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Abstract

In order to study the viral etiology of human brain tumors, attempts were made to isolate cytopathogenic agents from human brain tumors by the tissue culture of tumor tissues and by the mixed culture of tumor tissues with HeLa cells. Five glioblastomas, a mixed form of glioblastoma and fibrosarcoma, two astrocytomas, two ependymomas, two meningiomas, an oligodendroglioma, a spongioblastoma polare and a choroid plexus papilloma were studied. In the tissue culture, besides the cells which appeared to be the tumor parenchymal cells, varying amounts of fibroblastic cells appeared in all the tumors tested and they increased with the prolongation of the culture period. In any of the tumors tested, no cytopathogenic agents were detected by either the culture of tumor tissues or the mixed culture of tumor tissues with HeLa cells. From the virological point of view, the significance of these negative results was discussed.

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ATTEMPTS TO ISOLATE VIRUS FROM HUMAN BRAIN TUMORS

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Abstract: In order to study the viral etiology of human brain tumors, attempts were made to isolate cytopathogenic agents from human brain tumors by the tissue culture of tumor tissues and by the mixed culture of tumor tissues with HeLa cells. Five glioblastomas, a mixed form of glioblastoma and fibrosarcoma, two astrocytomas, two ependymomas, two meningiomas, an oligodendroglioma, a spongioblastoma polare and a choroid plexus papilloma were studied. In the tissue culture, besides the cells which appeared to be the tumor parenchymal cells, varying amounts of fibroblastic cells appeared in all the tumors tested and they increased with the prolongation of the culture period. In any of the tumors tested, no cytopathogenic agents were detected by either the culture of tumor tissues or the mixed culture of tumor tissues with HeLa cells. From the virological point of view, the significance of these negative results was discussed.

The brain has a high sensitivity to many viruses (1). When inoculated intracranially into animals, as reviewed by IKUTA and KUMANISHI (2), many tumor viruses induce brain tumors. As for human brain tumors, there are several reports on the electron microscopic observation of virus-like structures, though some of them are atypical for virus particles, in tumor tissues or in tissue culture cells of tumors (3–10).

It is known, on the other hand, that several types of human adenovirus induce tumors in laboratory animals (11, 12). Of the various opinions on the origin of the tumors induced by human adenoviruses, the neuroectodermal origin has been proposed by OGAWA *et al.* (13, 14). The relationship between human adenoviruses and human tumors has been studied by the attempts to isolate adenoviruses from human tumors or cancer patients (15–17), to find T-antigen in human tumors and T-antibody in cancer patient sera (18–23) and to find adenovirus-specific mRNA in human tumors (24, 25). No definite results have been so far obtained, but MALMGREN *et al.* reported that brain tumors were one of the tumor types which seemed most often to be associated with adenovirus-T-antibody-positive sera (21). These results suggest that at least some of human brain tumors might be of adenovirus origin. It thus appeared significant to attempt the isolation of viruses, particularly adeno-

viruses, from human brain tumors. In the present study, the author tried to isolate viruses from human brain tumors by the tissue culture of tumors and by the mixed culture of tumor tissues with HeLa cells.

MATERIALS AND METHODS

Brain tumors: A part of human brain tumors removed surgically was immediately put in an ice box and transferred to our laboratory.

Tissue culture media: Earle's balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate (YLE) was prepared in our laboratory, and medium 199 was obtained commercially. Just before use, these media were supplemented with heat-inactivated bovine serum of varying concentrations.

HeLa cells: Wild type HeLa cells maintained in our laboratory were used. HeLa cells were cultured in YLE with 10% bovine serum.

Culture of tumor tissues: Tumor tissues were minced with sharp scissors, trypsinized and suspended in YLE or medium 199 supplemented with 50% bovine serum. The suspension was poured at 1 ml/tube into twenty test tubes, of which four contained coverslips for cell staining, stoppered and placed in an incubator of 37°C.

Mixed culture with HeLa cells: After 3-4 weeks' incubation, tumor tissue culture tubes were divided into two groups. The first group was maintained as before without any treatment except for decreasing the serum content of the medium to 10%—tumor group. In the second group, 10⁴ HeLa cells per tube were planted onto the tumor cell cultures and maintained in the same way as the first group—tumor-HeLa-mixed group. As the control to the second group, five test tubes were planted with 10⁴ HeLa cells per tube—HeLa group. All these groups were further maintained for 35-37 days and then stored at -70°C.

Tissue culture passage: Cells of the tumor and the tumor-HeLa-mixed groups stored for 1-7 days at -70°C were thawed, mixed and inoculated into three HeLa cell tubes (1 ml/tube). On the next day, the medium was changed with YLE containing 2% bovine serum, and maintained for 14-17 days.

Medium change and observation: Throughout all these tissue culture processes, medium change and light microscopic observation were done at least twice a week. Every one or two weeks, cells cultured on coverslips were fixed in formalin, stained in hematoxylin and eosin, and observed for the cytopathogenic effect.

RESULTS

As shown in Table 1, five glioblastomas, a mixed form of glioblastoma and fibrosarcoma, two astrocytomas, two ependymomas, two meningiomas, an oligodendroglioma, a spongioblastoma polare and a choroid plexus papilloma were studied. Varying degrees of cell growth were observed in all

TABLE 1 ATTEMPTS TO ISOLATE VIRUS FROM HUMAN BRAIN TUMORS

Patient Age Sex	Tumor	Cell Growth ¹⁾	Viral cytopathogenic effect in		
			Tumor tissue culture	Mixed culture of tumor and HeLa cells	Passage onto HeLa cells ²⁾
30 ♂	Glioblastoma	+++	—	—	—
40 ♂	Glioblastoma	+++	—	—	—
42 ♀	Glioblastoma	+++	—	—	—
48 ♀	Glioblastoma	+++	—	—	—
60 ♂	Glioblastoma	+++	—	—	—
59 ♂	Mixed form of glioblastoma and fibrosarcoma	+++	—	—	—
4 ♂	Astrocytoma	++	—	—	—
33 ♀	Astrocytoma	++	—	—	—
28 ♂	Ependymoma	+	—	—	—
29 ♂	Ependymoma	++	—	—	—
48 ♂	Meningioma	++	—	—	—
49 ♀	Meningioma	++	—	—	—
62 ♂	Oligodendroglioma	++	—	—	—
22 ♀	Spongioblastoma polare	+	—	—	—
69 ♂	Choroid plexus papilloma	++	—	—	—

1) Cell growth in tumor cell cultures at the end of 56-65 days' maintenance. + Cells occupy 20-30% of the culture surface, ++ 40-50%, +++ over 50%.

2) Frozen-thawed suspensions of the tumor tissue culture and the mixed culture of tumor and HeLa cells maintained for 56-65 days were inoculated onto HeLa cells, and observed for the cytopathogenic effect for additional 14-17 days.

these tumors, but it appeared better in glioblastomas than in others. Besides the cells which appeared to be the tumor parenchymal cells, varying amounts of fibroblastic cells appeared in all the tumors cultured. With the prolongation of the period of *in vitro* culture, fibroblastic cells increased, and, around the end of the culture period of 56-65 days, they constituted the majority. In the mixed cultures of tumor tissues and HeLa cells, HeLa cells overgrew tumor cells in the later stage of culture. During the whole period of 56-65 days of the primary culture, cells were observed for the cytopathogenic effect. In all tumors tested, as shown in Table 1, the cytopathogenic effect was not observed in any of the cultures of tumor tissues or the mixed cultures of tumor tissues with HeLa cells.

The cell cultures of these tumors were frozen-thawed, further inoculated into HeLa cells and maintained for 14-17 days, but no cytopathogenic effect was observed (Table 1).

DISCUSSION

Isolation of virus from tumor cells does not necessarily mean that the virus is the causative agent of the tumor. From the virological point of view, however, animal tumors may be divided into four groups: 1) tumors which usually yield the causative virus, *e. g.* tumors induced by many RNA tumor viruses and papilloma or pox viruses; 2) tumors which usually do not yield the causative virus but yield the virus under such appropriate conditions as in the tissue culture of tumor cells, in the mixed culture of tumor cells with virus-sensitive indicator cells or in a similar mixed culture in which cells are fused by cell fusion technique, *e. g.* hamster tumors induced by SV40 (26-28); 3) tumors which are evidently viral but do not yield the causative virus under the conditions so far tested, *e. g.* hamster tumors induced by polyoma virus or human adenoviruses (26, 29); and 4) tumors in which no causative relations with viruses have been detected by the methods available at present.

In the present study, only the culture of tumor tissues and the mixed culture of tumor tissues with HeLa cells were done, and the cytopathogenic effect was taken as the only indication for the presence of virus. Hence the significance of the negative results in the present study is very limited, and more informations will be obtained by further studies by such methods as the use of human embryonic brain cells for isolation of viruses, the cell fusion technique to enhance the production of viruses, inoculation of tumor extracts or tissue culture fluids into the brain of animals, particularly into that of non-human primates, and the electron microscopy of the cultured tumor cells.

It is known, however, that the tissue culture of adenoids or tonsils frequently discloses the latent adenoviruses, showing the specific cytopathogenic effect in the cultured cells (30). It is also known well that HeLa cells are sensitive to adenoviruses and show the specific cytopathogenic effect at their infection (30). Therefore, the negative results in the present study would imply at least that these fifteen human brain tumors tested did not harbor adenoviral gene rescuable by such conventional methods as employed in the present study. McALLISTER *et al.* reported the isolation of adenovirus type 1 from an astrocytoma by similar methods (17). Their experiments to determine the significance of the isolated virus, however, did not indicate whether the virus had the etiological significance or whether it was a laboratory contaminant or a passenger virus in the tissue. SABIN also briefly described negative results in a similar attempt to isolate viruses from neuroblastomas (31). Our repeated attempts to transform human embryonic brain cells *in vitro* by human adenovirus type 12 were unsuccessful (32).

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