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Neuronal Properties of Neuroectodermal Tumors *in Vitro*[1]

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ABSTRACT

Cell lines of medulloblastoma, retinoblastoma, and neuroblastoma, three childhood tumors derived from neuroectoderm, have been compared with respect to their neuronal properties. Neuroblastoma, a neural crest derivative, has been shown to express specific neuronal enzymes and the action potential sodium ionophore. Cell lines of medulloblastoma and retinoblastoma also express neuronal specific enzymes and therefore are considered to be of neuroblastic origin.

INTRODUCTION

Medulloblastoma, retinoblastoma, and neuroblastoma, 3 tumors thought to be derived from neuroectoderm, have been successfully cultivated *in vitro*. This has provided information relative to their growth characteristics and morphological features (1, 3, 4, 6-9, 11, 13-16). The determination of specific neuronal enzymes and the action potential sodium ionophore in these tissue culture systems has been reported only for neuroblastoma, a neural crest derivative. Recently, continuous cell cultures of retinoblastoma and medulloblastoma have become available. Although these 2 cancers are also derived from neuroectoderm, the cell of origin has not been well established. We have therefore attempted to identify specific neuronal enzyme activities and the presence of the action potential sodium ionophore in tissue culture preparations from these tumors. The presence of these specific neuronal markers would support the concept that these tumors arose from primitive neuroblasts.

MATERIALS AND METHODS

Cell Culture. Human neuroblastoma cell lines CHP-126, CHP-134, and CHP-212 have been described previously (10, 11). CHP-234, another neuroblastoma line, was subsequently cultured from a bone marrow aspirate. TE-671 is a cell line derived from a medulloblastoma (7) which was supplied by Dr. R. M. McAllister, Los Angeles Children's Hospital and University of Southern California (Los Angeles, Calif.). The retinoblastoma cell line WERI-RB1 (8) was obtained from R. McFall, Wills Eye Hospital, Philadelphia, Pa. Lines Y79 (9) and GM-1232, a variant of Y79, were received from Dr. C. Croce, Wistar Institute, Philadelphia, Pa.

All cell lines were grown in Roswell Park Memorial Institute Tissue Culture Medium 1640 containing 10% fetal bovine serum and 2 mM glutamine at 37° under 5% CO_2. Cells were serially subcultured at weekly intervals, and the medium was replenished every 3 days. The biochemical determinations were performed at several different passages.

Enzyme Analysis. Each of the cell lines was harvested in the stationary phase of growth (2) and washed twice with cold 0.15 M NaCl. The cells were centrifuged at 500 × g for 10 min at 4°. The specific activities of tyrosine hydroxylase, choline acetyltransferase, and acetylcholinesterase in the frozen cell pellet were measured as described by Schrier *et al.* (12).

Veratridine-dependent Lithium Uptake. Each of the cell lines was assayed for veratridine-dependent lithium uptake as described previously (10). Briefly, cells were removed from flasks and grown to a confluent monolayer in Linbro Model FB-16-24TC wells in growth medium, with the exception of the retinoblastoma cell lines Y-79, WERI-RB1, and GM 1232 which were grown as suspension cultures. CHP-126 has been adapted to grow in suspension as well as in monolayer and served as a control for the determination of the action potential sodium ionophore. After the cells were washed with PBS,[3] an incubation medium of PBS containing lithium chloride (5 mM) was added. In any experiment, veratridine (0.1 mM) and/or tetrodotoxin (0.01 mM) was added to some of the wells in addition to incubation medium to assess its effect on the uptake of Li^+. The cultures were incubated for 60 min at 37° under 5% CO_2. Subsequently, the medium was removed, and the cells were washed with cold PBS.

Cell protein and lithium were recovered from the wells by lysing the cells in cold distilled water followed by 2 additional washes of the wells. Trichloroacetic acid was added to the lysate to a final concentration of 9%. The solution was mixed and then centrifuged at 1700 × g for 10 min at 4°. The concentration of the lithium ion in the supernatant fluid was measured by atomic absorption spectrophotometry against standards prepared in 9% trichloroacetic acid. The cell pellets were resuspended in N NaOH, and protein was determined by the method of Lowry *et al.* (5). The lithium concentration in the cells at the start of the incubation was measured and subtracted from the final time measurement. All points were determined in triplicate for lithium and protein.

Suspension cultures of CHP-126, WERI-RB1, Y79, and GM1232 were placed in 12 × 75-sq mm glass tubes, pelleted at 500 × *g*, and washed. The cells were then treated as described above.

RESULTS

Neural Enzyme Content. Cells were inoculated into 75-sq mm plastic flasks and harvested three days into the stationary phase of growth. The specific activities (pmol per mg of cell

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[3] The abbreviation used is: PBS, phosphate-buffered saline (100 mM NaCl-5.3 mM KCl-1.8 mM $CaCl_2$-2.0 mM Na_2HPO_4-25 mM glucose-50 mM sucrose-20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer adjusted to pH 7.4).

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protein per min) of acetylcholinesterase, choline acetyltransferase, and tyrosine hydroxylase are given in Table 1. The numbers represent an average of 3 determinations done in triplicate on 3 separate subcultures. Acetylcholinesterase and choline acetyltransferase activity were found in the 3 types of tumors, but only the neuroblastoma displayed specific activities for all 3 marker enzymes. The enzyme activities found in mouse brain, human fetal brain, and bovine-adrenal are included as controls.

Effect of Veratridine and Tetrodotoxin on Li^+ in Neuroblastoma, Retinoblastoma, and Medulloblastoma Cells. Table 2 outlines the uptake of Li^+ as seen in human neuroblastoma cell lines (CHP-126, CHP-134, CHP-212, and CHP-234), a medulloblastoma cell line (TE-671), and retinoblastoma cell lines (Y-79, WERI-RB1, and GM 1232) in the presence and absence of veratridine and tetrodotoxin. In the absence of veratridine, there was relatively little uptake of Li^+ in neuroblastoma cells. When veratridine was added, the uptake of Li^+ increased severalfold over the control values in human neuroblastoma. Tetrodotoxin lowered the values for Li^+ uptake in the neuroblastoma cell lines as described previously (6). However, Li^+ uptake in the retinoblastoma and medulloblastoma cell lines was not sensitive to veratridine or tetrodotoxin.

DISCUSSION

A number of childhood neuroectodermal tumors are of special interest to investigators because it is thought that they have the ability to differentiate *in vivo* and *in vitro* along lines that follow stages of normal neural development. Evaluation of such differentiation has been based on morphological criteria, such as identification of Homer-Wright or Flexner-Wintersteiner rosettes, immature or mature ganglion cells, spongioblasts, mature astrocytes, or oligodendrocytes.

While it is generally agreed that peripheral neuroblastomas

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Table 1

Specific activities of tyrosine hydroxylase, choline acetyltransferase, and acetylcholinesterase in human neuroblastoma, medulloblastoma, and retinoblastoma cells

Cells in culture were removed from the flasks, washed twice with cold 0.15 M NaCl, pelleted, and frozen at −70°. The specific activities of the enzymes in the frozen pellets were measured as described in "Materials and Methods."

		mol product formed/mg protein/min		
Cell line	Disease	Tyrosine hydroxylase	Choline acetyltransferase	Acetylcholinesterase
CHP-126	Neuroblastoma	75	495	9,079
CHP-134	Neuroblastoma	54	270	13,800
CHP-212	Neuroblastoma	25	8	5,367
CHP-234	Neuroblastoma	671	12	3,500
TE-671[a]	Medulloblastoma	0	5	6,700
Y-79[b]	Retinoblastoma	0	8	732
GM-1232[b]	Retinoblastoma	0	19	2,989
WERI-RB1[c]	Retinoblastoma	0	5	600
Bovine adrenal		600	22	50,000
Mouse brain		0	400	51,000
Human fetal brain		0	28	49,000

[a] TE-671 cells kindly provided by Dr. R. McAllister.
[b] Y-79 and GM-1232 cells kindly provided by Dr. C. Croce.
[c] WERI-RB1 cells kindly supplied by R. McFall.

Table 2

Effect of veratridine and tetrodotoxin on Li^+ uptake into human neuroblastoma, retinoblastoma, and medulloblastoma cell lines

Stimulation of lithium uptake was studied in neuroblastoma, medulloblastoma, and retinoblastoma by veratridine and its inhibition by tetrodotoxin. Cultures of CHP-134, CHP-212, CHP-234, CHP-126, and TE-671 were seeded into Linbro Model FB-16-24TC plates at 1.5×10^5 cells/well and grown to confluency in 7 days in Roswell Park Memorial Institute Tissue Culture Medium 1640-10% fetal bovine serum-2 mM glutamine. The average amount of protein per well was 212 µg for CHP-126, 460 µg for CHP-234, 225 µg for CHP-134, and 450 µg for TE-671. Cultures of WERI-RB1, Y-79, and GM-1232 were seeded into 75-sq mm Falcon flasks containing Roswell Park Memorial Institute Tissue Culture Medium 1640-10% fetal bovine serum-2 mM glutamine and then harvested in 7 days when the cells had reached a density of 1.5×10^6 cells/ml. The average amount of protein for each cell line was 250 µg/tube.

		Li^+ uptake (nmol/mg protein/60 min)		
Cell line	Disease	Li^+ alone[a]	Li^+ plus veratridine[a]	Li^+ plus veratridine and tetrodotoxin
CHP-126	Neuroblastoma	11.3	46.2	21.0
CHP-234	Neuroblastoma	10.5	28.5	18.6
CHP-134	Neuroblastoma	9.7	50.3	15.0
CHP-212	Neuroblastoma	6.0	29.0	10.5
TE-671	Medulloblastoma	12.4	17.7	16.6
Y-79[b]	Retinoblastoma	5.0	6.0	13.0
WERI-RB1[b]	Retinoblastoma	12.0	12.0	13.0
CHP-126[b]	Neuroblastoma	27.1	41.0	28.0

[a] Average of 2 experiments done in triplicate.
[b] Suspension cultures.

are of neural crest-sympathetic nervous system origin, the precise cell of origin of the medulloblastoma and the retinoblastoma has been a matter of debate for many years.

In view of the confusion relative to the cell of origin of the medulloblastoma and retinoblastoma and the fact that these 2 tumors are morphologically similar in several respects to neuroblastoma, it was reasoned that it might be possible to identify activities of specific neural enzymes and the action potential sodium ionophore in retinoblastoma and medulloblastoma similar to those characteristic of neuroblastoma at a stage when no histological features of putative differentiation are present.

Our studies indicate that biochemical differentiation *in vitro* is most highly developed in the neuroblastoma, although both medulloblastoma and retinoblastoma exhibit activity for acetylcholinesterase and choline acetyltransferase. Neither showed tyrosine hydroxylase activity. Moreover, of the three tumors, only neuroblastoma demonstrated evidence of the action potential sodium ionophore as measured by the effect of veratridine and tetrodotoxin on the uptake of the lithium ion.

It appears then that the medulloblastoma cell line, TE-671, and the 3 retinoblastoma cell lines, WERI-RB1, GM 1232, and Y79, are derived from primitive neuroblasts since they display specific neuronal enzyme markers, However, absence of the action potential sodium ionophore may be a reflection of the stage of differentiation of the tumor cells. This suggests that cells derived from undifferentiated neuroectodermal tumors of the central nervous system such as retinoblastoma and medulloblastoma, may serve as good *in vitro* model systems for the study of cellular differentiation.

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