Characteristics of human optic gliomas in tissue culture

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Nine human optic gliomas were examined in tissue culture. Typically, growth from the explants revealed well differentiated bipolar cells with abundant 9 to 10 nm fibers similar to those observed in the surgical specimens. Multinucleation was rare except for one culture, which had as many as 20 nuclei arranged in a palisading fashion along the periphery of some of the cells. Degenerative changes of the 9 to 10 nm fiber bundles with the production of amorphous electron-dense deposits were observed both in vivo and in vitro, and were thought to represent the formation of Rosenthal fibers. A distinctive feature of some of the optic gliomas was the ability of their long, thin cellular processes to form fibrous tangles in tissue culture. The correlation of these fibrous tangles in culture with Rosenthal fibers in vivo is still uncertain.

KEY WORDS • optic glioma • astrocytoma • tissue culture • Rosenthal fibers

The proper management of patients with optic gliomas is controversial. Some optic gliomas have been considered potentially aggressive and certain authors have advocated complete excision or radiotherapy. Others have suggested that most of these tumors should be considered as benign hamartomas which, on the whole, are best managed conservatively. A major therapeutic dilemma is therefore presented to the clinician who must treat a patient with this tumor. Unfortunately, a study of standard histopathological sections of these tumors is of little help in resolving this dilemma. Although such sections can be used to make the diagnosis, they cannot differentiate between those optic gliomas that may be potentially aggressive and those which seem to behave in a more benign fashion. To obtain further knowledge of the cellular biological features of these tumors and to aid in the eventual solution of these therapeutic problems, we have studied human optic gliomas in tissue culture. In this report, we describe the morphological and ultrastructural characteristics of nine human optic gliomas grown in tissue culture in the past 9 years.

Materials and Methods

Tissue Culture

Biopsies obtained at operation from patients ranging in age from 7 months to 19 years were divided into three pieces. One piece was fixed with formalin and stained with hematoxylin and eosin or with phosphotungstic acid-hematoxylin (PTAH) for routine pathological examination. Using Kernohan's terminology, we found all these tumors to be Grade I astrocytomas of the anterior optic pathways. The second piece was minced into 1-mm fragments that were allowed to adhere for 30 minutes to the sur-
face of a tissue culture vessel (Falcon plastic flask or a Leighton tube containing a glass cover-slip). The F-10 nutrient medium* with 10% heat-inactivated (at 56°C for 30 min) fetal bovine serum was then added and the cultures were incubated at 35°C. The medium was changed every 7 days and the growth was observed with phase microscopy. One to 2 weeks after explantation, the specimens were fixed with Zenker’s solution and stained with Jenner-Giemsa or hematoxylin-Giemsa. Trypsin-Versene solution (0.25%)* was used for subculturing. These cultures did not contain mycoplasma as determined by multiple tests using standard mycoplasma isolation media and the Fortner plate method.

Ultrastructural Studies

Cold phosphate-buffered 2.5% glutaraldehyde was used to fix the third fragment of the surgical specimen as well as those cells grown in Leighton tubes on carbonized coverslips. After fixation, these specimens were rinsed in phosphate buffer and osmicated in Dalton’s chrome-osmium for 1 hour. They were then stained en bloc with 2% uranyl acetate in 50% ethanol for 1 to 12 hours, dehydrated in a graded ethanol series, and embedded with propylene oxide in Epon 812. The samples were sectioned on a LKB Ultramicrotome III,† stained with lead citrate, and examined on a Siemens Elmiskop I electron microscope‡ at 80 kV.

For metal cast replicas, the cells were grown on a non-carbonized glass cover-slip, fixed as above, dehydrated in a graded ethanol series, and then critical-point dried with Freon 13 and Freon 113. They were shadowed with platinum-carbon at a 45° angle by using spectrographic carbon rods wrapped with platinum wire. After reinforcing this specimen with carbon, the glass substrate and the cells were dissolved with hydrofluoric acid. The resulting replica was rinsed with distilled water and examined on a nked 75-mesh grid at 80 kV.

*F-10 nutrient medium and Trypsin-Versene solution supplied by Grand Island Biological Company, Grand Island, New York 14072.
†LKB Ultramicrotome III manufactured by LKB Produckter, Bromma 1, Sweden.
‡Siemens Elmiskop I electron microscope manufactured by Siemens Corporation, 186 Wood Avenue, South Iselin, New Jersey 08830.
FIG. 3. Photomicrograph of section from a surgical specimen of an optic glioma showing several Rosenthal fibers (arrows). H & E, × 540.

FIG. 5. Photomicrograph of bipolar cells with long, thin piloid processes growing in culture from an explant of an optic glioma. Hematoxylin-Giemsa, × 340.

FIG. 4. Electron micrograph of section from a surgical specimen of an optic glioma demonstrating amorphous electron-dense deposits (D) adjacent to abundant 9 to 10-nm fibers within an astrocytic process. × 38,400.

FIG. 6. Electron micrograph of section from a tissue culture monolayer of an optic glioma demonstrating an amorphous electron-dense deposit (D) in the vicinity of abundant 9 to 10-nm fibers. Also shown are micropinocytotic vesicles (V) often found adjacent to substrate in cultured cells. × 49,000.
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Light microscopy of the surgical specimens typically revealed pilocytic astrocytes that were sometimes arranged in perivascular rosettes (Fig. 1). Electron microscopy demonstrated that these rosettes were composed of numerous astrocytes with foot-processes attached to the basement membrane of blood vessels by hemidesmosomes (Fig. 2). Rosenthal fibers were visible by light microscopy in some of these tumors (Fig. 3). Ultrastructural studies demonstrated amorphous intracellular deposits in the vicinity of numerous 9 to 10 nm fibers (Fig. 4). Such deposits have been considered the ultrastructural counterparts of the Rosenthal fibers seen on light microscopy.

In tissue culture, the predominant growth from the tumor explants was composed of bipolar cells with long, thin processes (Fig. 5), whereas only a few flattened stellate cells or small round cells were observed. The bipolar cells were similar to the piloid astrocytes seen in the surgical specimens, and ultrastructural studies of these cultured cells revealed abundant 24 nm microtubules in addition to numerous bundles of 9 to 10 nm fibers.

Electron-dense deposits similar to those observed in the surgical specimens could also be seen in vitro in the vicinity of these 9 to 10 nm fiber bundles (Fig. 6). Multinucleation was seen in only one case. This tissue culture specimen exhibited an interesting form of multinucleation with as many as 20 nuclei arranged in a palisading fashion at the periphery of some of the cells (Fig. 7).

Metal cast replica studies of the cultured optic gliomas demonstrated a generally smooth cell surface with numerous tiny processes that projected from the cell membrane to form an interlacing meshwork adjacent to the cells (Fig. 8). Three of these primary cultures formed fibrillary tangles that were visible by light microscopy (Fig. 9).
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FIG. 9. Photomicrograph shows tangles of cell processes developing in a cultured optic glioma. PTAH, × 540.

Under our growth conditions, these tangled fibers did not coalesce to form structures definable by light microscopy as Rosenthal fibers.

Three of these specimens were maintained in culture past the stage of initial explantation. After several passages, the predominant cell type was stellate rather than piloid. Whether this represents an adaptation of the piloid cells to the culture conditions, or an overgrowth of the smaller number of stellate cells initially present could not be ascertained. When examined ultrastructurally, however, these stellate cells still contained numerous 9 to 10 nm fibers as well as 24 nm microtubules.

Discussion

Relatively few optic gliomas have been studied previously in tissue culture. In this paper, we report the characteristics of nine human optic nerve gliomas grown in tissue culture from our series of 1200 consecutively cultured brain tumors. The well differentiated bipolar cells observed growing from the explants resemble the piloid astrocytes of the original tumors and are similar to the cells grown from optic gliomas and other related tumors reported by previous investigators. Moreover, these cells differ markedly from the pleomorphic cells that often grow in tissue culture from malignant cerebral astrocytomas. Only one of our cases of optic glioma exhibited significant multinucleation in tissue culture. This tumor was from the youngest of all the patients (7 months), and even by this early age, the tumor had grown to involve both optic nerves and the chiasm, suggesting a fairly aggressive character. Despite these clinical features, the routine pathology sections of this tumor did not differ from those of the other tumors in this report. Whether the bizarre, multinucleated cells seen in the tissue-culture study of this tumor are peculiar to this one case, or perhaps are characteristic of more aggressive forms of optic glioma merits further study.

Rosenthal fibers are amorphous, hyaline, globular bodies commonly found in optic gliomas and in the other low-grade midline gliomas collectively referred to as spongioblastomas. Although the mechanism of development of these bodies has been somewhat unclear, their staining characteristics with PTAH have suggested that they may be derived from the glial fibers, which are abundant in the cells of these tumors. Further evidence for this idea is provided by the demonstration of amorphous electron-dense deposits in the vicinity of bundles of glial fibers as both we (Fig. 4) and others have shown. Tissue-culture studies have allowed further investigation of the development of these structures. In this study, growth from the explants of optic gliomas typically produced bipolar cells with long, thin processes that contained numerous 9 to 10 nm fibers, as well as amorphous electron-dense deposits similar to those observed in the surgical specimens. Although we did not observe in vitro the development of structures definitely identifiable by light microscopy as Rosenthal fibers, this could be due partly to our growth conditions, since, under different growth conditions, the development of Rosenthal-like fibers in other spongioblastic tumors has been reported. It appears, therefore, that the cells of spongioblastic
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tumors, such as the optic glioma, contain abundant intracellular 9 to 10 nm fibers that can be observed both in vivo and in vitro and that, in both instances, may undergo degenerative changes to form Rosenthal fibers.

Another interesting feature of these fiber-laden cells in tissue culture is that their long, thin cellular processes can split and whirl to overlap the processes of other cells and form fibrous tangles (Fig. 9). Of note in our cases, however, is that the in vitro production of such tangles did not necessarily correlate with the presence of Rosenthal fibers in the original surgical specimen. For example, tumors from several patients who had Rosenthal fibers in their surgical specimens did not form these tangles when grown in tissue culture, and, conversely, the tumor that formed the mass of tangles in vitro (Fig. 9) did not have any Rosenthal fibers in vivo. Although this could, in part, be due to sampling differences between the several different pieces of tumor used for the different phases of this study, another possibility is that these disorganized, tangled masses of cell processes seen in vitro may not necessarily occur in vivo. In fact, rather than being disorganized, the astrocytic processes of some optic gliomas seen in pathology sections are often quite well organized, especially in areas adjacent to blood vessels (Figs. 1 and 2). Therefore, although these fibrous tangles are characteristic of the growth in tissue culture of optic gliomas and related tumors, such tangles may not occur in vivo nor may they be necessary for the production of Rosenthal fibers in the surgical specimen. It is also possible that certain cytarchitectural features in vivo may encourage these long cellular processes to coalesce into Rosenthal fibers, whereas similar features may not be present in a tissue-culture environment. Nonetheless, the formation of such tangles by these tumors in vitro is still of significance for we have not observed the development of similar tangles in over 1200 brain tumors of other types grown in our laboratory under similar conditions. Despite some uncertainty about the relationship of these fibrous tangles in vitro and Rosenthal fibers in vivo, both are commonly found in only a few types of tumors and therefore may represent some underlying characteristics that are helpful in differentiating optic gliomas and other midline, low-grade gliomas from the majority of astrocytomas occurring in the cerebral hemispheres. Although these tumors have been thought to originate from cells of the astrocytic series, Gullotta and Fliedner have suggested that these morphological features and patterns of gliofibrillar changes could indicate that spongioblastic tumors such as the optic glioma might originate from a different cell population than do most astrocytomas.

Summary

We have described the morphological and ultrastructural characteristics of nine human optic gliomas grown in tissue culture. Multinucleation was rare except for one culture that showed as many as 20 nuclei palisading along the periphery of some of the cells. Whether this feature is unique to this particular case or is perhaps indicative of certain types of more aggressive optic gliomas remains for further study. More typically, growth from the explants revealed numerous piloid astrocytes with abundant intracellular 9 to 10 nm fibers similar to those seen in the surgical specimens. Degenerative changes of these fiber bundles with the production of amorphous electron-dense deposits could be observed both in vivo and in vitro and may represent the early formation of Rosenthal fibers. A distinctive feature of the piloid cells of optic gliomas (and other spongioblastic tumors) is their ability to form fibrous tangles in tissue culture. The relationship of these tangles present in culture to the Rosenthal fibers present in vivo is still unclear. Yet, these distinctive morphological features have suggested the possibility that the cells of origin of optic gliomas may have significant differences from the cells comprising most cerebral astrocytomas. Further studies of the in vitro characteristics of these tumors will hopefully add more to our understanding of their biology.

Acknowledgments

The authors are indebted to Dr. William H. Sweet for his advice and encouragement during this work; to Drs. Edward P. Richardson, Jr., and George M. Kleinman for their assistance in examining the pathology specimens; to Miss Beverly O. Whitman for her technical assistance; and to Drs. Arthur S. Grove and F. Curtis Dohan, Jr., for their advice in the preparation of the manuscript.

J. Neurosurg. / Volume 46 / January, 1977 83
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This work was presented in part at the 26th Annual Meeting of the Tissue Culture Association, on June 4, 1975, in Montreal, Canada.

This investigation was supported in part by Public Health Service research grants NS-05406 and CA-07368 from the National Institutes of Health.

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