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DEMONSTRATION OF SV40-RELATED TUMOUR ANTIGEN IN HUMAN MENINGIOMAS BY DIFFERENT HAMSTER SV40-T-ANTISERA

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Summary. — Three of 5 human meningiomas tested in cell culture expressed SV40-related tumour (T) antigen as measured by indirect immunofluorescence with four different hamster anti-SV40 tumour sera. The per cent of T-antigen-positive nuclei varied depending on the antisera used. In one case (meningioma G. S.), only 25 % of the T-antigen-positive nuclei detected with one antiserum could be demonstrated with another serum. The possible explanations are discussed. None of the meningioma cell cultures tested expresed SV40-related viral capsid (V) antigen.

Key words: Papovavirus; SV40; meningioma; immunofluorescence

Introduction

In the past few years several papovaviruses have been isolated from humans (Padgett and Walker, 1976). They can be divided into three classes and resemble more (SV40-PML) or less (JC virus, BK virus) the papovavirus SV40. The viruses induce intranuclear T antigen which cross-reacts immunologically with SV40 T antigen (Dougherty, 1976).

Although the oncogenic potential of these viruses in animals has been demonstrated there is no real evidence that they are associated with any known disease. However, several reports have been published on the possible role of papovaviruses in human tumours (Soriano *et al.*, 1974; Smith *et al.*, 1969; Takemoto *et al.*, 1974; Scherneck *et al.*, 1979*b*; Geissler *et al.*, 1980). Weiss *et al.* (1975) detected SV40-related T or U antigen in the nuclei of in vitro cultured human meningioma cells by an indirect immunofluorescence test. This observation was confirmed by May *et al.* (1978) and at our institute (Scherneck *et al.*, 1979*a*; Geissler *et al.*, 1980). On the other hand some authors failed to find expression of SV40-related T antigen in many different human brain tumours (Becker *et al.*, 1976; Merletti *et al.*, 1976; Cikes *et al.*, 1977; Mason and Takemoto, 1978 as quoted by Takemoto, 1978; Israel *et al.*, Greenlee *et al.*, 1978). The cause of these discrepancies remains obscure. They might be due to either factors which influence the variation in the

appearance of SV40 T antigen in transformed cells, or the methods used for detecting T antigen in cells and serological reagents, or statistical and epidemiological factors (Merletti *et al.*, 1976; Cikes *et al.*, 1977; Robb, 1977; Tabuchi *et al.*, 1978).

Some years ago we started a screening programme aimed at detecting by immunofluorescence SV40-like antigens in human meningiomas. SV40-related T antigen was found in 12 out of 37 meningiomas tested in cell culture.

In the present study we report on the screening by immunofluorescence of 5 human meningiomas for the presence of SV40 T antigen with 4 different anti-SV40 tumour sera. Three meningiomas revealed a positive immunofluorescence reaction for T antigen in fresh cultured tissues. But the appearance of the SV40 T antigen varied depending on the antiserum used.

Materials and Methods

Tumours and cell cultures. Primary meningiomas were obtained from 5 patients at the Neurosurgical clinic of the Municipal Hospital Berlin-Buch. Some details of the patients and histological characteristics of the tumours are given in Table 1. Primary tumour cell cultures were prepared as described (Scherneck *et al.*, 1979*a*). Briefly, freshly obtained tumour biopsy material was comminuted with scissors, and small pieces of the material were seeded into 50-ml glass bottles containing about 1 ml of Eagle's minimal essential medium (MEM; Staatliches Institut für Immunpräparate und Nährmedien, Berlin GDR) supplemented with 10 % foetal bovine serum (Flow Laboratories). Two days later 10 ml of medium was added. All primary cell cultures were tested for presence of SV40-related antigen in the first passage.

Immunofluorescence. Cell cultures grown on coverslips were washed with phosphate-buffered saline (PBS), air-dried, fixed in ice-cold acetone for 10 min. and finally washed in PBS before indirect immunofluorescence staining. The cells were incubated at 37 °C for 1 hr with SV40 T-antiserum and, after washing, incubated for another 45 min with fluorescein-isothiocyanate-(FITC-) conjugated antiglobulin prepared against the antisera. The control cells used as a reference for T-antigen testing were primary cultures of nontumorogenic tissues (galea) of the same patients. Cell cultures were tested for absence of mycoplasma by cultivation on PPLO-agar (Difco) and electron microscopic examination. At least 3 indirect immunofluorescence tests on each meningioma cell culture were done.

Hamster SV40 tumour antisera. Four different hamster anti-SV40 tumour sera were used. Serum No. 1 originated from our laboratory and was prepared as described by Scherneck *et al.* (1979*a*). Sera No. 2 and No. 3 were provided by Dr. H. Fischer, German Cancer Research Center, Heidelberg, F. R. G., and serum No. 4 was prepared by Dr. M. Cikes, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland. All sera were prepared from tumour-carrying hamsters. The tumours had been induced by transplantation of cultured SV40-transformed hamster cells. All sera were used at a dilution of 1 : 5.

Table 1. Histological characteristics of the screened meningiomas

Meningioma	Patient		Histological characteristics
	Age	Sex	
G. S.	65	F	Endotheliomatous meningioma
R. G.	37	F	Endotheliomatous meningioma, partially fibromatous
E. H.	19	F	Endotheliomatous meningioma, partially fibromatous
R. V.	62	M	Endotheliomatous meningioma
G. M.	56	M	Endotheliomatous meningioma

FITC-conjugated rabbit antiserum against hamster serum was obtained from the Institute of Sera and Vaccines, Prague (dilution 1 : 3) and GIBCO (dilution 1 : 4). In all experiments, sera taken from hamsters injected with 0.5 ml PBS were used as a control. To test for SV40-related viral capsid antigen, rabbit antiserum against SV40 virions and FITC- conjugated goat antiserum prepared against rabbit serum (obtained from Institut Pasteur, Paris) were used. The rabbit immune serum against SV40 virions was diluted 1 : 4, the antiserum against rabbit serum 1 : 10 (all in PBS). All sera were tested with CV-I monkey cells lytically infected with SV40 and SV40-transformed and -untransformed 3T3 cells.

Results

Detection of SV40-related T antigen in cell cultures of human meningiomas

During a screening programme for detection of SV40-related T antigen in human brain tumours, 5 human meningioma cell cultures were examined for the presence of SV40 antigens. Three tumours revealed a positive SV40 T-antigen reaction by indirect immunofluorescence, although SV40-related capsid (V) antigen was not detected in any of the 5 tumours. These results confirmed former observations in our laboratory (Scherneck *et al.*, 1979*a*) and correspond with the data obtained by other groups (Weiss *et al.*, 1975; Zang *et al.*, 1979). The characteristics of the meningiomas tested for the presence of SV40 antigens are shown in Table 1. The limited number of tumours tested in this study did not permit any conclusion that any particular type of meningioma or group of patients is preferentially accompanied by SV40-related T antigen. But tests on more than 50 meningiomas revealed no preference in expression of SV40-related T antigen (Scherneck *et al.*, 1979*a*; and unpublished results).

Use of different anti-SV40 tumour sera for screening SV40-related T antigen in cell cultures of human meningiomas

The results obtained with four different hamster SV40 T-antisera are presented in Table 2. The first remarkable finding was that a tumour cell culture recognized as T-antigen-positive with one serum was also positive with the other sera. Similarly, the T-antigen-negative cell cultures were negative with all four sera used. In contrast, Zang *et al.* (1979) found that the T-antigen negative or positive reaction depended on a specific antiserum in some meningiomas. Otherwise, as shown in Table 2, the percentage of T-antigen-positive nuclei varied with the different antisera used. For example, cell cultures, derived from meningioma G. S., showed 80 %, 40 %, 50 % and 20 % T-

Table 2. Per cent of T-antigen-positive cells demonstrated by different hamster anti-SV40 tumour sera

Meningioma	% of T-antigen-positive cells with serum				
	No. 1	No. 2	No. 3	No. 4	control
G. S.	80	40	50	20	0
R. G.	0	0	0	0	0
E. H.	0	0	0	0	0
R. V.	70	30	30	20	0
G. M.	50	20	20	20	0

antigen-positive nuclei with T-antisera 1—4 respectively (Figs 1—4). In another case (meningioma G. M.), 50 % T-antigen-positive nuclei were detected with serum No. 1 but only 20 % positive nuclei with the other 3 sera. These data and the results obtained with meningioma R. V. suggest a high T-antigen resolution power of serum No. 1 for all the meningiomas tested. In general, the fluorescent pattern of the T antigen was granular and filled the whole nucleus. In some case with sera Nos 2—4, the fluorescent nuclei were rather pale.

Discussion

Meningiomas belong to the most frequent intracranial tumours in man and seem to be a good subject for examining a relative large number of tumours for the presence of virus or virus antigen. In extending previous data (Scherneck *et al.*, 1979*a*) we were able to show that, in 3 out of 5 meningiomas tested in cell culture, SV40-related T antigen could be demonstrated by indirect immunofluorescence. Contamination of the cell cultures with SV40 or another human papovavirus can be excluded with a high degree of probability because (*a*) 80 % of the meningioma cell culture G. S., known to be semipermissive for SV40, were positive for T antigen and such a high percentage of T-antigen-positive cells obtained after only one passage would never be achieved by an accidental laboratory infection; and (*b*) V antigen was absent in all the tumour cells tested. In general human cells are semipermissive for SV40 replication and one would expect a certain number of V-antigen-positive cells after a laboratory infection with SV40. Our results agree with those of others (Weiss *et al.*, 1975; Zang *et al.*, 1979) and indicate at least the expression of early functions of a SV40-like papovavirus.

Neither SV40-related capsid antigen nor infectious virus was detected in any of the present meningioma cell cultures. But some other authors failed to detect SV40-related T antigen in human meningiomas by indicrect immunofluorescence staining, direct and indirect enzyme labelling and testing sera from selected cancer patients for T antibody (Merletti *et al.*, 1976; Cikes *et al.*, 1977; Takemoto, 1978; Greenlee *et al.*, 1978; Israel *et al.*, 1978).

The causes of these discrepancies remain obscure. Many factors may influence the experiments done in different laboratories. The present data and those reported by Zang *et al.* (1979) clearly show that the quality and specificity of an anti-T-serum used for T antigen screening is a important factor in detecting the antigen. E. g., only 25 % of the fluorescent positive nuclei of meningioma G. S., detected with serum No. 1, could be demonstrated with serum No. 4. These findings, and the negative results mentioned above, might result from the fact that the regulation of synthesis and appearance of SV40 T antigen in transformed cells are poorly understood (Weil, 1978), and that there are variations in the appearance of T antigen in transformed (mouse 3T3) cells (Robb, 1977). These variations depend on the growth state of the cells, the temperature of incubation and the type of transforming virus. Futhermore, the number of T-antigen-positive cells seems to decrease

at high passage levels of meningioma cell cultures (Scherneck *et al.*, 1979*a*; Zang *et al.*, 1979; Tabuchi *et al.*, 1978) and no ready explanation has been found for this observation.

Obviously, a better understanding of these processes, including statistical and epidemiological factors, may help to explain the different results obtained in detecting SV40-related T antigen in human meningiomas and open the way for studying the possible role of papovavirus in human brain tumours.

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Explanation of Micrographs (*Plate XXXV*):

Figs 1–4. Demonstration of SV40-related T antigen in cell cultures of human meningioma G. S. by different hamster SV40 T-antisera.

1 — serum No. 1
2 — serum No. 2
3 — serum No. 3
4 — control serum.