**"Detection of SV40 like viral DNA and viral antigens in malignant pleural mesothelioma." M. Ramael, J. Nagels, H. Heylen, S. De Schepper, J. Paulussen, M. De Maeyer and C. Van Haesendonck. *Eur Respir J* 1999; 14: 1381–1386.**

*From the authors*:

We reported in a previous paper on the possible association of simian vacuolating virus 40 (SV40)-like DNA and protein in malignant mesothelioma using the primed *in situ* labelling (PRINS) method with SVrev and SV2for, as well as a general polyomavirus primer PYVrev and immunohistochemistry using the antibody PAb280 [1]. As some concerns were raised regarding the specificity of the methods used, we felt it necessary to provide an explanatory clarifying note, including some new figures.

We used in the original experiments several SV40 specific primers, including SVrev and SV2for, as well as a general polyomavirus primer PYVrev, in order to rule out the potential pitfall of aspecific binding of an oligonucleotide DNA primer (figure 1). PRINS signals were only observed in neoplastic mesothelioma cells. No PRINS signals were observed in stromal cells [1]. Positive control reaction is shown in a malignant epithelial mesothelioma with pan-chromosome primer in PRINS reaction (figure 1, insert).

None of the negative controls, including placental tissue, nor the negative mesothelioma controls where primer was omitted, showed any reactivity, confirming the specificity of the reaction (figure 2). Additionally, 30 specimens of pleural carcinoma metastasis of bronchial cancer and 30 specimens of reactive mesothelium did not display any nuclear or cytoplasmic PRINS signal with the SV40 primers [1].

PRINS has been used in clinical medical genetics [2], as well as for detecting single copy genes [3] and viral sequences [4, 5], proving a reliable technique. We used the technique for detecting other viruses such as HPV in paraffin-embedded formalin-fixed tissues and found it a highly reliable technique [5].

Our findings concerning the presence of SV40 or SV40-like DNA in Belgian malignant mesothelioma cases are corroborated by the findings of Dhaene *et al.* [6]. They described SV40 DNA or SV40-like DNA with a classical PCR technique (SVfor3 and SVrev primers) and found SV40 PCR amplicons in 13 of the 28 (46%) Belgian mesothelioma cases. Cytoplasmic, but no nuclear, immunoreactivity staining was found in 10 of these 13 cases in the study of Dhaene *et al.* [6], using the PAb419 and the PAb101 SV40 LTag antibodies [6]. Very strangely, only 2 years later this study was challenged by Hübner and Van Marck [7], who only investigated 12 mesothelioma cases and did not find SV40 or SV40-like DNA in any of the 12 investigated mesothelioma cases.

We used in our study the monoclonal antibody PAb280, which is directed against a specific epitope of the SV40 small t-antigen. Darmon and Jat [8] describe cross-reactivity with cellular proteins BAP37 and prohibitin using the monoclonal antibodies PAb419 and PAb210, which are directed against an epitope specific to SV40 large T-antigen. They add that other anti-SV40 T-antigen monoclonal antibodies do not

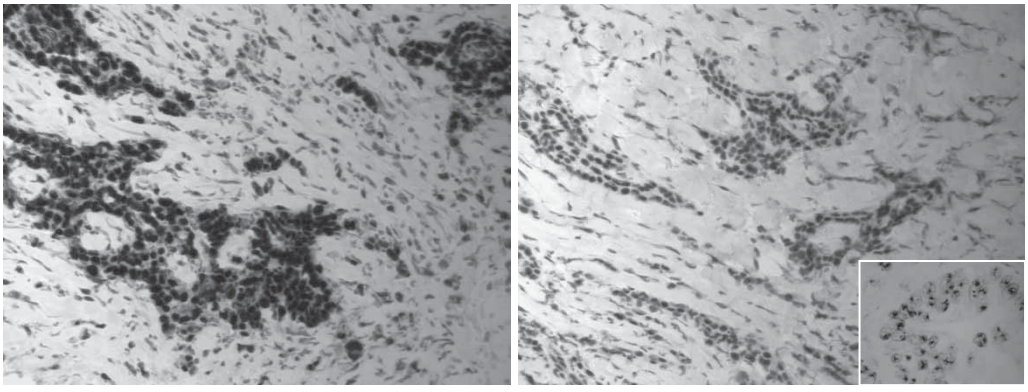

FIGURE 1 Most nuclei of neoplastic mesothelial cells display nuclear staining for simian vacuolating virus 40 (SV40) DNA with the primed *in situ* labelling (PRINS) method in an epithelial mesothelioma (SV2 for primer). Insert: Positive PRINS control reaction with the pan-chromosome primer specific for all human chromosomes showing multiple nuclear signals indicating the detection of chromosomal DNA in neoplastic mesothelial cells of an epithelial mesothelioma.

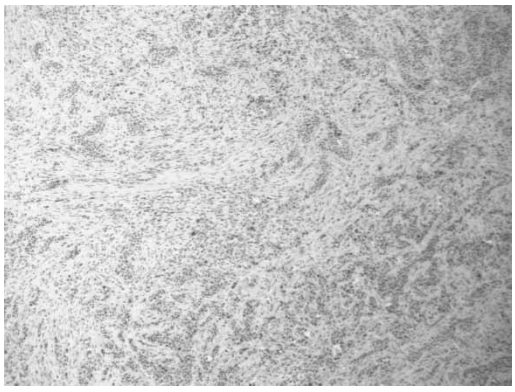

FIGURE 2 Negative control reaction for SV40 of the epithelial mesothelioma for simian vacuolating virus 40 (SV40) DNA with the primed *in situ* labelling (PRINS) method in an epithelial mesothelioma. No specific staining was observed in neoplastic cells nor in stromal cells.

display this feature. We used the monoclonal antibody PAb280, which is completely different from the aforementioned monoclonal antibodies PAb419 and PAb210 used by Darmon and Jat [8]. The monoclonal antibody that we used is directed against another epitope at another SV40 protein (small t-antigen) [9]. The conclusions of Darmon and Jat [8] may not simply be extended to the PAb280 antibody used in our study.

None of the negative controls including placental tissue, nor the negative mesothelioma controls where anti-SV40 PAb280 was omitted and replaced by another non-related antibody in the same concentration and of the same isotype, showed any immunoreactivity, confirming the specificity of the reaction. Additionally, 30 specimens of pleural carcinoma metastasis of bronchial cancer and 30 specimens of reactive mesothelium did not display any nuclear or cytoplasmic immunoreactivity with the PAb280 monoclonal antibody.

The observation of both nuclear and cytoplasmic immunoreactivity with the PAb280 monoclonal antibody used in our study has been clearly discussed and addressed in our paper. Fixation times that are longer than 60–120 min seem to influence the subcellular localisation of nuclear proteins, such as c-myc protein, resulting in both nuclear as well as cytoplasmic immunoreactivity [10]. If the observed immunoreactivity was only a cross reaction with cellular proteins, such as BAP37 and prohibitins (localised in the cytoplasm), one would expect only cytoplasmic immunoreactivity and not nuclear immunoreactivity as observed in our study. Fixation times varied in our study from 6 to 24 h, where classically most histopathology laboratories accept fixation times for formalin tissue from 6 h up to 72 h.

We must therefore also take into account that we were working on paraffin-embedded formalin-fixed tissue and not on fresh SV40 virus-infected cell lines. Infected cell lines *in vitro* are not completely comparable as they show lytic infection, in contrast to the *in vivo* neoplastic mesothelioma cells in formalin-fixed paraffin-embedded neoplastic mesothelioma tissue that shows no lytic characteristics [8].

The possible association between the presence of SV40 virus DNA or SV40 virus-like DNA and human tumours remains an interesting and controversial issue with exciting and sometimes emotional discussions between "believers" and "non-believers". Further research will hopefully clarify this enigma.

## References


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