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DIFFERENTIATION OF HUMAN NEUROBLASTOMA CELLS IN CULTURE

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SUMMARY

Modulation of a membrane glycoprotein, approximate molecular weight 200,000, in concert with active ionic flux has been shown in a human neuroblastoma cell line. The modulating agent was 2% dimethyl sulfoxide. Other neuronal properties, acetylcholinesterase and choline acetyltransferase, were also modulated but to a lesser extent. The appearance of this glycoprotein on the surface of both human and mouse neuroblastoma cells only under conditions of differentiation leads to the suggestion that it is directly involved with the active Na^+ channels.

Human neuroblastoma cell lines obtained from different tumors preserve a near diploid chromosomal number when maintained in culture (1-4). These human cells circumvent the disadvantages of the mouse neuroblastoma cell lines which were all derived from a single tumor, C-1300, and which show anaploidy constantly changing in culture.

In this report we show that the neuronal properties of a human cell line are modulated to a high degree as a function of time in culture even without the use of external inducing agents such as DMSO[1] (5) and HMBA[1] (6).

METHODS

Cell culture. Human neuroblastoma cells CHP-134 (3) were seeded at 3 x 10^6 cells in 75 cm^2 flasks and cultured for 72 h in RPMI-1640 with 10% fetal calf serum as described (7). The medium was changed and fresh medium with or without 2% DMSO or 2.5 mM HMBA was added for 48 h and changed thereafter every 48 h for the duration of the experiment. The cells were made radioactive by growth in L-[^{3}H]fucose (5 μC/flask) 48 h prior to harvest. For the ion flux, CHP-134 cells were seeded at 1 x 10^6 cells on 60 mm culture dishes and cultured as above. Cell counts and viability determinations were with the use of a Biophysics Counter.

Preparation of membranes. CHP-134 cells were harvested by shaking from the monolayers after washing three times with 0.16 M NaCl. The cells in suspension were washed three times with 0.16 M NaCl and membranes prepared by the Zn ion technique (8). The surface membranes and cell bodies were collected from the sucrose gradients, pelleted and used for further analysis.

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[1]DMSO, dimethyl sulfoxide; HMBA, hexamethylene bisacetamide; AChE, acetylcholinesterase; CAT, choline acetyltransferase; SDS, sodium dodecyl sulfate.

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Ion flux. The efflux of ^{86}Rb was as described (9). The data were calculated and plotted by computer. Veratridine (100 μM) and scorpion venom (0.75 μM) were used to stimulate the efflux of ^{86}Rb and tetrodotoxin (1 μM) to inhibit the efflux.

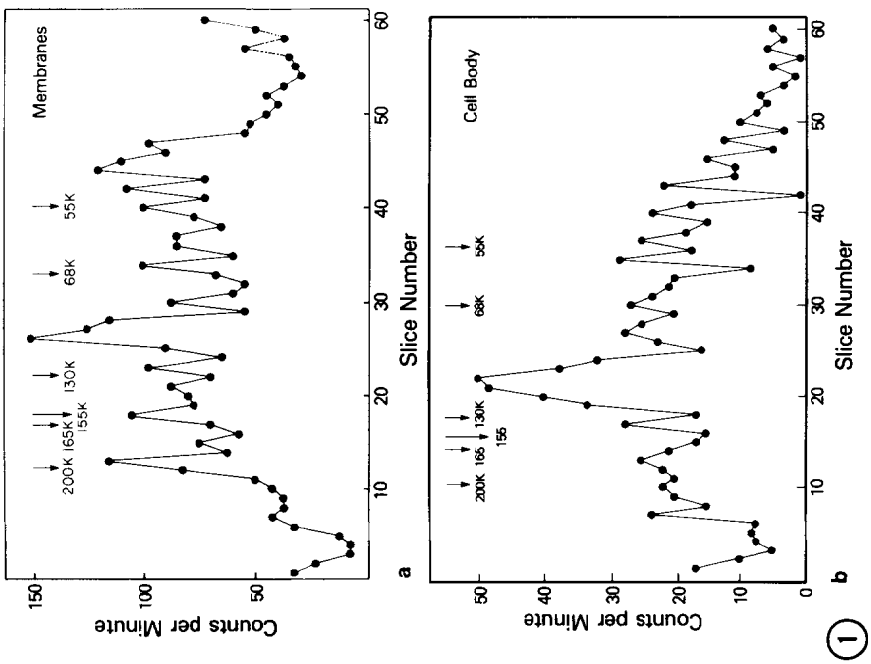
Other methods. Gradient polyacrylamide gel electrophoresis (7-14% or 5% polyacrylamide) was as described (10). The radioactivity was determined by slicing and counting the gels in a scintillation counter. The activities of AChE[1] and CAT[1] were measured as previously (5). Precipitation of the radioactive culture medium with heparin and all other methods have been described (11).

Materials. Veratridine was obtained from Aldrich. Scorpion venom and tetrodotoxin were obtained from Sigma. L-[^{3}H]fucose and ^{86}Rb (Gl 4.3 C/mmol and 6 mC/mg, respectively) were from New England Nuclear Corporation.

RESULTS AND DISCUSSION

High molecular weight glycoprotein from surface membranes. Surface membranes were isolated from human neuroblastoma cells (CHP-134) which were induced to differentiate in media containing 10% fetal calf serum and 2% DMSO. The membrane glycoproteins were labeled metabolically with L-[^{3}H]fucose and subjected to SDS[1] polyacrylamide gel electrophoresis. Figure 1 shows the distribution of the fucose-containing glycoproteins in the surface membranes and remaining cell preparation. A glycoprotein of approximate molecular weight of 200K was observed in the membrane preparations which was not seen to the same extent in the cell remains (2.2% vs 1.0% of the total radioactivity). Moreover, a glycoprotein of this molecular weight was found only in small amounts in the growth medium of these cells (less than 1200 cpm/100 ml of medium from 4 x 10^{7} cells). The presence of the protease inhibitor (0.5 mM p-toluene sulfonic acid) during the isolation procedures did not affect these results. This is in accord with our previous findings that a glycoprotein of similar molecular weight was present in membrane preparations of differentiated mouse neuroblastoma cells (11). Thus, by the criteria of the expression of the 200K glycoprotein, CHP-134 seems to be a highly differentiated human cell line. Adding further support is the face that a similar glycoprotein was found in only small amounts in the culture medium of either CHP-134 or the differentiated mouse neuroblastoma cells, but was present in large quantities in the medium of the non-differentiated mouse neuroblastoma cells (11).

Enzymes. CHP-134 became confluent at Day 8 to Day 10 after subculturing with the growth conditions used (Figure 2). At the same time, cells treated with



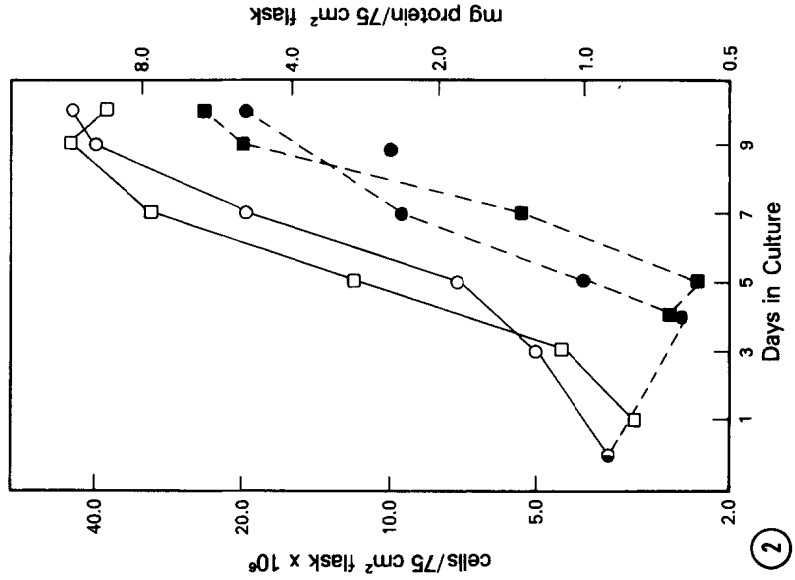


Figure 1. Polyacrylamide gel electrophoresis of (a) surface membranes and (b) cell bodies of human neuroblastoma cells (CHP-134) metabolically labeled with L-[^{3}H]fucose. The gels (7-14% polyacrylamide in 0.1% SDS and 2.5% mercaptoethanol) were sliced and the radioactivity determined in the scintillation counter. The markers were: chicken breast muscle myosin, 200K; RNA polymerase, 165K and 155K; β-galactosidase, 130K; phosphorylase A, 100K; and tubulin, 55K.

Figure 2. Growth curve of CHP-134 expressed as cell count with (●---●) or without (○ ○) 2% DMSO added to the complete medium at Day 3, expressed as mg of cell protein with (■---■) or without (□---□) 2% DMSO.

Table I

Effect of Veratridine, Scorpion Venom, and Tetrodotoxin on ^{86}Rb Efflux in Human Neuroblastoma Cells

Condition of culture[a]			Additions to the efflux assay[b]				
FCS	Inducer	Incubation	None	V	S	V+S	V+S+T
		Days	Rate constant (min^{-1}) x 10^{-2}				
10%	2% DMSO	1	0.6	1.2	0.8	2.7	0.8
		7	0.8	2.7	3.4	6.8	1.1
		8	0.7	--	--	7.9	--
		12	0.6	--	--	11.0	--
3%	2% DMSO	8	0.6	1.6	2.6	6.0	1.5
		12	0.6	--	--	6.9	--
3%	2.5 mM HMBA	4	0.6	--	--	3.1	--
		12	0.6	--	--	6.4	--
3%	none	4	1.1	--	--	5.1	--
10%	none	4	0.7	--	--	4.2	--

a. All cultures were grown for 3 days after plating in RPMI-1640 and 10% fetal calf serum (FCS); thereafter, the medium was changed and the incubation continued with conditions as indicated.

b. V, veratridine; S, scorpion venom; T, tetrodotoxin

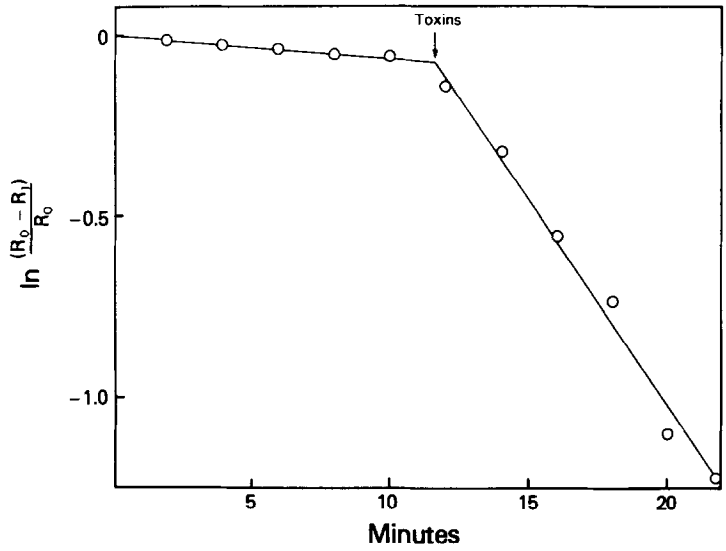


Figure 3. ^{86}Rb efflux through the Na^+ channel of differentiated human neuroblastoma cells (CHP-134). The ordinate is the fraction of isotope remaining in the cells at a particular time shown on the abscissa. Veratridine (100 μM) and scorpion venom (0.75 μM) were added as shown by the arrow. The points before the addition of the toxins represent the passive efflux and after the toxin addition, the active efflux.

DMSO were growth-arrested for 4-5 days. After that time, at least some of the cell population resumed growth. The amount of protein followed the cell numbers (Figure 2). During the period of growth arrest, the activity of AChE was the highest (1.6 nmol product/min/mg cell protein), while at latter stages the values dropped when growth began. The activity of the enzyme was inversely proportional to the growth rate. In the presence of 2% DMSO, the AChE activity was approximately 50% higher.

The activity of CAT, which was already high at the initial period, increased slowly with time. In the presence of 2% DMSO the activity was similar. We conclude that high levels of the enzyme existed during logarithmic growth and were elevated only to a small extent by the state of cell growth. Furthermore, this activity was independent of the presence of externally added inducing agents.

^{86}Rb efflux. Measurement of passive and active ionic flux through cell membranes is a useful tool to monitor the presence of Na^+ channels in the excitable membranes of cultured cells (9,12,13). Veratridine, scorpion venom and batrachotoxin activate ionic fluxes via tetrodotoxin-inhibited channels. A convenient way to monitor the development of Na^+ channels is by measurement of the efflux of ^{86}Rb activated by these neurotoxins (9). The active efflux of ^{86}Rb was measured in CHP-134 in the presence of veratridine and found to be two to three times higher than the passive efflux. Scorpion venom stimulated the efflux to a greater extent, while the action of both toxins was slightly synergistic (Table I). The active flux was inhibited by 1 μM tetrodotoxin and therefore supports the concept that ^{86}Rb efflux is mediated by the Na^+ channel (9). Figure 3 shows an example of the passive efflux and, in the presence of both toxins, the active efflux. The rate of the active efflux increased with time in culture and was even more pronounced when 2% DMSO was added to the cultures, particularly in medium containing 10% fetal calf serum (Figure 4).

The use of ^{86}Rb efflux as an indicator of membrane maturation in cells is shown further in Table I. Induction with HMBA appeared to be a longer

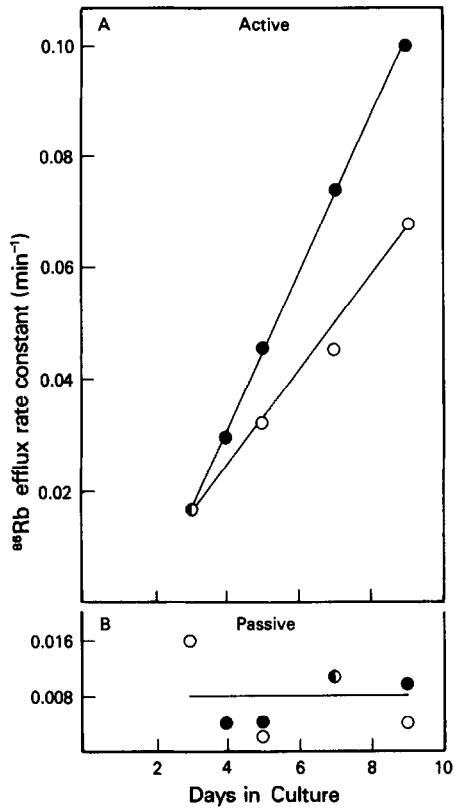


Figure 4. Development of toxin-induced ^{86}Rb efflux in human neuroblastoma cells with time in culture. The cells were cultured for 3 days, 2% DMSO was added to one-half the cultures and growth was continued with fresh medium containing 10% fetal calf serum added every 48 h with (●——●) or without (o---o) DMSO. The rate constants were derived from the efflux of ^{86}Rb measured on the specified days as described in Figure 3. A, active efflux; B, passive efflux.

process than with DMSO or serum alone. In 10% fetal calf serum and 2.5 mM HMBA, the cells did not remain attached to the culture dish so it was not possible to measure the efflux. Scorpion toxin may activate the Na^+ channel via a regulatory site (14); therefore it is interesting to note that the synergistic effect of veratridine and scorpion toxin was more pronounced in 3% fetal calf serum than in the 10% serum, suggesting a complementary effect of the serum.

Thus it seems that this human neuroblastoma cell line was well differentiated, and that the properties characteristic of differentiation were expressed even when the cells were actively growing. Finding the 200K glycoprotein in the human cells as well as the mouse neuroblastoma cells (11) suggests that this

glycoprotein may be neuronal-specific and thus important in the expression of the differentiated functions. Moreover, the appearance of this glycoprotein in concert with the active channels suggests that it could be directly involved with the ion flux. Of further significance from these studies is that cells in culture from a human tumor which maintained tumorigenic properties (3) also showed a high degree of neuronal characteristics, suggesting that the concept that a highly differentiated cell loses its tumor potential should be reconsidered.

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