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Simian Virus 40-Related Antigens in Three Human Meningiomas with Defined Chromosome Loss

(cell culture/chromosome 22 loss/simian virus 40 T, U, and V antigens/immunofluorescence/ Sendai virus fusion)

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ABSTRACT **Two out of seven meningiomas tested in early cell cultures by indirect immunofluorescence staining showed simian virus 40 (SV40)-related tumor (T) antigen. In one tumor 90% of the cells were positive. An additional SV40-related antigen (U) was found in 10% of cells of a third tumor. These findings indicate that the meningioma cells showing a positive reaction are transformed by a papova virus that has at least partly the same antigenic properties as SV40 virus. SV40-related viral capsid (V) antigen was absent in all the meningiomas tested. No virus infectious for African green monkey kidney (AGMK) cells could be isolated. The tumors positive for T and U antigens showed the chromosome aberration typical for human meningiomas, i.e., the loss of one chromosome, G-22. The T-antigen-positive tumors showed further hypodiploidization. Experiments to rescue virus from the T-antigen-positive meningioma cells were performed: fusion of cells pretreated with 8-azaguanine with cells permissive for SV40 led to a low percentage (0.01–0.05%) of V-antigen-positive nuclei in heterokaryon cultures. On the basis of these results, the possibility of a correlation between the meningioma, a relatively common intracranial tumor in man, and an SV40-related papova virus must be considered. It remains to be shown whether this virus is a causative agent for human meningiomas.**

Meningiomas are tumors deriving from the spiderweb-like tissue of the middle of the three meninges (m. arachnoidea). Normally they grow relatively slowly in a nodular form, displacing the underlying brain and without the usual signs of malignancy. They appear preferentially in the late decades of life. Taking into account the small meningiomas only detected *post mortem*, these tumors are the most frequent of intracranial tumors in man.

In a consecutive series of more than 100 human meningiomas we were able to establish characteristic chromosome aberrations beginning with the loss of a small acrocentric G group chromosome. In about half the tumors studied the only chromosome aberration was the loss of G-22, either completely or in a mosaic form having a cell line with 22 monosomy. In other tumors an additional loss of up to five chromosomes could be observed (Table 1). The degree of hypodiploidization was clearly correlated with the histological structure of the meningiomas in question, changing from typical arachnotheliomatous through fibromatous to poorly structured tumors (1–4).

In human cell cultures transformed by simian virus 40 (SV40) nonrandom chromosome aberrations were found that showed striking similarities to our findings in human meningiomas (5–7). The aberrations consisted of a significant loss of acrocentric, especially small acrocentric, chromosomes. Furthermore, an enhanced association between the nucleolus-organizing regions of the acrocentric chromosomes was noticed (5). These corresponding chromosome aberrations in SV40-transformed cells and human meningiomas motivated us to look for a relation between SV40 and the meningioma, which up to now, is the only solid tumor with definite chromosome aberrations in man.

SV40 was first detected and isolated in 1960 from primary rhesus monkey kidney cells that were to be used for poliovirus vaccine production (8). It has been shown that humans

TABLE 1. A. *Chromosomal findings in a consecutive series of 100 human primary meningiomas*

No. of tumors	Karyotype
21	Normal karyotype or mosaicism 46/45, −G
72	G-chromosome loss (in all or almost all cells)
44	45, −G 22
4	46, 22q− *
24	G-monosomy and further chromosome loss (44–40 chromosomes)
3	Hypodiploidy without G-chromosome loss
4	Hyperdiploidy

B. *Correlation between chromosomal and histological findings*

Karyotype†	Histological structure
46–45	Arachnothelial meningioma with or without secondary structures. Isomorphic nuclei.
45–43	Fibromatous meningioma. Isomorphic nuclei.
43–40	Endotheliomatous poorly structured meningioma. Occasionally anisomorphic nuclei.

* Partial loss of the long arm of chromosome 22 corresponding to the Philadelphia-chromosome in chronic myeloic leukemia.

† Also in mosaic forms. The predominant chromosome numbers are underlined.

Abbreviations: AGMK, African green monkey kidney; HAU, hemagglutination units; SV40, simian virus 40; T antigen, tumor antigen; U antigen, a further SV40-related antigen; V antigen, viral capsid antigen.

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TABLE 2. *Correlation between SV40-related antigens, karyotype, and histological structure in seven human meningiomas*

Meningioma no.	Patient age/sex	Histological characteristics	Karyotype of the stemlines	SV40-related antigens T	U	V
T 1546	44 ♀	Arachnothelial	45, −G 22	−	+ (10%)†	—
T 1558	63 ♀	Poorly structured epitheloid	40/41, (−G 22)*	+ (30%)†	−	—
T 1623	56 ♀	Arachnothelial	46	−	−	—
T 1625	61 ♀	Arachnothelial	46	−	−	—
T 1647	63 ♂	Poorly structured epitheloid	40/41, (−G 22)*	+ (90%)†	−	—
T 1695	34 ♀	Arachnothelial	46	−	−	—
T 1720	61 ♀	Arachnothelial	46	−	−	—

* T 1558 and T 1647 in the great majority of investigated mitoses have 41 or 40 chromosomes. The loss of one chromosome 22 is constant, whereas there is some variation in the loss of further chromosomes. In both meningiomas the short arm of one chromosome, A-1, is missing.

† Percentage of cells showing positive fluorescence.

can undergo SV40 infections, but only in a subclinical form (9), a fact that may have contributed to the late detection of the virus. Human cells *in vitro* are semipermissive for SV40, i.e., a cell can be either lytically infected or transformed. In rodents, SV40-specific tumors can be experimentally induced. For these reasons, SV40 provides a good model to study the mechanisms by which viruses transform cells in culture and produce tumors in certain animals (10). The detection of SV40-related T and V antigens in the metastases of a human malignant melanoma has recently been reported (11). With the exception of this case, no relation between SV40 and human tumors has been described.

Here we report on the detection by immunofluorescence of SV40-specific antigens in human meningioma cells and in heterokaryon cultures of meningioma and African green monkey kidney (AGMK) cells.

MATERIALS AND METHODS

Tumors and Cell Cultures. Meningiomas were obtained from seven patients. The relevant details of these patients and the histological and chromosomal features of their tumors are entered in Table 2. Except for T 1720, a third recidivation tumor of a 61-year-old woman, the tumors are all primary meningiomas.

Freshly obtained tumor biopsy material was minced with scissors and trypsinized. The resulting cells were seeded in milk dilution bottles in a cell concentration of 2×10^7/10 ml of medium. A good cell proliferation was obtained in Dulbecco's modified Eagle's medium with 10% bovine serum, which allowed subcultivation every week in early passages. Tumors T 1546 and T 1558 were studied between the fifth and twelfth culture passages, whereas, T 1623, T 1625, T 1647, T 1695, and T 1720 were examined between the first and fifth passages. The SV40 permissive cell line, MA 134, derived from African green monkey kidney, kindly provided by B. Hirt, Lausanne, was used for cell fusion experiments with meningioma cells. The control cells for antibody assays were primary cultures of adult human fibroblasts and of two human glioblastomas and the established cell lines MA 134, CV 1, 3T3, and HeLa.

SV40-Related Antigens. Viral Specific Capsid Antigen and Tumor Antigen. Monolayers of meningioma and control cell cultures were examined for the presence of SV40-related antigens by indirect immunofluorescence staining. For each antibody assay, coverslips (size 12 × 32 mm, average cell count of 2.4×10^5) were removed from four culture flasks at the same passage level. To test for SV40-related viral capsid antigen (V antigen), rabbit antiserum against SV40 and fluorescein-conjugated goat antiserum prepared against rabbit serum were used. The coverslips were washed in phosphate-buffered saline (pH 7.2) and fixed in acetone at 0°. After further washing they were incubated at 37° for 1 hr with specific antiserum and for an additional hour with fluorescein-isothiocyanate-conjugated antibodies prepared against the first. The rabbit antiserum against SV40 was used at a dilution of 1:4. It was tested with CV 1 cells lytically infected with SV40 before use in these experiments, and was found to give a strong fluorescence for SV40 viral specific capsid antigen.

To determine the presence of SV40-related tumor (T) antigen, a hamster anti-T serum was prepared from adult hamster inoculated with virus-free tumor homogenates derived from baby hamsters bearing SV40-specific tumors. The anti-T serum was tested in a number of control systems and was shown to give a negative reaction with primary cultures of human adult fibroblasts and of two human glioblastomas as well as with the established cell lines MA 134, CV 1, and HeLa. It gave a specific positive fluorescence with SV40-transformed 3T3 cells. Anti-T serum was used at a dilution of 1:1. Except for T 1546 and T 1558, which were assayed for SV40-related T and V antigens in the fifth culture passage, all the meningioma cultures were tested in the first passage.

8-Azaguanine Incubation and Sendai Virus-Induced Cell Fusion. Three meningiomas, two (T 1558 and T 1647) with detectable T antigen and one (T 1720) without, were used in the cell fusion studies. Immediately prior to these experiments, positive antibody assays were obtained for T 1558 and T 1647, now at their tenth and fourth passage, respectively. The tumor cells were fused with cells from the SV40 permissive AGMK line, MA 134. Three cell fusion experiments were done for each tumor tested (Table 3), with β-propiolactone-inactivated Sendai virus as a fusion factor and by a monolayer hybridization method (12). Tumor cells were exposed to 100 μg of 8-azaguanine/ml of medium for 24 hr before fusion experiments with MA 134, as 8-azaguanine was shown to improve the percentage of virus-producing het-

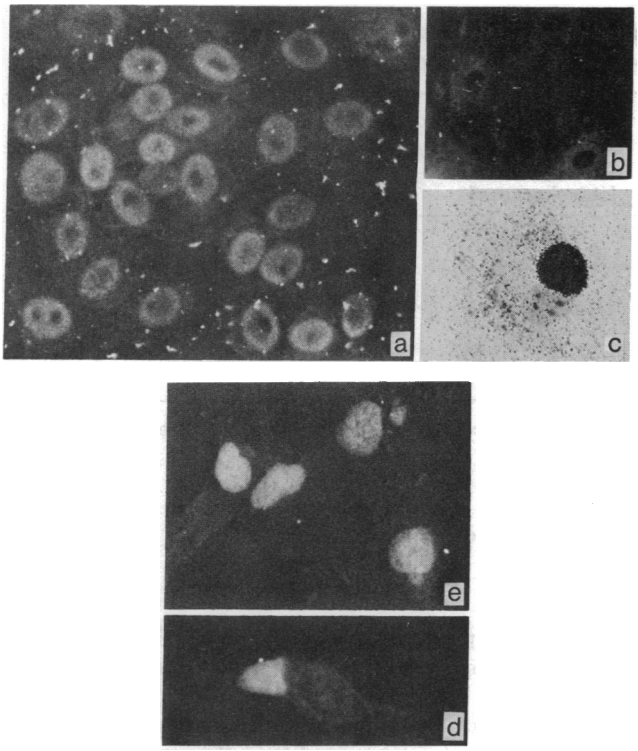

FIG. 1. (a) SV40 T-antigen-positive cells of meningioma T 1647 in the first culture passage. (b) SV40 V-antigen-negative cells of T 1647 in the first culture passage. (c) Heterokaryon with labeled T 1647 and unlabeled MA 134 nucleus. (d) Heterokaryon with SV40 V-antigen-positive (MA 134) and nonfluorescent (T 1647) nucleus (cell fusion 5; 7 days after fusion). (e) Group of SV40 V-antigen-positive fluorescent nuclei (cell fusion 5; 7 days after fusion). Magnifications: a, c, d, and e, ×278; b, ×154.

erokaryocytes after fusion of SV40-transformed cells with AGMK cells (13). As controls beside meningioma T 1720 showing no fluorescence for SV40 T antigen, human fibroblasts and human fetal brain cells were each fused with MA 134. The ratio of heterokaryon cells was determined through labeling of meningioma cells with [^{3}H]thymidine (specific activity, 50.7 Ci/mmol; concentration used, 1.0 μCi/ml). Autoradiography indicated that 75–85% of the tumor cell nuclei were labeled. A heterokaryon cell was determined by the presence of at least one labeled nucleus in the same cell with one unlabeled nucleus (Fig. 1c).

SV40-Related Viral Capsid Antigen in Heterokaryon Cultures. The percentage of V-antigen-positive nuclei was determined by indirect immunofluorescence 7 days after fusion experiments. A minimum of 10^6 cells were examined in each cell fusion culture.

Chromosomal Preparation and Giemsa Banding Staining. Chromosomes of the meningioma cell cultures were prepared in the usual way (14) and identified by Giemsa banding staining according to Kato and Yosida (15), with slight modifications. At least 30 metaphases from each cell population were karyotyped.

RESULTS

Presence of SV40-Related T Antigen in Meningioma Cell Cultures. A positive fluorescence reaction characteristic for SV40 T antigen was found in cells of two out of seven tumors

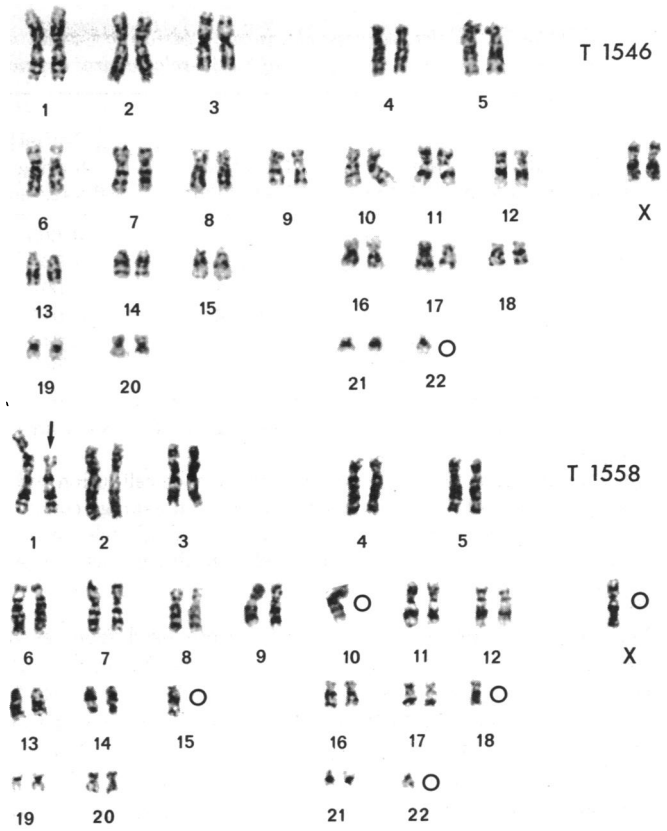

FIG. 2. (*Top*) Stemline karyotype of T 1546. (*Bottom*) Stemline karyotype of T 1558.

examined (Table 2; Fig. 1a). It corresponded to that found in SV40-transformed 3T3 cells.

The percentage of T-fluorescent nuclei varied with the culture passage. Monolayers from tumor T 1647 had 90% fluorescent nuclei in the first passage, whereas in the fourth passage, just before the cell fusion, only 40% of the nuclei were T-fluorescence-positive. For tumor T 1558, the percentage of positive nuclei decreased from 30% in the fifth to 20% in the tenth passage, when the assay was repeated prior to cell fusion.

One tumor, T 1546, tested in the fifth passage, presented a perinuclear fluorescence similar to the SV40 U-fluorescence (16) in about 10% of the cells (Table 2). None of the control cells (primary cultures of human adult fibroblasts and of two human glioblastomas; established cell lines MA 134, CV 1, and HeLa) gave a positive reaction in the antibody assays. Anti-T serum diluted 1:1 with phosphate-buffered saline was used in the experiments. The generation time was significantly reduced in the T-fluorescence-positive tumors in contrast to the others showing a negative reaction. None of the cell cultures showed V-fluorescence (Fig. 1b), and infectious virus was not found by cultivation along with MA 134 cells.

Chromosomal Pattern and Histological Findings of the Examined Tumors. Karyotypes and histological structure of the meningiomas examined are shown in Table 2. The arachnothelial meningioma T 1546 (U-fluorescence) had lost only chromosome 22. In T 1558 and T 1647 (T-fluorescence), four or five further chromosomes were missing (Fig. 2).

Ratio of Heterokaryon Formation in Fusion Experiments. The cell fusion experiments were carried out to induce virus.

TABLE 3. *Heterokaryons obtained by fusion of human meningioma cells with AGMK cell line MA 134 and appearance of SV40-related viral capsid antigen in heterokaryon cultures*

Fused parental cells* Meningioma × AGMK line	No. of cell fusion exp.	% Nuclei† showing V-fluorescence	Average % nuclei in polykaryons‡		Average nuclei count per heterokaryon
			Homokaryons§	Heterokaryons¶	
	CF 1	0.008			
T 1558 × MA 134	CF 2	0.006	45.2	24.0	3.0
	CF 3	0.007			
	CF 4	0.015			
T 1647 × MA 134	CF 5	0.048	46.0	20.4	2.5
	CF 6	0.007			

* Inoculum size: 10^7 cells of each cell type in 4 × 8-cm flasks. Each cell type to be fused with MA 134 was pretreated with 8-azaguanine.

† A minimum of 10^6 cells were examined in each cell fusion culture 7 days after fusion.

‡ Counted on 12 × 32-mm coverslips with an average count of 2.4×10^5 cells.

§ Homokaryon: a multinucleated cell containing nuclei of only one parental cell type.

¶ Heterokaryon: a multinucleated cell containing nuclei of both parental cell types.

For these experiments 1×10^7 cells were used from each parental line. Table 3 indicates that with the fusion method used, 24.0% of the nuclei of cell fusion cultures 1 to 3 (T 1558 × MA 134) and 20.4% of the nuclei of cell fusion 4 to 6 (T 1647 × MA 134) were found in heterokaryons. The average count of 2.75 nuclei per heterokaryon is rather favorable in view of the fact that heterokaryons with a large number of nuclei are reported to show a significantly reduced virus production (12).

SV40-Related Viral Capsid Antigen in Heterokaryon Cultures. In the SV40 viral capsid antigen assay 7 days after fusion, the heterokaryon cultures showed a positive reaction (Fig. 1d and e). The percentage of fluorescent nuclei on the stained coverslips was determined for each heterokaryon culture (cell fusions 1 to 6) (Table 3). One coverslip contained an average number of 2.5×10^5 cell nuclei. The cells tended to die on the ninth day after fusion.

A cocultivation without fusion of cells of the T-fluorescence-positive tumor T 1647 with MA 134 gave no positive V-fluorescence after 7 and 14 days in spite of the pretreatment of the tumor cells with 8-azaguanine. The cells did not die and appeared normal 14 days after cocultivation. Heterokaryon cultures of T 1720 (T-fluorescence-negative) with MA 134, of human fibroblasts (primary cultures) with MA 134, and of human fetal brain cells with MA 134, showed no positive V-fluorescence reaction 7, 14, and 18 days after fusion.

DISCUSSION

Two out of seven meningiomas tested in cell culture by indirect immunofluorescence staining showed SV40-related T antigen in a large proportion of cells. SV40-related U antigen was found in 10% of the cells of a third tumor. These findings indicate that the meningioma cells showing a positive reaction are transformed by a papova virus that has at least partly the same antigenic properties as SV40.

An accidental infection or transformation of these cultures *in vitro* can be ruled out for the following reasons: (*i*) Ninety percent of cells of the primary cell culture of the meningioma T 1558 were positive for T antigen. (*ii*) V antigen was absent in this tumor as well as in all the other tumors tested. (*iii*) No virus infectious for AGMK cells could be isolated. (*iv*) No work with SV40 or polio vaccine has ever been done in this laboratory.

From cells experimentally transformed by SV40 it is known that T antigen, an early function, is expressed but no V antigen and no virus particles are produced. This indicates a stable integration of the viral DNA in the host genome. However, virus can often be rescued from these transformed cells by cocultivation or fusion of the transformed cells with permissive cells (17).

Experiments to rescue virus from T-antigen-positive cells were performed. Fusion of cells of the tumors T 1558 and T 1647 (pretreated with 8-azaguanine) with MA 134 cells led to a low percentage (0.01–0.05%) of V-antigen-positive nuclei. Late viral genes are therefore expressed, and from this one can conclude that viral DNA does replicate in the heterokaryons. In recent years SV40-related papova viruses have been isolated from humans. Three viruses belonging to this group were found in brain autopsy material from different patients with progressive multifocal leucoencephalopathy, a rare demyelinating disease in man. These were the DAR and EK viruses (18–20) and the JC virus (21), the former two of which were indistinguishable from SV40 in fluorescence antibody assays. Another papova virus, BK virus (22), antigenically also related to SV40, could be isolated from the urine of a patient after a kidney transplantation. Screening for antibodies against BK virus in stored human sera revealed that such antibodies were already present in humans before the use of contaminated polio vaccines. It is not clear at the present time whether the virus responsible for the T and V antigens shown in the present investigation is identical to one of the already described SV40-related viruses.

Of the tumors that were examined and found to contain SV40-related antigens, T 1546 (SV40-related U antigen) was shown to have a typical loss of only a chromosome 22, while T 1558 and T 1647 (SV40-related T antigen) exhibited a further hypodiploidization also characteristic for meningiomas. It cannot be definitely excluded that human cells *in vivo*, after having lost a chromosome 22, may be more easily transformed by a virus. However, this chromosome loss could also be explained as a secondary phenomenon typical for cells transformed by this virus.

We were able to show that in two out of seven human meningiomas with definite chromosome aberrations, SV40-related T antigen could be demonstrated and that after fusion of these cells with SV40-permissive AGMK cells, SV40-related

V antigen was induced. On the basis of these results the possibility arises that a correlation may exist between the meningioma, a relatively common tumor in man, and the presence of an SV40-related papova virus.

Note Added in Proof. In the meantime (November, 1974) we succeeded in detecting by electron microscopy of cell fusion cultures of MA 134 cells with cells of T 1546, T 1558, and a new T antigen positive meningioma (T 1709) not described in this paper, virus particles corresponding in size and structure to papova viruses. This study was performed in cooperation with Drs. G. Birkmayer and F. Miller, Institut für Zellbiologie der Universität München.

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