Commentary

Simian virus 40 and the human mesothelium

George Klein

Microbiology and Tumor Biology Center, Karolinska Institute, S-171 77 Stockholm, Sweden

DNA tumor viruses have evolved to replicate, not to cause tumors. Their carcinogenic effect depends on the blocking of the lytic cycle and the ability of latently infected, potentially proliferative cells to escape immune surveillance. These conditions can be satisfied by accidental "experiments of nature" or by the artifices of laboratory experimentation.

Potentially tumorigenic papovaviruses and adenoviruses stimulate host cell DNA synthesis. The induction of an S phase appears to be essential for the integration of the viral genome into host cell DNA. The induction of host cell proliferation is a secondary consequence. Under natural conditions, the risk of malignant growth is counteracted by the viral oncogene-dependent triggering of apoptosis, e.g., through the ARF-p53 pathway, and/or by the immune response of the host. Efficient rejection responses are preferentially directed against MHC class I-associated peptide derivatives of the virally encoded transforming proteins.

Convergent evolution has endowed several of the DNA tumor viruses like simian virus 40 (SV40), the cancer-associated human papilloma viruses, and the oncogenic adenoviruses, with the ability to target the cellular retinoblastoma and p53 proteins. The papilloma viruses and adenoviruses use two different latency and transformation-associated proteins for this purpose, whereas two different domains of the single SV40 large T protein can perform this double function. It leads to the inactivation of both the retinoblastoma protein-dependent growth arrest pathway and the p53-dependent apoptotic mechanism.

SV40 causes lytic infection in the cells of its natural primate host where it is not believed to induce any tumors. It can be highly tumorigenic in rodents, where the lytic replication of the virus is blocked, particularly in the hamster. This is consistent with the requirement of nonpermissiveness for the lytic cycle as a prerequisite for cell transformation.

The infection of human cells with SV40 is semipermissive. Human fibroblasts (HFs) can be transformed but only at a low rate. This has been confirmed in the paper of Bocchetta *et al.* (1) in this issue of PNAS. They also report, for the first time, that the interaction of the virus with human mesothelial (HM) cells is nonlytic. It has led to T-antigen synthesis in 100% of the cells. Transformed foci appeared at a more than 1,000-fold higher efficiency than in HF cultures, infected in parallel. Most of the HM foci could grow at low serum concentration and in soft agar and contained immortalized cells, in contrast to the HF foci. It would be interesting to know whether the difference in the immortalizability of SV40-infected HM cells compared with HF cells is paralleled by a difference in telomerase activity.

Bocchetta *et al.* attribute the high transformability of the HM cells to their much higher level of p53, compared with HFs. This hypothesis was supported by inhibition experiments. Antisense p53 increased viral DNA replication and induced cell lysis in SV40-infected HM cells.

Are the SV40-induced HM foci but not HF foci tumorigenic? This is an important question for two different reasons. One is the notorious difficulty to transform normal HFs or epithelial cells by SV40 and other means. The frequently reported presence of SV40 DNA in about two-thirds of malignant human mesotheliomas is the other. According to microdissection experiments, SV40 is present only in the malignant cells and not in the surrounding normal cells (see ref. 2 for a review).

Asbestos exposure shows a strong epidemiological association with human mesotheliomas. Bocchetta *et al.* therefore also have tested the effect of asbestos in their HM *in vitro* system. Crocidolite asbestos caused only a moderate increase in the frequency of HM and HF foci, respectively, in cells transfected 3 days earlier with plasmids that carried both large and small T antigen (T+t+). T+t− plasmids induced no foci in either HM or HF cells but asbestos addition rescued focus formation, raising the efficiency of the T+t− plasmids to a level that was nearly comparable to the double positives. Importantly, the high toxicity of asbestos for HM (but not HF) cells was counteracted by the transforming SV40 plasmids.

Interesting as they are, the relatively minor contributory effects of asbestos to SV40 transformation *in vitro* can hardly account for mesothelioma induction *in vivo*. Bocchetta *et al.* suggest that asbestos also may act by immunosuppression, or alternatively or in addition, the production of oxygen radicals by asbestos-activated macrophages may play a cocarcinogenic role. Both possibilities are perfectly conceivable. SV40-induced tumors are highly immunogenic in rodents. In mice and rats, they rarely develop at all, unless the host is immunosuppressed. In hamsters, they may "sneak through," outrunning the rejection response, particularly if newborn animals are inoculated. Tumor development can be prevented by protective vaccination, administered during the latency period, however. Further analysis of the postulated immunosuppressive role of asbestos in human mesothelioma is therefore of both analytical and possible immunotherapeutic significance.

The postulated role of asbestos-induced genetic changes as an additional requirement for SV40-induced malignization of mesothelial cells is also interesting. The recent work of Hahn *et al.* (3) may be relevant in this context. They have shown that HFs and epithelial cells can be transformed and become tumorigenic upon combined transfection with SV40 large T oncogenic H-ras, and the catalytic subunit of human telomerase. Given the fact that SV40 large T inhibits both retinoblastoma and p53 proteins, they suggested that a minimum of four distinct signaling pathways need to be affected to transform normal human cells. It would not be surprising if the SV40-transformed HM cells of Bocchetta *et al.* would still need a strong proliferation-driving oncogene, akin to H-ras, for tumorigenicity. Could asbestos exposure raise the probability of corresponding genetic changes *in vivo*?

In conclusion, the predominantly nonlytic interaction of SV40 with normal HM cells, possibly a consequence of their high p53 level, emphasizes the role of the cell type within the same species for the choice between lytic and nonlytic, potentially transforming, virus-cell interactions. The induction of focus formation, agarose clonability,

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and immortalization in SV40-transfected HM but not HF cells is consistent with, although it does not prove, the postulated role of SV40 in the etiology of some human mesotheliomas. In contrast to ubiquitous, potentially oncogenic DNA viruses like polyoma in mice or Epstein–Barr virus in humans, SV40 is not a recognized inhabitant of human populations. The question of how it gains access to the human mesothelium *in vivo* or, indeed, whether the SV40 sequences found in malignant mesotheliomas are identical with the simian prototype or derive from a distinct variant, remains to be clarified.

1. Bocchetta, M., Di Resta, I., Powers, A., Fresco, R., Tosolini, A., Testa, J. R., Pass, H. I., Rizzo, P. & Carbone, M. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10214–10219.
2. Carbone, M. J. (1999) *Cell Biochem.* **76,** 189–193.
3. Hahn, W. C., Counter, C. M., Lundberg, A. S., Beijersgern, R. L., Brooks, M. W. & Weinberg, R. A. (1999) *Nature (London)* **400,** 464–468.