Cultivation of Medulloblastoma Cells Derived from Simian Adenovirus SA7-induced Hamster Brain Tumor

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SUMMARY

In vitro cultivation of medulloblastoma cells was successfully established from hamster brain tumors induced by simian adenovirus SA7. These tumor cells possess morphological features in cultures that are very similar to those of the original tumor. Both tubular and rosette growth patterns were evident. At the ultrastructural level only small numbers of cytoplasmic organelles could be detected in the tumor cells, typical of generally immature and/or undifferentiated cells. The proliferation of these tumor cells depended upon properly dense initial platings. In addition, it was found that single medulloblastoma cells could be stimulated to produce colonies if treated for 10 days with conditioned medium. Inoculation of these cells into syngeneic animals resulted in 100% uptake. Survival of the tumor cells, typical of generally immature and/or undifferentiated cells. The proliferation of these tumor cells was directly correlated with the number of cells inoculated. This new medulloblastoma cell line may prove to be a useful model for experimental brain tumor studies.

INTRODUCTION

A number of oncogenic viruses including polyoma virus, Rous sarcoma virus, and SV40 virus have been reported to induce brain tumors in hamsters (7, 8, 14–16). The simian adenovirus type 7 (SA7) was recently reported to be highly oncogenic in the brains of hamsters (5, 12, 17), the inoculation generally producing medulloblastoma (17). Although SA7-induced brain tumors have been maintained in serial s.c. or intracranial passages, their adaptation to tissue culture conditions have not yet been fully described. This report describes the successful establishment of one such hamster tumor cell line. To a large extent, these tumor cells maintained their characteristic morphology and oncogenicity in vitro.

MATERIALS AND METHODS

Virus. The SA7 was obtained from Dr. Theodore Burnstein of Purdue University. The virus was passed through a stable line of African green monkey kidney cells (BS-C-1 cells) in our laboratory.

Experimental Animals and Induction of Brain Tumors. Newborn Syrian hamsters obtained from Engle’s Laboratory Animals, Inc., Farmersburg, Ind., were given intracerebral injections of 10⁵ 50% tissue culture infective dose of SA7 virus contained in 0.05 ml of Puck’s Saline A. When clinical illness became evident after 3 to 4 weeks, the animals were immediately sacrificed and the tumor tissues removed aseptically for culture. Portions of tumor tissue were also processed for histological examination. Microscopically, most of the samples possessed the characteristic features of medulloblastoma. Tissues were extremely cellular in appearance and composed of small, darkly staining nucleated cells in which very little cytoplasm was recognizable (Fig. I). Mitotic figures were numerous. Although most of the tumor cells were arranged randomly in no particular structure, occasionally there were groups of cells formed around small blood vessels which resembled pseudorosettes.

Cultivation of Tumor Cells. Eagle’s basal medium supplemented with 2 mM glutamine and 10 to 20% fetal calf serum was used as the standard medium. (These materials were all purchased from Grand Island Biological Co., Grand Island, N. Y.) Parts of the removed hamster tissues which appeared to be medulloblastoma were minced into small fragments and incubated with 0.05% trypsin plus EDTA, according to a technique previously described (2). The trypsin-dissociated cells were collected by centrifugation and approximately 2 x 10⁷ cells were suspended in 2 ml Eagle’s basal medium plus 20% fetal calf serum, plated in each 35-mm Falcon plastic dish, and incubated in standard humidified conditions with 5% CO₂ in air and media changes every 2 to 3 days.

Growth Studies of Tumor Cells in Culture. At the time of the 5th subculture, the tumor cells were adjusted to 3 different cell densities, 10⁸, 5 x 10⁷, and 10⁶ cells/ml, and plated in Falcon plastic flasks. After incubation for different periods of time, the cells in each flask were individually trypsinized and counted. 

Cloning Experiments. For cell cloning, dissociated tumor cells were suspended in standard Eagle’s basal medium at a low density (200 cells/ml). Two ml of this cell suspension were then plated in each Falcon dish. After overnight incubation, the medium was replaced with conditioned medium. The conditioned medium was obtained by preincubating standard medium for 1 day with a monolayer

1 Supported by USPHS Research Grant CA-06145 from the National Cancer Institute.
2 Recipient of Research Career Development Award PHS KO4 CA-50315 from the National Cancer Institute.

Received May 2, 1975; accepted August 19, 1975.
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After this 1-day conditioning, the medium was harvested, pooled, and centrifuged at 650 × g for 5 to 10 min to sediment detached cells. When the cultures were changed to this conditioned medium, stimulation of single cells resulted and many colonies developed after 10 days.

Transplantation of Tumor Cells. Tumor cell suspensions were adjusted to 3 different densities. An inoculum of 0.01 ml of each suspension was injected intracerebrally into young Syrian hamsters. All the animals were kept in an air-conditioned room on a standard diet. After each animal died, evidence of tumor growth was confirmed by gross and/or histological examination.

Electron Microscope Studies. The tumor cells were removed from the flask with 0.25% trypsin as soon as a monolayer had formed. The cells were collected in conical centrifuge tubes containing 1% osmium tetroxide acid and kept at 4° for 1.5 hr (11). The cells were then centrifuged and rinsed with neutral formalin. The cell pellets were transferred to small vials for conventional dehydration with graded alcohol and propylene oxide and were then embedded in the epoxy medium of Luft (10). Specimen sections were doubly stained with 3.5% aqueous uranyl acetate and lead citrate (18).

RESULTS

Morphology of Hamster Tumor Cells in Culture. After 24 hr in culture the tumor cells still remained rounded, but many mitotic divisions could be seen. The divided cells stayed packed together without separation of cytoplasm, and the rate of proliferation increased after 2 days of incubation. Typical morphology of a fixed and stained 2- to 3-day-old culture is shown in Fig. 2. Most of the cells were generally small and dense with both round and irregularly shaped nuclei and small amounts of poorly staining cytoplasm. The cultured cells strongly resembled the original tumor cells seen in histological section. Some of the tumor cells had grown in columnar arrangements and others displayed tubular or rosette formations. A few multinucleated giant cells were seen occasionally. After the cultures had formed a monolayer, the cells then developed more cytoplasm. These characteristic features were stable throughout all subsequent subcultures.

Growth Properties of the Tumor Cells. The growth rate of the tumor cells was closely dependent upon the cell number originally plated. The higher the number of cells plated, the better the growth (Chart 1). If the initial density was as low as 10⁴ cells/ml, no net growth at all was seen; but a higher density produced uniformly excellent growth. Under standard conditions, the cloning of individual tumor cells was unsuccessful unless conditioned medium was applied. This conditioned medium stimulated compact, dense colonies within 10 days (Fig. 3). If growth of the tumor clone was allowed to continue, cells began to pile up on top of each other, many soon becoming necrotic. A hole eventually formed in the middle of the colony while peripheral cells continued to divide and spread laterally.

Transplantation of the Tumor Cells. Two experiments were conducted to determine the cell number present and the time of the onset of death of the hamsters after transplantation of the tumor cells had been made. As shown in Table 1, all animals given injections of a total number of 1 to 2 × 10⁴ cells died within 2 to 3 weeks; the median survival time being approximately 15 days. The duration of survival seemed to be proportional to the number of cells originally injected. When the transplanted tumor from the hamsters was later fixed and stained, it was histopathologically proved to be the same type tumor as the primary tumor tissue (Fig. 4). These tumor cells have been preserved at −70° without loss of transplantability.

Electron Micrographs of Cultured Cells. The tumor cells had round to oval or highly lobulated nuclei surrounded by fairly poor cytoplasm (Fig. 5). The cytoplasm contained few mitochondria and freely distributed ribosomes. Occasional cells were seen which contained 5 to 6 nuclei arranged in a...
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Table 1
Survival of hamster with intracerebrally implanted medulloblastoma

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of tumor cells inoculated</th>
<th>No. of animals inoculated</th>
<th>Days of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.5 \times 10^4$</td>
<td>3</td>
<td>25.3 ± 2.0°</td>
</tr>
<tr>
<td></td>
<td>$5.0 \times 10^4$</td>
<td>3</td>
<td>20.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>$1.0 \times 10^4$</td>
<td>3</td>
<td>17.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>$2.0 \times 10^4$</td>
<td>3</td>
<td>14.6 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>$2.5 \times 10^4$</td>
<td>3</td>
<td>25.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>$5.0 \times 10^4$</td>
<td>3</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>$2.0 \times 10^4$</td>
<td>3</td>
<td>15.0 ± 1.2</td>
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</tbody>
</table>

*Mean ± S.E.

The successful propagation of these hamster medulloblastoma cells in culture is significant for several reasons. The biological and biochemical behavior of these tumor cells can now be investigated at the cellular and molecular level. Transplantation experiments gain reliability because inocula of the cultured cell suspensions can be accurately adjusted to uniformity. Relationships between inoculum cell number and survival time of the animal hosts have proved to be very predictable; this fact alone indicates that this medulloblastoma cell line, in addition to other cell lines described (19), can be considered to be an excellent in vivo model for experimental brain tumor studies.

REFERENCES

12. Panzarella, S. P., Rawls, W. E., Melnick, J. C., Pina, M., and Green, an efficient means to clone individual tumor cells and also to conduct quantitative cloning experiments using these cells. Currently, chemotherapy experiments in vitro are underway to determine the effects of several drugs on these cells based on colony survival assay. Although loss of neoplastic properties is considered to be a great threat in the in vitro propagation of tumor cells, periodic animal passage, cloning of tumor cells, and preservation of early subcultures in ultracold conditions are useful steps that have been taken to prevent this loss from occurring.

DISCUSSION

In this paper, we have reported successful in vitro propagation of medulloblastoma cells derived from SA7 virus-induced hamster brain tumors. In culture, these tumor cells possessed the same morphology seen in the original tissue sections and demonstrated characteristic malignant behavior when implanted in animal hosts. Growth behavior, particularly, appeared to be typical of medulloblastoma. Many cells adhered to each other while others were continuously dividing and migrating toward the periphery, resulting occasionally in the development of a ring-like arrangement or rosette pattern of growth (Fig. 3). This same pattern of migration and formation has been described in a human medulloblastoma cell culture (9).

The success of our cultivation of these cells can be attributed to correct judgment of necessary cell density. As clearly shown in the study of growth rate (Chart 1), the cells were heavily dependent upon the cell density originally plated. No growth was obtained when the cells were plated with less than $10^4$ cells/ml. When the cells are crowded enough, the mutual feeding of certain nutrients synthesized by the cells can be achieved effectively and maintain an adequate level of intracellular concentration for cellular growth and division (6). At low population densities under our culture system, these nutrients are probably lost from the cells into the media in amounts that exceed the biosynthetic capacity of the cells. We determined, in contrast to other reports (17), that most importantly the number and survival time of the animal hosts have proved to be very predictable; this fact alone indicates that this medulloblastoma cell line, in addition to other cell lines described (19), can be considered to be an excellent in vivo model for experimental brain tumor studies.
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Fig. 1. Photomicrograph of a histological section of a primary medulloblastoma. Most tumor cells are small, with darkly staining nuclei and little cytoplasm. H & E, × 170.

Fig. 2. Photomicrograph of a 3-day-old culture of medulloblastoma cells in the 10th subculture. Small tumor cells are densely packed and arranged in tubular or rosette formations. Giemsa, × 170.

Fig. 3. Colony formation of single medulloblastoma cells incubated with conditioned medium (left) and standard medium (right).

Fig. 4. Photomicrograph of a histological section of tumor that developed after transplantation of cultured medulloblastoma cells to a hamster. The morphology of these cells is very similar to that of cells in Fig. 1. H & E, × 170.
Fig. 5. Electron micrograph of hamster medulloblastoma cells showing large nuclei with relatively scanty cytoplasm. The cells have round to oval or lobulated nuclei. Lead citrate and uranyl acetate, × 4500.

Fig. 6. Electron micrograph of hamster medulloblastoma cells showing a tubular or pseudorosette formation. Notice the 6 nuclei arranged in a ring structure with no clear cytoplasmic partitions between them. Lead citrate and uranyl acetate, × 4500.
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