Acta Neuropathol. (Berl.) 46, 51 – 55 (1979)

Acta Neuropathologica

Establishment of a Human Oligodendroglial Cell Line*

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Summary. A human oligodendroglial cell line has been established from a mixed glioma. Over a period of 22 months, the cell consisted of cells with the morphologic features of such a strong perinuclear halo, dichotomous branching, and a tendency to aggregate and float in the medium. In small aggregates, most cells are bipolar. The cell line also continues to synthetize the S-100 protein specific to neural tissue. The response of this cell line to dibutyryl-cyclic AMP is striking, resulting in a remarkable narrowing and elongation of the cell processes.

This cell line could be used for studies on glial differentiation and neuron-glia interaction.

Key words: Oligodendroglia – Established cell line – Myelin formation – S-100 protein – Glial differentiation

Permanent human cell lines from malignant gliomas have been established in several laboratories (Manuelidis, 1965, 1969; Wilson, 1966; Pontén et al., 1968; Westermark, 1973). These cell lines were obtained only from glioblastomas and consist of undifferentiated glial cells. On the other hand, it has been very difficult to establish oligodendroglial cell lines from either animal or human tissue cultures. It has been generally accepted that oligodendroglial cells will disappear or transform to astroglial cells in long-term tissue culture. The establishment of oligodendroglial cell line would be highly desirable for the study of neuroglial differentiation and central myelination. This report described the features of an oligodendroglial cell line obtained from a human mixed glioma.

* This work was supported by a Grant-in Aid for Scientific Research from the Japan Ministry of Education, Science, and Culture

Materials and Methods

The specimen was surgically obtained from a cerebral mixed glioma of a 13-year-old boy on January 22, 1977. Part of the tumor tissue was submitted to histological examination with hematoxylin and eosin (H & E), Bodian, and phosphotungstic acid hematoxylin (PTAH) stains. For in vitro culture, the tumor tissue was minced by scissors and trypsinized with a mixture of 200 i. u. trypsin (Mochida, Japan) and 0.05% EDTA for 30 min.

Contaminating erythrocytes were removed by hypotonic shock with 0.85% NH_4Cl, and the tumor cells were cultured in TD 15 flasks (Ikemoto, Japan) in humidified atmosphere of 95% air and 5% CO_2 in medium 199 (DIFCO, U.S.A.) or MEM (Nissui, Japan) or F 12 (GIBCO, U.S.A.) or RPMI (Nissui, Japan) supplemented with 20% fetal bovine serum (MBA, U.S.A.). Subcultures were performed by treatment with 200 i. u. trypsin and 0.05% EDTA. Eight months after the initial culture, the processes of cell possessing strong perinuclear halos formed connections with each other on a layer of epithelial and fibroblastic cells. The glial cells were isolated by pipetting and subcultured serially. After establishment of the cell line, the cells were easily subcultured by pipetting. This cell line was designated KG-1.

Morphology of the Primary Tumor

Histological appearances of the primary tumor varied from area to area and consisted of epedymomatous, astrocytomatous and oligodendrocytomatous elements. The tumor was diagnosed as a mixed glioma.

In Vitro Properties

Growth curve: Tumor cells harvested by trypsinization were adjusted to 1×10^5 cells/ml in culture medium and 1 ml each of these suspensions was poured into cubic tubes (16 mm × 160 mm, Ikemoto, Japan). These triplicate culture tubes were placed in a CO_2 incubator at 37° C and the total cells were counted at two-day intervals.

Plating Efficiency

Single cell suspensions consisted of 50 or 100 cells obtained by trypsinization were placed in triplicate Falcon plastic dishes (3002), and incubated in a CO_2 incubator at 37° C. After 14 days, the cultured cells were fixed in absolute methanol, and stained with Giemsa solution. Four or more cells growing closely were counted as a colony.

0001-6322/79/0046/0051/$ 1.00

Immunofluorescence

Purified IgG from rabbit anti-bovine S-100 protein was kindly provided by Dr. Uyemura, Saitama Medical School, Saitama, Japan. The total protein concentration at the appropriate dilution was 1 mg/ml. The rabbit antiserum to bovine S-100 protein showed a single band with bovine S-100 protein and crude rat brain extract in agar by double diffusion method (Ouchterlony, 1958).

Rabbit anti glial fibrillary acidic protein (GFAP, Astroprotein) serum was kindly provided by Dr. Mori, Osaka University Medical School, Osaka, Japan. The rabbit anti-GFAP (1:20) serum was used. These rabbit antisera to bovine S-100 protein and GFAP were used for our immunofluorescence study.

Cultured cells on LabTek tissue culture chamber slides were fixed in absolute methanol for 30 min and washed with phosphate buffered saline (PBS), then rabbit antiserum to bovine S-100 protein or rabbit antiserum to human GFAP was added to the cells. After 30 min at room temperature, the cells were washed with PBS, and FITC-goat anti rabbit IgG serum (F/P = 1.1, total protein protein concentration in applied dilution = 0.58 mg/ml, Medical Biological Laboratories, Nagoya, Japan) was added to the cells for 30 min at room temperature. After washing, the cover slips were mounted in PBS glycerin and examined with an Olympus fluorescence microscope using a UG-1 excitor filter and L 420 barrier filter. Photographs were taken using Kodak Tri-X films.

Ouchterlony Method

The confluent cultured KG-1 and rat C_6 glial cells were homogenized and centrifuged at 3,000 rpm for 10 min. 60 µl of each extract or purified bovined S-100 protein was reacted with rabbit anti-bovine S-100 protein in 1% agar plates.

Electron Microscopy

The cultured cells on the Falcon plastic dish was fixed in 4% phosphate buffered glutaraldehyde for 1 h and postfixed in 1% OsO_4. After dehydration with ethanol, the fixed cells were embedded in Epon. A thin section was stained with uranylacetate and lead citrate without using propylene oxide. Electron micrographs were taken by an Hitachi HS-9 electron microscope.

Response to Dibutyryl Cyclic AMP

$(But)_2$-cAMP (Boehringer, West Germany) was dissolved in MEM supplemented with 10% fetal bovine serum and preserved until use. 1 mM of $(But)_2$-cAMP was added to the cell suspension and the cells were seeded. $(But)_2$-cAMP was allowed to remain in the medium through the entire period thereafter. Control cells were cultured in the medium without $(But)_2$-cAMP.

Chromosome Analysis

The cultured cells were treated with colchicin (Boehringer, West Germany) at a final concentration of 0.5 µg/ml for 5 h and then detached by shaking. The cells were treated with 0.075 M KCl for 10 min and fixed in Carnoy solution. The cells were then dropped on a slide glass and stained with Giemsa solution for 40 min.

Results

Morphology. The cells are globose or bipolar or multipolar (Figs. 1, 2, 3). They have refractile halos and possess slendar processes which arise sharply from the

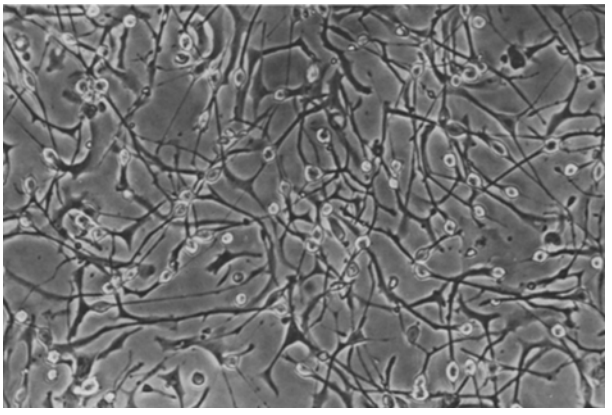

Fig. 1. Three days after seeding, the cells are bipolar or multipolar and have slender processes which arise sharply from the perikaryon. Phase contrast, × 160

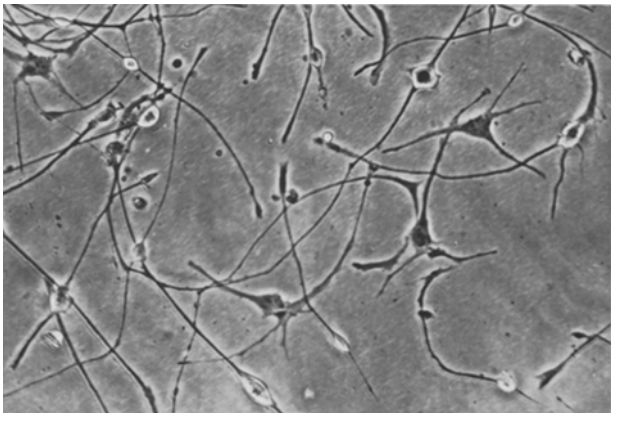

Fig. 2. Seven days after seeding, the cells are almost bipolar and possess refractile halos and very long processes. Phase contrast, × 320

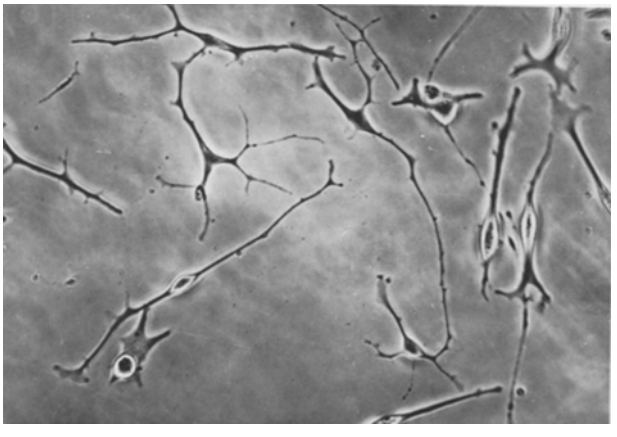

Fig. 3. The processes of cells form connections with each other. Phase contrast, × 320

perikaryon. The processes branch only once or twice and dichotomously (Fig. 4).

Growth Rate. Doubling time calculated from the growth curve at the 23rd passage (12 months after the initial cultivation) was approximately 41 h. Plating efficiency was 6% or 14% after seeding of 50 or 100 cells, respectively. The growth properties are summarized in Table 1.

Table 1

Designation	KG-1
Cell morphology	Bipolar or multipolar or Globose
Doubling time	Approximately 41 h
Saturation density	Approximately $5 \times 10^4/cm^2$ (in MEM)
Plating efficiency	6% (50 cells seeding) 14% (100 cells seeding)

Immunofluorescence. Detection of S-100 protein: The cytoplasms and cell processes of the KG-1 cells were strongly stained (Fig. 5). Specificity controls of the reaction included incubation without test serum and blocking the reaction with non-fluorescent goat anti-rabbit IgG serum before addition of the fluorescence antibody. Human fibroblast was used as a control cell. These control results were negative.

Investigation of glial fibrillary acidic protein: No positive staining was found in KG-1 cell, whereas strongly positive reaction was found in astrocytic cell.

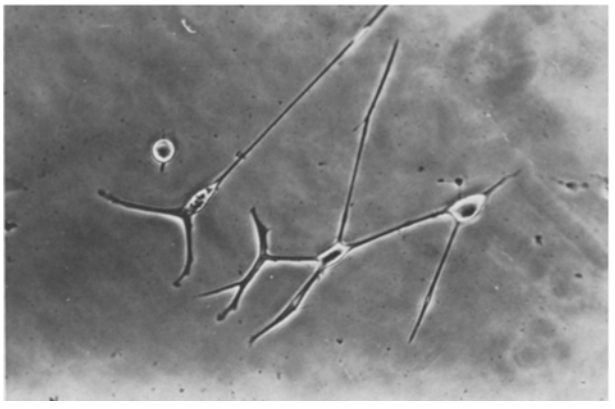

Fig. 4. Cells from a single cell clonal line, remarkable dichotomous branching and straight processes. Phase contrast, × 320

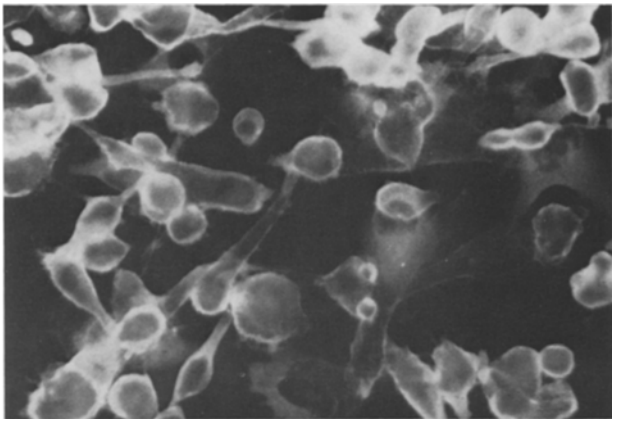

Fig. 5. Indirect immunofluorescence method for the demonstration of nervous tissue specific (S-100) protein, the cytoplasms and cell processes show strong positive staining. Olympus fluorescence microscope, × 640

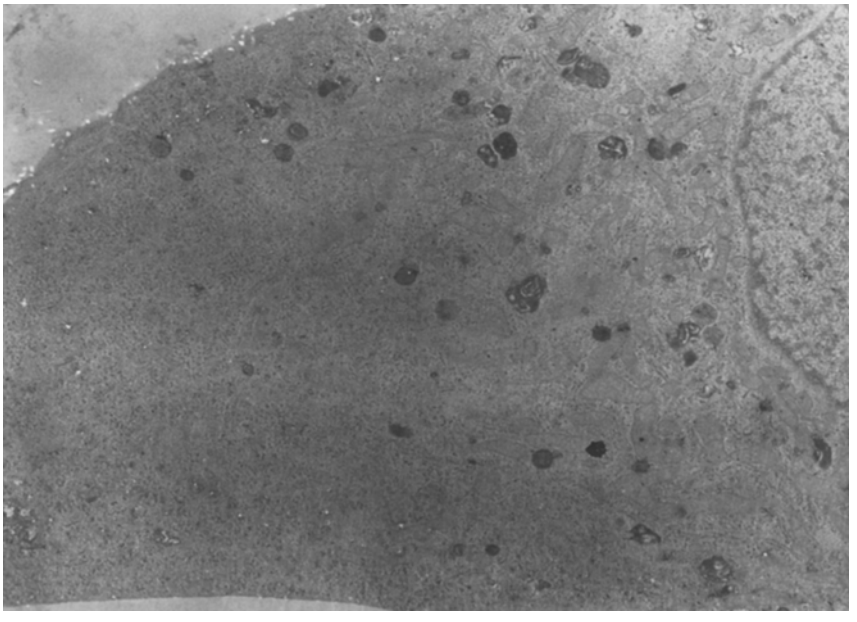

Fig. 6. Electronmicrograph of KG-1 cell. The cytoplasm is rich in mitochondrias and polysomes. Microtubules are observed. Epon-embedded section stained with uranylacetate and lead citrate, × 12,000

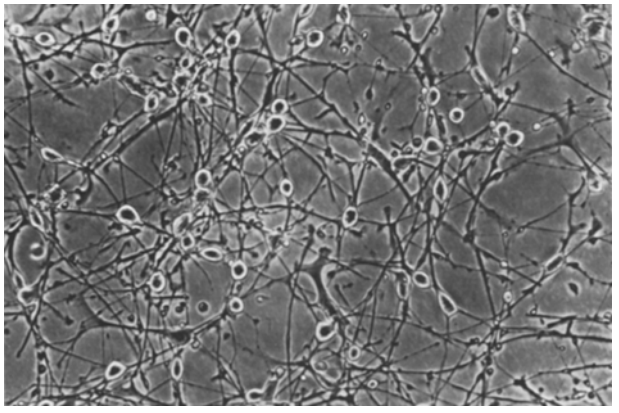

Fig. 7. Two days after the treatment with dibutyryl-cAMP (1 mM/ml). Remarkable narrowing and elongation of cell processes. Perinuclear halos are clear. Phase contrast, × 320

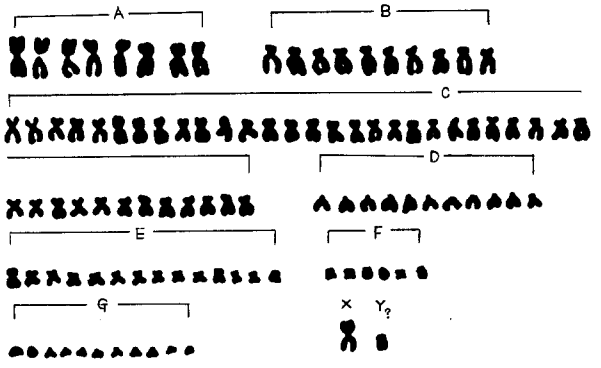

Fig. 8. Karyotype of KG-1 cell, species specific of human origin, Giemsa staining

Electron Microscopy. The cells have many short microvilli without any coating substance. The nuclei usually lies in an eccentric position. The cytoplasm was rich in organelles, especially true in both free ribosome and rough-surfaced endoplastic reticulum. The glial filaments observed in astricytic cells were not seen (Fig. 6).

Response to $(But)_2$-cAMP after 2 days: The cell processes were very narrow and long. Perinuclear halos were clear (Fig. 7).

Chromosome Analysis. Species specificity of the cells was monitored by chromosome analysis. The chromosome number ranged from 78 to 139. The modal number was 102 (Fig. 8).

Discussion

The term oligodendroglia was introduced by Del Rio Hortega in 1921 (Hortega, 1921). The functions of oligodendroglial cells include formation and maintenance of myelin and, presumably, nutrition of neurons. However, for the further studies of oligodendroglial cells, it is essential to establish an oligodendroglial cell line and to characterize it. In primary culture of normal and neoplastic explants, several investigators have reported characteristic appearances, e. g., refractile halo, pulsation, or tug-or-war movements in vitro (Lumsden et al., 1951; Pomerat et al., 1964; Nakai et al., 1963; Nakazawa et al., 1963; Kersting, 1968; Unterharnsheidt, 1972; Liss, 1972; Russell and Rubinstein, 1977). The KG-1 cells posses slender processes which arise sharply from the perikaryon and the processes branch once or more, forming attachments with processes of other cells. In the fluid medium, the KG-1 cells tend to aggregate and float. This tendency has been observed by other investigators. Biochemically, since Moore reported the existence of a nervous tissue specific protein (S-100), this protein has been utilized for the identification of nervous tissue tumors (Moore, 1965; Benda et al., 1968; Pfeiffer et al., 1972; Haglid et al., 1973).

In our study, S-100 protein was found in the cytoplasm of the cultured cells by the indirect immunofluorescence method. Glial fibrillary acidic protein (GFAP) has proved to be specific for the astrocyte (Mori, 1970; Eng et al., 1971; Bignami et al., 1972). In KG-1 cells no GFAP was found by indirect immunofluorescence method. The absence of electron microscopic evidence of glial filaments and no detection of glial fibrillary acidic protein in the KG-1 cells ruled out the possibility that this cell line originated from astrocytic cell. Also, there were very abundant free and associated ribosomes, and few glycogen granules in the KG-1 cells. This was in accord with the observation of oligodendroglial cell. Cyclic AMP has a defined role as a "second messenger" in the action of many hormones, and morphological changes induced by $(But)_2$-cAMP have been reported in a variety of cell types (Hsie et al., 1971; Johnson et al., 1971; Prasad et al., 1971; Edström et al., 1974; Moonen et al., 1975). The response of KG-1 cells to $(But)_2$-cAMP was very striking, resulting in remarkable narrowing and elongation of the cell processes.

As discussed above, KG-1 cells are most likely oligodendroglial cells and could be used as a model for the study of glial differentiation and neuron-glial interaction. The KG-1 cells have been subcultured over 22 months, up to 40 passages. This cell line is morphologically very homogenous. Cloning of the line has been successful.

Acknowledgement. The author is grateful to Prof. J. Nakai, Tokyo University and Prof. K. Kitamura, Kyushu University, for their kind advice. The author also thanks Dr. Ohta and Dr. Matsushima for their collaboration on this electron microscopical study. Skillful technical assistance was given by Dr. Yamashita, Miss K. Hatanaka, and Miss K. Beppu.

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Received July 17, 1978/Accepted November 10, 1978