

In Fig. 1A, we show how the sensitivity of the detection method can drastically change the scoring of SV40 large T antigen (Tag) expressing malignant mesotheliomas (from 0% to ~50%) in immunoprecipitation/Western blots. Note that A1 to A3 contain the same tumor lysates, and that the same membrane (A2) was stripped and rehybridized (A3). This experiment shows how apparently conflicting results can be resolved by optimizing the technical approach (see figure legend).

In Fig. 1B, we show a double immunohistochemistry for SV40 Tag and CD44 of a formalin-fixed, paraffin-embedded tumor that developed in a severe combined immunodeficient mouse 1 month after injection of 5 × 10^6 cells derived from an SV40-positive malignant mesothelioma. This example shows how immunoreactivity for Tag can be influenced by the method. Tag staining was detected only when hybridizing the Tag-specific antibody first, developing the reaction, and then proceeding with the anti-CD44 hybridization. Using CD44 first and then Tag or mixing the two together resulted in loss of Tag nuclear staining, suggesting that the anti-CD44 ready-to-use, prediluted antibody (see legend) contains an inhibitor for the Tag reaction.

The described issues are in addition to other variables known to affect SV40 detection results, such as DNA extraction protocols and the relative content of cancer cells in a tumor specimen (reviewed in ref. 5). Our data support Cristau do et al.'s message that only when rigorous controls are used is it possible to reliably evaluate the presence and expression of SV40 in human specimens. The examples shown underscore how small technical details can produce opposite results that at first glance could erroneously appear in conflict.
References


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