Int. J. Cancer: **20**, 206-212 (1977)

ESTABLISHMENT OF A HUMAN MEDULLOBLASTOMA CELL LINE

R. M. McAllister [1], H. Isaacs [2], R. Rongey [3], M. Peer [1], W. Au [4], S. W. Soukup [4] and M. B. Gardner [3]

[1] *Division of Infectious Diseases and Virology,* [2] *Department of Pathology, Children's Hospital of Los Angeles, Calif.;* [3] *Department of Pathology, USC School of Medicine; and* [4] *Department of Pediatrics, Children's Hospital Research Foundation, Cincinnati, Ohio, USA*

A cell line consisting of polygonal and fusiform cells was derived from a cerebellar medulloblastoma. Cells grew to high population density in liquid medium and formed colonies in agar medium. No neural or glial elements could be demonstrated in the cultured cells by electron microscopy nor were virus particles detected. The cells formed tumors in nude mice and in antithymocytic serum (ATS)-treated hamsters. The tumors had a microscopic appearance similar to that of the medulloblastoma from which the cell line was derived. The cell line and lines derived from the transplant tumors in two hosts had four common "marker" chromosomes as well as non-specific cytogenetic changes.

Since 1925 a number of investigators have attempted to culture human brain tumor cells *in vitro* (Manuelidis, 1965; Pontén and MacIntyre, 1968; Manuelidis, 1969; Pinkerton, 1971; Barker *et al.*, 1972; Westermark *et al.*, 1973; Fogh and Trempe, 1975; Pontén, 1975). Although some permanent lines of human glial tumor cells have been established (see review by Pontén, 1975) these have been from glioma grades III and IV and only a small number of medulloblastoma cultures have survived for prolonged periods (Manuelidis, 1965; Barker *et al.*, 1972). Manuelidis reported that two of eight medulloblastoma cultures grew well and that one survived for 131 days. Barker reported that six medulloblastomas survived *in vitro* from 42-1,355 days. Pontén suggests that the reason why no permanent lines of medulloblastoma have been established is that too few biopsies have been cultured and that the tumor cells do not attach to the surface of the culture vessels.

We describe here a cell line with interesting growth and cytogenetic properties that was derived from a cerebellar medulloblastoma.

MATERIAL AND METHODS

TE-671: a biopsy was obtained and cultured, before X-ray or chemotherapy, from a tumor of the left cerebellum of a 6-year-old Caucasian girl. The pathological diagnosis was cerebellar medulloblastoma.

Tissue culture techniques and nutrient media

The tumor specimen was minced with scalpel blades and fragments approximately 1 mm in diameter were cultured in plastic culture flasks (25 cm^2) in minimum essential Eagle's medium supplemented with 10% fetal bovine serum. The culture medium was replaced with fresh medium twice weekly. As described for the sarcoma lines we established previously (Mc Allister *et al.*, 1969, 1971, 1975) the medulloblastoma cell line described here was isolated by picking, with Pasteur pipettes, six foci of multilayered growth of round or polygonal cells interspersed in monolayer sheets of stellate cells, presumed to be stromal glial cells, 6 weeks after the culture was initiated. These picked cells were seeded into culture medium in 1-cm wells in plastic dishes. Five of the six foci formed confluent cell sheets apparently without glial cells and were subcultivated by trypsin dispersion into 25 cm^2 plastic flasks. Subline No. 2 of the five sublines was studied further and the results are the basis of this report.

The agar suspension culture technique has been described (Macpherson and Montagnier, 1964). Plating efficiency on solid substrate was determined by counting colonies of five or more cells 14 days after plating 5×10^2 and 5×10^3 cells in 5-cm plastic Petri dishes. To determine the dependence of the cell line upon serum concentration, cell replication in 1% fetal bovine serum was measured.

Other techniques

The pathology and electron microscopy techniques have been previously described (McAllister *et al.*, 1969) as have the techniques and dosage schedule used to inoculate hamsters with antithymocyte serum (ATS) and cells (McAllister *et al.*, 1975). Tumorigenicity testing of the cells in nude mice was performed according to the methods of Giovanella *et al.* (1974). When tumors formed in both hamsters and mice, the animals were killed and tumors removed aseptically for cell culture, karyological and histological examination.

Chromosome techniques

Cultured tumor cell lines were harvested in the log phase for chromosome analysis. Cells were

Received: February 23, 1977 and in revised form May 25, 1977.

treated 24-48 h after subculture with 0.1 μg/ml colcemid for 2-4 h, trypsinized, treated with 0.7% sodium citrate hypotonic solution for 15 min and fixed. At least 30 orcein-stained metaphases were counted in routine preparations. Chromosome banding methods used were Quinacrine (Q) banding (Lin *et al.*, 1971) and Giemsa (G) banding (Seabright, 1972).

plasmic ratio was high and cells with multiple nuclei were observed (Fig. 2B). Table I summarizes the results of *in vitro* studies with this line. As noted, the cells divided rapidly even in 1% fetal bovine serum and reached a high saturation density. They exhibited independent cell growth on solid substrate and were anchorage-independent, forming colonies in agar medium.

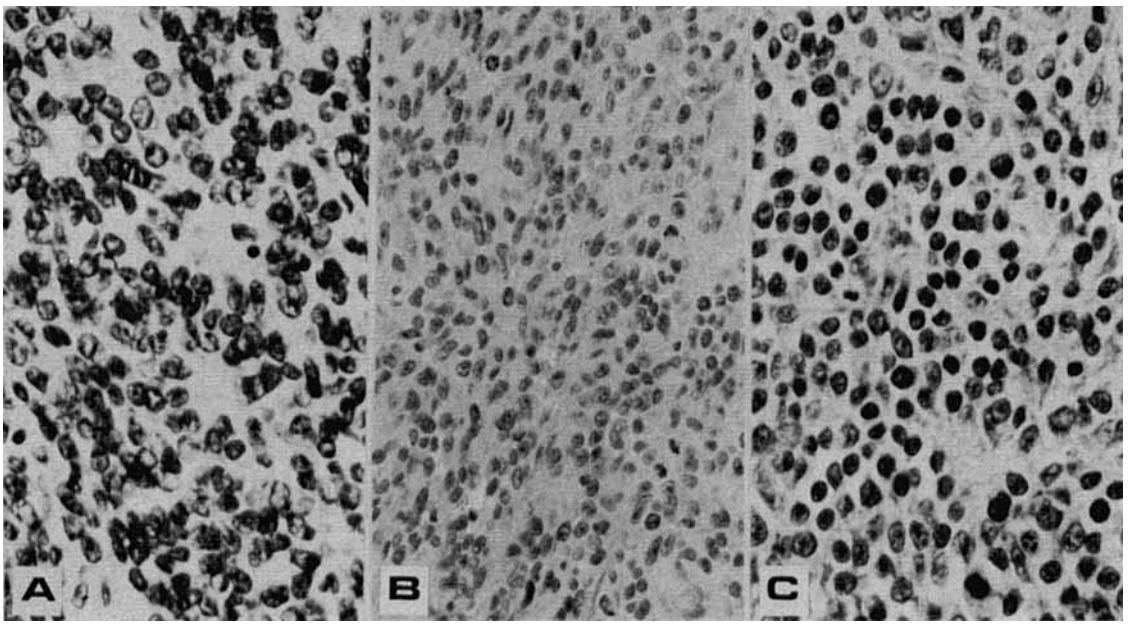

FIGURE 1

(A) Original cerebellar medulloblastoma (TE-671) is composed of small round or oval cells with scant cytoplasm forming occasional pseudo-rosettes. (H and E, ×390). (B) Tumors formed by TE-671 cells in hamster are composed of small round or oval cells. Many mitoses are evident. (H and E, ×390). (C) Tumor formed by TE-671 cells in nude mouse is composed of rather regular, small, round cells forming pseudo-rosettes with central fibrillar material. (H and E, ×390).

RESULTS

Histopathology

The primary tumor (Fig. 1A) had the microscopic appearance of a medulloblastoma. It consisted of sheets of small, uniform, darkly stained cells. Pseudo-rosettes were present in some areas.

Properties of the cell line

Subline No. 2 derived from the tumor consisted mainly of piled-up pleomorphic, often polygonal or fusiform cells with two, three or four short unbranched processes (Fig. 2A). The nuclear to cyto-

Ultrastructural features of cell line

The common ultrastructural characteristic of these cells was the absence of special differentiation features (Fig. 3). Neural or glial elements such as neurotubules, parallel arrays of neurofilaments or secretory vesicles were not observed. Cells were mostly round or oblong with large nuclei and prominent nucleoli. A few blunt microvilli were noted at the cell surface. The cytoplasm contained mostly free ribosomes and relatively few unremarkable appearing mitochondria, lysosomal vacuoles, glycogen deposits, and Golgi zones. No virus particles were seen.

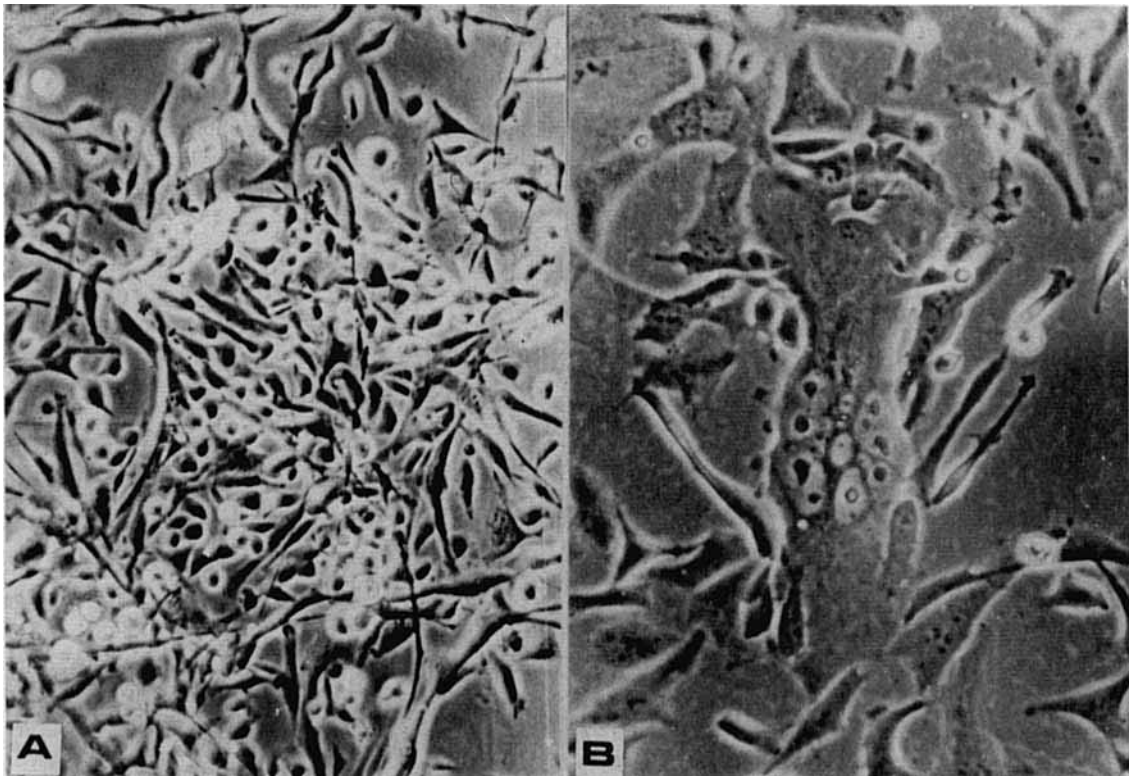

FIGURE 2

(A) TE-671 medulloblastoma cells growing as monolayer, passage 8. (Unstained, ×100). (B) Multinucleated medulloblastoma cell in culture, passage 8. (Unstained, ×100)

TABLE I

PROPERTIES OF CELL LINE [1]

Cell line designation	TE-671, subline No. 2
Cell morphology	Piles of pleomorphic, polygonal or fusiform cells with short, unbranched processes
Ultrastructural appearance	Undifferentiated cells
Population doubling time (hours)	27, (passage 22)
Saturation density (cells/cm^2)	2×10^6 (passage 22, 10% fetal bovine serum)
Plating efficiency on solid substrate	10.5% (passage 13) 25.8% (passage 27)
Plating efficiency in agar medium	14% (passage 13) 16.4% (passage 27)
Increase in cell number in medium with serum (15 days)	27.6-fold in 10% fetal bovine serum 12.7-fold in 1% fetal bovine serum

[1] Studies conducted by Dr. W. D. Peterson, Jr., Child Research Center of Michigan, indicated that TE-671, subline No. 2, had human cell surface antigens and LDH pattern as well as type-B G-6-PD.

Tumorigenicity of cells in ATS-treated hamsters and in nude mice

Following inoculation with 10^7 cells of subline No. 2, all eight surviving hamsters developed tumors (average dimensions $18\times12\times9$ mm) within 16 days. Of 8 nude mice inoculated with 10^6 cells, 7 developed gross palpable tumors in 20-34 days; the tumors in the hamsters and mice had the microscopic appearance of the parent tumor (Fig. 1B and C). Cultured cells derived from these tumors yielded cell lines with the cytological appearance of the parent cell line and with a human karyotype (see below).

Chromosome analyses of tumor cell lines

TE-671, subline No. 2, was studied at passages 8 and 13. A cell line derived from a tumor formed in a hamster was studied at passage 9 and a line derived from a tumor formed in a nude mouse was studied at passage 1.

Polyploid, especially tetraploid cells, ranging from 25-45% of total metaphases, were observed in

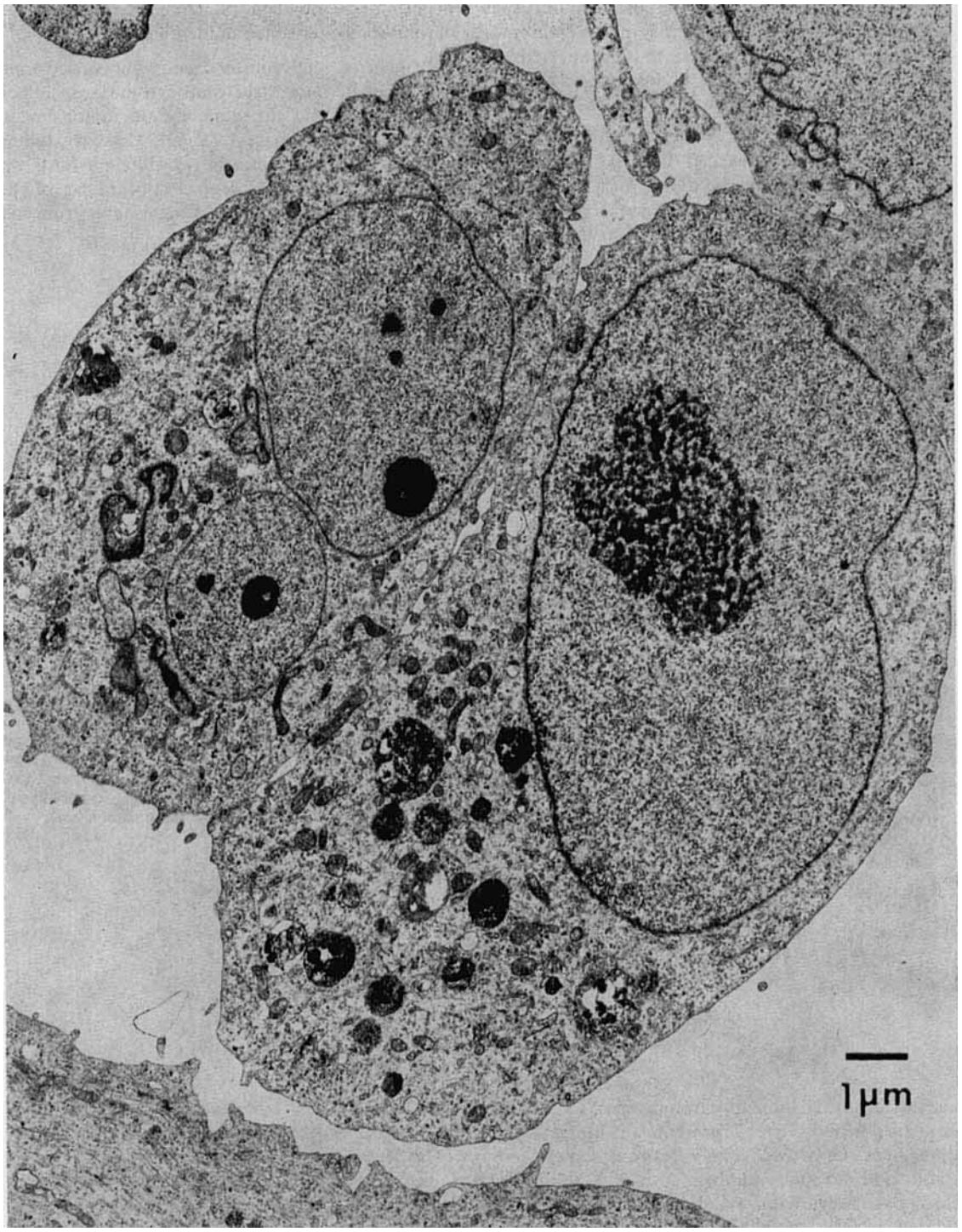

FIGURE 3

Fine structural features of medulloblastoma cells in culture, passage 10, have a primitive undifferentiated appearance. Both cells measure about 14-17 μm in length and have large nuclei; the cell on the right has a prominent nucleolus. The cytoplasms contain mostly free ribosomes with sparse endoplasmic reticulum, a moderate number of mitochondria and fairly numerous lysosomes. Only a few blunt microvilli extend from the plasma membrane and no junctional or synaptic complexes are noted. No neurotubules, secretory granules or virus particles are seen. Uranyl acetate and lead citrate fixation, $\times$9,500.

all three tumor lines. Of the near-diploid cells analyzed in all lines the majority had 47 chromosomes which was considered to be the stemline number. A Bq+ and one to two Gq− markers were consistently observed in unbanded diploid as well as polyploid metaphases of all lines.

Giemsa banding revealed a large number of structural rearrangements. Four marker chromosomes were found in all three lines (Fig. 4). They were arbitrarily classified according to their resemblance to normal human chromosomes, *i.e.*, 4q+, 9q+, 17p+ and Gq−. Trisomy 8 was found in the three lines. Only two pairs of chromosomes were stable and normal, numbers 3 and 5. Fourteen chromosomes varied from diploid, and were considered unstable, numbers 2, 4, 6, 8, 9, 12, 13, 14, 15, 17, 19, 20, 21 and X. The remaining seven chromosomes varied in only one or two lines. Q-banding confirmed the absence of the Y chromosome. Complex rearrangements indicated that four other chromosomes were present in excess, *i.e.*, numbers 1, 2, 13, and 16; three chromosomes were deficient, *i. e.*, numbers 14, 21 and X. A few marker chromosomes remained unidentified.

In general, the hamster tumor line varied more in numerical and structural chromosome abnormalities than did the nude mouse tumor line in comparison to the parent TE-671. However, tumorigenicity of the cell lines in two different hosts was similar and the microscopic appearance of the tumor in two hosts was indistinguishable from that of the original tumor.

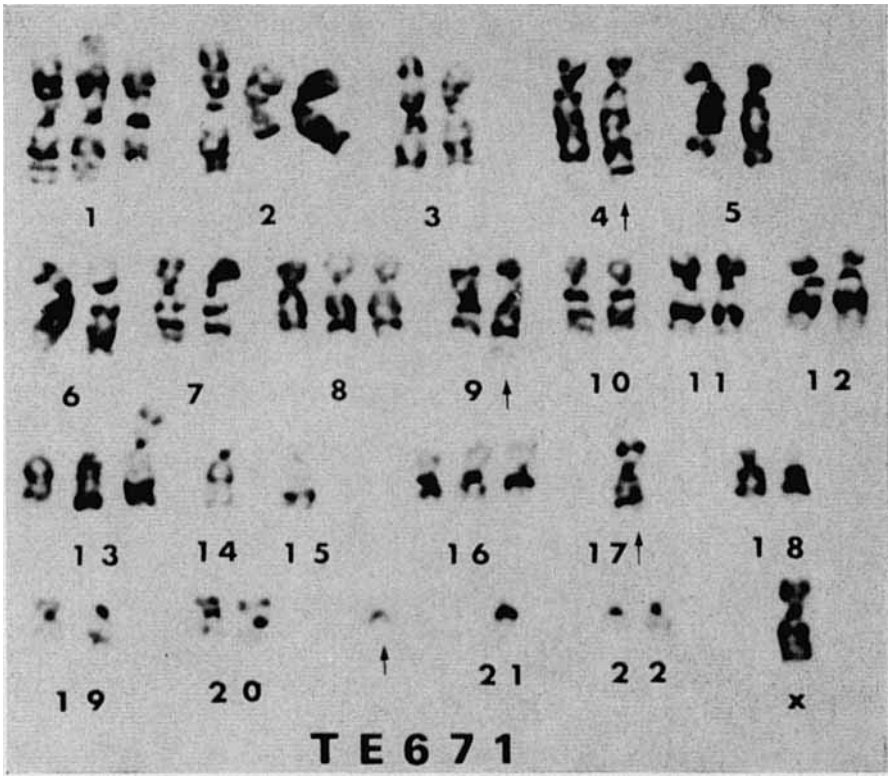

FIGURE 4
Giemsa banding karyotype from the original tumor line TE-671 showing 47 chromosomes. See text for description. Arrow indicates consistent markers found in all three tumor lines.

DISCUSSION

The technique described here and our previous studies of holding primary cultures until piles of round or polygonal cells form and then picking out these piled cells into smaller vessels, is occasionally successful in isolating tumor cells from stromal cells. Since 1967 we have cultured *in vitro* 70 surgical biopsy specimens of childhood brain tumors (Table II). Although 58 yielded glial cells and 11 exhibited foci of piled-up cells that were picked out to smaller vessels, only one, described here,

TABLE II

SOURCES OF BRAIN TUMOR BIOPSY SPECIMENS FOR TISSUE CULTURE

Diagnosis	No. of specimens cultivated
Medulloblastoma	21
Astrocytoma grades I and II	20
Astrocytoma grades III and IV	13
Ependymoma	8
Oligodendroglioma	2
Craniopharyngeoma	2
Malignant tumor cerebellum (not otherwise specified)	1
Microglial sarcoma	1
Carcinoma of choroid plexus	1
Papilloma of choroid plexus	1
Total	70

apparently yielded a cell line. Surprisingly, none of the grade III or IV gliomas yielded cell lines as reported by other authors, although the conditions of culture used in our studies appear similar.

The absence of differentiation features by electron microscopy is consistent with the postulated origin of this type of tumor from the most primitive neuroectodermal cells (Rubinstein, 1972). An ultrastructural similarity to embryonic tissue, *i.e.*, no special differentiation, was also found to be the principal feature of biopsied medulloblastomas (Matakas *et al.*, 1970).

A comparison of chromosome findings from a medulloblastoma cell line before and after passage through two different host animals, *i.e.*, hamster and nude mouse, is reported. With banding, only two of 23 pairs of human chromosomes were seen to be normal and stable in all three lines. Four apparently identical marker chromosomes were consistently found in all lines. It is of some interest that trisomy 8 was found in the three lines since simple trisomies were rare in other chromosomes, and trisomy 8 has also been found to be one of the chromosome abnormalities associated with acute myelocytic leukemia (Rowley and Potter, 1976).

The *in vitro* and *in vivo* growth properties of TE-671 subline No. 2 cells as well as their ultrastructural and chromosome composition suggest that they are malignant brain tumor cells that may prove useful for further biological and biochemical study.

ACKNOWLEDGEMENTS

This study was conducted under Contract NO1-CP-53500 within the Virus Cancer Program of the National Cancer Institute, National Institutes of Health. William Au is supported by Grant No. CA18588A, awarded by the National Cancer Institute, DHEW, and Grass Foundation for Neurobiological Research.

We are indebted to Dr. G. Vladimir, Division of Hematology and Oncology, Children's Hospital Research Foundation, Cincinnati, for permitting us the use of his nude mouse colony.

The authors thank Drs. W. Peterson and C. Stulberg for their contribution to this work and Ms. E. Goodman for preparing the manuscript.

ÉTABLISSEMENT D'UNE LIGNÉE DE CELLULES DE MÉDULLOBLASTOME HUMAIN

Une lignée consistant en cellules polygonales et fusiformes a été obtenue à partir d'un médulloblastome cérébelleux. Les cellules ont atteint une forte densité dans un milieu liquide et ont formé des colonies dans la gélose. Aucun élément neural ni glial n'a pu être mis en évidence au microscope électronique dans les cellules en culture; on n'a pas non plus décelé de particules virales. Les cellules ont formé des tumeurs chez les souris nues et les hamsters traités au sérum antithymocyte (ATS). Ces tumeurs avaient un aspect microscopique semblable à celui du médulloblastome d'où provenait la lignée. La lignée initiale et les lignées provenant de tumeurs transplantées chez deux hôtes avaient quatre chromosomes marqueurs communs et présentaient des altérations cytogénétiques non spécifiques.

REFERENCES

BARKER, M., WILSON, C. B., and HOSHINO, T., Tissue culture of brain tumors. *In:* W. M. Kirsch, E. G. Paoletti and P. Paoletti (ed.), *The experimental biology of brain tumors*, pp. 57-84, Charles C. Thomas Publisher, Springfield (1972).

FOGH, J., and TREMPE, G., New human tumor cell lines, *In:* J. Fogh (ed.), *Tumor cells* in vitro, pp. 115-159, Plenum Press, New York and London (1975).

GIOVANELLA, B. C., STEHLIN, J. S., and WILLIAMS, L. J. JR., Heterotransplantation of human malignant tumors in "nude" thymusless mice. *J. nat. Cancer Inst.*, **52**, 921-930 (1974).

LIN, C., UCHIDA, I., and BYRNES, E., A suggestion for the nomenclature of the fluorescent banding patterns in human metaphase chromosomes. *Canad. J. genet. Cytol.*, **13**, 361-363 (1971).

MACPHERSON, I., and MONTAGNIER, L., Agar suspension culture for the selective assay of cells transformed by polyoma virus. *Virology*, **23**, 291-294 (1964).

MANUELIDIS, E. E., Long-term lines of tissue cultures of intracranial tumors. *J. Neurosurg.*, **22**, 368-373 (1965).

MANUELIDIS, E. E., Experiments with tissue culture and heterologous transplantation of tumors. *Ann. N.Y. Acad. Sci.*, **159**, 409-431 (1969).

MATAKAS, F., CERVOS-NAVARRO, J., and GULLOTTA, F., The ultrastructure of medulloblastomas. *Acta Neuropath. (Berlin)*, **16**, 271-284 (1970).

MCALLISTER, R. M., GARDNER, M. B., GREENE, A. E., BRADT, C., NICHOLS, W. W., and LANDING, B. H., Cultivation *in vitro* of cells derived from a human osteosarcoma. *Cancer*, **27**, 397-402 (1971).

MCALLISTER, R. M., MELNYK, J., FINKLESTEIN, J. Z., ADAMS, E. C., JR., and GARDNER, M. B., Cultivation *in vitro* of cells derived from a human rhabdomyosarcoma. *Cancer*, **24**, 520-526 (1969).

MCALLISTER, R. M., NELSON-REES, W. A., PEER, M., LAUG, W. E., ISAACS, H., JR., GILDEN, R. V., RONGEY, R. W., and GARDNER, M. B., Childhood sarcomas and lymphomas: characterization of new cell lines and search for type-C virus. *Cancer*, **36**, 1804-1814 (1975).

PINKERTON, H., Histology and ultrastructure of cultured human tumor cells exposed to antisera to the nerve growth factor. *Cancer Res.*, **31**, 1483-1487 (1971).

PONTÉN, J., and MACINTYRE, E. H., Long term culture of normal and neoplastic human glia. *Acta path. microbiol. scand.*, **74**, 465-486 (1968).

PONTÉN, J., Neoplastic human glia cells in culture, *In:* J. Fogh (ed.), *Tumor cells* in vitro, pp. 175-206, Plenum Press, New York and London (1975).

ROWLEY, J. D., and POTTER, D., Chromosome banding patterns in acute nonlymphocytic leukemia. *Blood*, **47**, 705-719 (1976).

RUBINSTEIN, L. J., Cytogenesis and differentiation of primitive central neuroepithelial tumors. *J. Neuropath. exp. Neurol.*, **31**, 7-26 (1972).

SEABRIGHT, M., The use of proteolytic enzymes for the mapping of structural rearrangements in the chromosomes of man. *Chromosoma (Berl.)*, **36**, 204-210 (1972).

WESTERMARK, B., PONTÉN, J., and HUGOSSON, R., Determinants for the establishment of permanent tissue culture lines from human gliomas. *Acta path. microbiol. scand., Section A*, **81**, 791-805 (1973).