SV40-Related T-Antigen Expression in Human Meningiomas with Normal and G-22-Monosomic Karyotype

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SUMMARY

Six of 16 meningiomas tested in early subcultures by indirect immunofluorescence showed SV40 T-antigen. Two different antisera specific for T-antigen were used. One serum gave a positive reaction with six tumours and the other with only two. In one T-antigen positive meningioma, the typical nuclear fluorescence changed, beginning with the second subculture, into an unusual brilliant granular pattern irregularly distributed over the nuclei. In six meningiomas, a specific chromosome aberration (monosomy G 22) was established. However, up to now, no clear correlation between karyotype and T-antigen expression could be found: cells from three meningiomas with positive reactions had normal karyotypes, whereas those from three tumours with typical chromosome loss showed no T-antigen.

A number of observations have been reported regarding the association of papovaviruses with human tumours. In one case, SV40 T-antigen was observed in human melanoma cells (Soriano et al. 1974). BK-virus information was demonstrated in different human tumours (Fiori & Di Mayorca, 1976). Our group presented data on SV40-related T-antigen in meningiomas (Weiss et al. 1975), the most common intracranial tumour of man. Our findings were recently confirmed by S. Scherneck et al. (personal communication), who found the antigen in several meningiomas of a relatively large series of tumours tested. Still another group (Tabuchi et al. 1978), screening a series of intracranial tumours, found a negative T-antigen reaction with meningiomas but positive reactions with an ependymoma and a plexus papilloma, both tumours deriving from intracranial tissues which, like meninges, have a large surface exposed to the cerebrospinal fluid. Two other laboratories failed to confirm a T-antigen reaction in meningioma cell cultures (Corallini et al. 1976; Cikes et al. 1977). In order to clarify these discrepancies, we have screened a further series of meningiomas under our laboratory conditions but with anti-T sera of different origins and with cells exchanged between different laboratories (Padgett & Walker, 1976). In this paper, we report the results of testing of meningioma cell cultures, by means of the indirect immunofluorescence technique, for the presence of nuclear fluorescence specific to SV40 T-antigen.

The biopsy material from 16 meningioma patients (10 females and 6 males) was prepared immediately after the removal of the tumours. The biopsy specimens were mechanically minced and trypsinized. The cells were seeded in plastic culture bottles with coverslips in a cell concentration of 2 x 10^5 cells/ml in Dulbecco's modified Eagle's medium with 10% foetal calf serum and antibiotics.

Chromosomes were studied for every culture, beginning with the primary culture or the first subculture. The monolayers were examined for the presence of SV40-related T- and V-antigens by the indirect fluorescence technique (for details of the technique, see Weiss et al. 1975). Two hamster anti-T sera of different origins, both prepared in the usual way, were used in parallel (serum L, batch HaH 50 VI, Schweizer Krebsforschungszentrum,
Lausanne; serum H, batches A 10/11, A 10/9, A 10/12, A 10/17, Institut für Virusforschung am Deutschen Krebsforschungszentrum, Heidelberg). Each batch of anti-T serum was checked for its ability to cause the appearance of characteristic and specific fluorescence in transformed and lytically infected cell systems (SV3T3/3T3; SV80; CVI/infected CVI).

FITC-labelled rabbit anti-hamster serum (Miles) was used to test for the presence of T-antigen. The V-fluorescence was tested with rabbit anti-SV40-serum (Gibco); FITC-labelled goat anti-rabbit serum served as the conjugate. In every test, one part of the coverslip was incubated with anti-T serum and the other part with normal hamster serum used as a negative control.

The karyotypes were normal in seven of the 16 meningiomas. Chromosomal aberrations were found in six cases, each of which showed the loss of one chromosome, G 22; in three of these cases, there were additional numerical and/or structural aberrations. In three cultures, the proliferation was too poor to permit exact karyotyping.

Anti-T serum L gave a clear positive reaction in four tumours and a weak positive reaction in two other tumours. Only two meningiomas showed a corresponding positive reaction with anti-T serum H (T2038 and T2057). Both tumours and T2059, which was not studied with antiseraum H in the first subculture, were subcultured over a longer period. In all three cases the percentage of T-positive nuclei decreased during the first subcultures considerably in T2057 and T2059 and slightly in T2038, when studied with anti-serum L. With anti-serum H, T2038 gave positive reactions in only the third and seventh subcultures, with a different percentage of cells reacting each time; T2057 gave its only positive reaction, a weak one, in the eighth subculture.

In T2038, the percentage of T-positive nuclei varied between subcultures. We found between 5 and 90 % of the cells positive with serum L and 0 and 80 % with serum H (most subcultures tested with serum H gave a negative reaction). Beginning with the second subculture the pattern of the T-fluorescence differed clearly from that usually observed in SV40-transformed cells. The atypical reaction consisted of small brilliant granula irregularly distributed over the relatively pale nuclei (Fig. 1 c, d).

As demonstrated earlier (Weiss et al. 1975), fibroblasts from the connective tissue of the T-positive meningioma cultures were always T-antigen negative, as were fibroblast cultures from skin biopsies of the same patients. This phenomenon may be regarded as an intra-individual control demonstrating that virus information seemed to be present only in the tumour cells.

All cell cultures from the SV40 T-antigen-positive meningiomas were negative for SV40-related V-antigen.

We found papovavirus antigens in six of 16 human meningiomas cultured in vitro and studied by indirect immunofluorescence staining. A contamination of the meningiomas studied is unlikely as virological work had never been done before in the laboratories of the Institute of Human Genetics in Homburg. The T reaction differed quantitatively, depending on the tumour and the antiseraum H or L, from the fluorescence of simultaneously studied transformed mice cells (DBA-B). With one exception (T2038), the fluorescence was most intense in the primary culture or the first subculture and disappeared completely by the tenth (T2057) or fourteenth (T2038) subculture.

Similar fluctuations in the expression of T-antigen have been observed in another meningioma study (S. Scherneck, personal communication). In animal cells experimentally transformed by SV40 and an SV40 mutant, the T-antigen expression was reported to depend not only on the type of transformed cells and the incubation temperature, but also on the type of transforming virus and the growth state of the cell culture (Robb, 1977).

An unexpected and, to our knowledge, hitherto unreported observation in papovavirus-
Fig. 1. (a, b) SV40-T-antigen-positive cells from meningiomas T2038 and T2057, both in the 3rd subculture. (c, d) Cells with the atypical granular fluorescence pattern, from the 3rd subculture of T2038.

transformed cells was the change in the patterns of fluorescence distribution over the T-antigen-positive nuclei in higher subcultures of T2038. In these nuclei there were many brilliant granula irregularly distributed over the relatively dark nuclei. The nuclear fluorescence between the granula was weaker than the typical T-fluorescence of the same tumour (Fig. 1). The granular pattern of fluorescence was always limited to circumscribed regions within the monolayers.

The fluctuation of the percentage of positive nuclei as well as the change in the pattern of the T fluorescence may be due to the different expression of the virus genome or different cellular conditions or both.

The second new finding was that the negative or positive T-antigen reaction may not depend on a specific karyotype, unlike the findings in our first series of meningiomas (Weiss...
et al. 1975). Three tumours (T2040, T2057, T2059) showed positive T-reactions even though no chromosomal aberrations were detected. Moreover, in one meningioma with very typical and definite numerical and structural aberrations in the first subcultures (T2038), the positive T-reaction persisted in higher subcultures, in which only a stemline with a normal karyotype survived. As already discussed, we can rule out the possibility that the normal karyotype was the result of an overgrowth of the meningioma cells by (normal) fibroblasts, as under our conditions the fibroblasts of the tumour patients were always T-antigen-negative. On the other hand, three meningiomas with a typical monosomy G 22 (T2028, T2068, T2070) gave no positive T-fluorescence reaction.

The data presented here give further evidence for the presence of papovavirus information in some human meningiomas. Pilot experiments using blot hybridization also indicate that virus information is present.

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