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CELL CYCLE PHASES OF A NOVEL HUMAN NEURAL CELL LINE AND THE EFFECT OF EXOGENOUS GANGLIOSIDES

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Cell cycle phases of a new cell line (10-1) derived from a gemistocytic astrocytoma were analysed with computer-generated curves fit to the percentage of labeled metaphases at various times after a [^{3}H]-thymidine pulse. These large slowly growing tumor cells *in vitro* had a mitotic index of 2.8%, a confluent density of 34400 cells/cm^2 and an average DNA content of 7.91 pg/cell. Ganglioside treatment (50 uM in the culture medium) prolonged the generation time (26 hrs to 33 hrs) as well as all the phases of the cell cycle. The most noticeable effect of ganglioside exposure was to increase the G_1 phase by 40%. Therefore we conclude that exogenously added membrane components, such as gangliosides, can lengthen the gemistocytic astrocytoma cell cycle *in vitro* and modulate the proliferation of these neural cells.

INTRODUCTION

We already reported cell cycle studies of a normal fetal neural cell line (CH_I) and also a glioblastoma multiforme derived cell line (12-18) with a high growth fraction (1). In the present study, we measured for comparison the lengths of the cell cycle phases of a gemistocytic astrocytoma cell line (10-1) which we characterized as a slowly growing tumor *in vitro*.

There is some *in vitro* evidence that gangliosides (sialic acid - containing glycosphingolipids) which are components of the plasma membrane of many types of cells, are involved in cell contact recognition processes (2,3). When exogenously added to the culture medium, gangliosides become incorporated into the plasma membrane (4,5,6) and reduce both cell proliferation (7,8,9) and the rate of thymidine incorporation (9,10). We also report a lengthening of the 10-1 cell cycle phases by gangliosides exogenously added to the culture medium.

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MATERIALS AND METHODS

Cell culture. A cell line (10-1) was developed from a gemistocytic astrocytoma biopsy specimen of a 30 year old man, and maintained in culture in Eagles's Minimal Essential Medium (MEM) and 5% NCTC-135 supplemented with 10% fetal calf serum (FCS), 10 ml/l antibiotic - antimycotic mixture, 10 mg/l insulin and 2mM glutamine. Confluent cultures were split at 1:2 ratio in 75 cm^2 flasks after being washed once with phosphate buffered saline (PBS) containing 1mM EDTA and treated with 0.25% trypsin in MEM-PBS (1:1) for 5 minutes at room temperature. Cell counts were performed using a hemocytometer. After seeding, the medium was changed every 3 days.

DNA quantitation. DNA content was measured by a fluorometric technique using 3,5 -diamino benzoic acid dihydrochloride (DABA) as previously reported (1). Results are the means of 4 determinations per time point.

Ganglioside preparation. A mixture of gangliosides was isolated from normal human cerebral cortex as described elsewhere (3). This ganglioside preparation was taken to dryness, dissolved in MEM to a final concentration of 50 uM and filtered through a 0.2 um filter. Under these conditions, 80% of the gangliosides pass through these filters (10). Control medium was also filtered. Gangliosides did not affect the pH of the medium. The ganglioside concentration was chosen to reflect the physiological serum concentration observed in patients with some malignancies (11-12).

Cell cycle analysis. Cells were grown on coverslips in 60 mm diameter petri dishes. The percentage of labeled metaphases (PLM) method (13) was used following a 20 minute pulse label with 1 uCi/ml [^{3}H]-thymidine, (spec. act. 6.7 Ci/mmol.). Cultures were incubated for up to 40 hrs. in chase medium containing 2 uM unlabeled ThdR, 10% FCS and 50 uM gangliosides for the treated cells only. The autoradiographic procedure has been described elsewhere (1). Values are the mean of 2 slides (25 metaphases per slide).

RESULTS

When 10-1 cells, passage 6, were plated at 5000 cells/cm^2, they reached confluency in 13 days at a density of 34400 $\pm$ 850 cells/cm^2. During the log phase, the population doubling time was approximately 76 hrs. and the mitotic index (Mi) 2.8%. DNA content analysed at 5 time points along the growth curve was 7.91 $\pm$ 0.88 pg/cell.

The 10-1 cell cycle analysis was done with an asynchronous cell population at a density of 19380 $\pm$ 830 cells/cm^2. In order to obtain the best fit cell cycle curve of the 10-1 cells, two analyses were used: (1) the number of labeled metaphases was regressed on time elapsed and the curve with the largest R-square and the smallest mean square values were chosen, and (2) a parabola was fit to the first wave of labeled metaphases by multiple regression analysis using a quadratic model, the curve with the largest R-square was picked and the equation set was solved for the value of 50%

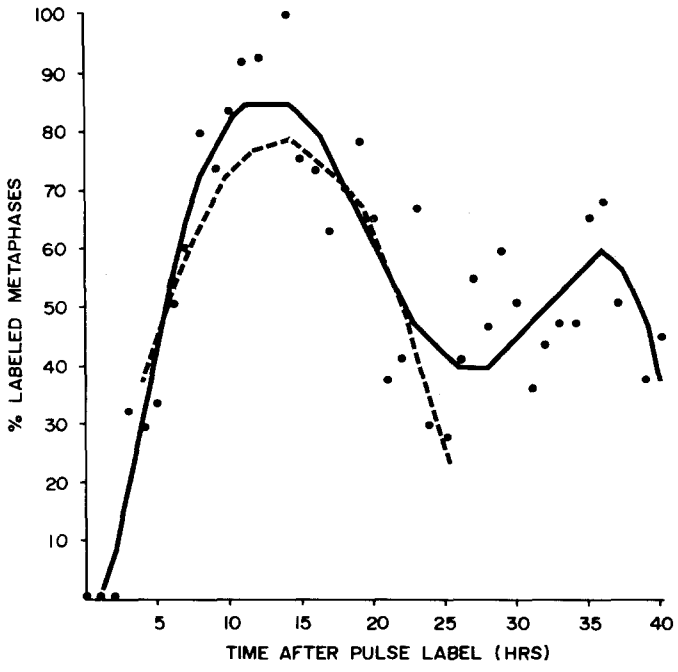

Figure 1 = The percentage of labeled metaphases of 10-1 cells were regressed on time elapsed. A 5th order model was fit to the data R^2=0.8131 (——). Multiple regression analysis of the PLM curve up to 26 hrs was determined using a quadratic model R^2=0.4094 (- - - -).

maximum labelling. Using the first method, the generation time (GT) of the 10-1 cells not exposed to ganglioside was 26.0 hrs, GT is considered to be the time between the addition of the label and the lowest point of the descending limb of the PLM curve (Figure 1). The second method was used to calculate the length of the S phase. The best fit parabola was obtained when the PLM values up to 26.0 hrs were analysed. The equation for the 50% value gave an estimate of 16.4 hrs for the S phase. The G_2 phase, time between the addition of the label and the 50% value of the first ascending limb of the PLM curve, was 5.5 hrs. The mitotic length (tm) was 1.0 hrs, calculated as follows: $t_m = \frac{Mi \times GT}{0.693}$ (14). The G_1 length was 3.2 hrs calculated by the difference between GT and the sum of S, G_2 and tm. The PLM curve of the gangliosides treated 10-1 cells is shown Figure 2, the lengths of the phases of the cell cycle were the following: GT = 33.0 hrs; S = 20.4 hrs; G_2 = 6.0 hrs; tm = 1.3 hrs; and G_1 = 5.3 hrs.

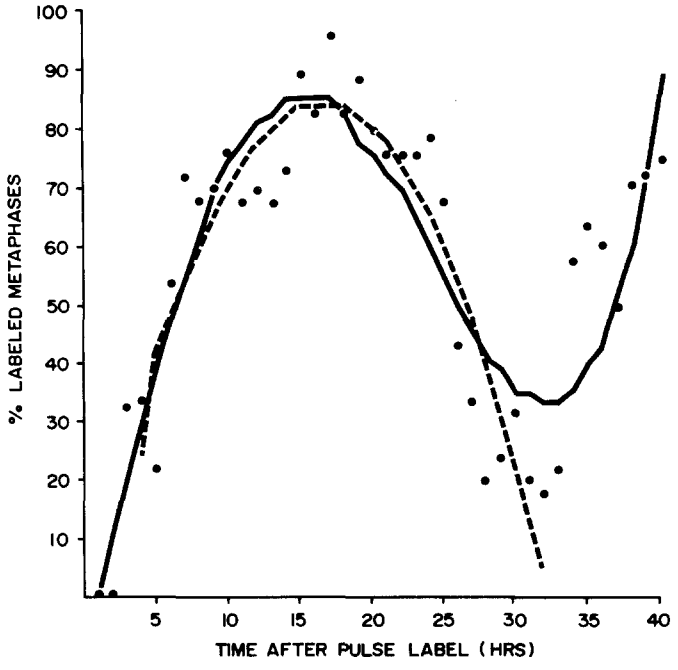

Figure 2 = The percentage of labeled metaphases of 10-1 cells treated with gangliosides were regressed on time elapsed. A 4th order model was fit to the data R^2=0.7827 (——). Multiple regression analysis of the PLM curve up to 33 hrs was determined using a quadratic model, R^2=0.7756 (- - - -).

DISCUSSION

Johnson et al. (15) have observed in vivo that glioblastoma multiforme cells were apparently dividing more slowly than were non-neoplastic neural cells. An astrocytoma-glioblastoma comparative study of human patients showed that the astrocytoma cell population increased at a slower rate with a lower labeling index and a longer turnover time (16). These authors have characterized an astrocytoma by its low growth fraction and insignificant movement of G_0 cells into the proliferating pool. In vitro studies also confirmed that astrocytoma cultures proliferated at a lower rate than glioblastomas (17) suggesting that many of the astrocytoma cells are in a nonproliferative pool (16). Our results have shown that cells derived from an astrocytoma (10-1) not only have a lower percentage of cells in the proliferative pool (data not shown) but a lower mitotic index (0.028 vs 0.043) compared to glioblastoma cells (1). However we observed that the G_1 periods of the two cell lines (10-1 and glioblastoma multiforme 12-18) were very

similar to the normal fetal cell line (CH_I) and were respectively, 3.1, 3.3 and 3.4 hrs (1) implicating that all these human neural cell lines retained a degree of topoinhibition in culture. We have previously reported that the generation time and S phase in glioblastoma multiforme cells were longer than in the fetal brain cells (respectively 30.0 vs. 25.0 hrs and 21.0 vs. 17.0 hrs) (1) in agreement with Westermark (18). Thus the length of S phase (DNA synthesis) in these cells may be related to the DNA content since 10-1 cells and fetal brain cells have about half the amount of DNA of a glioblastoma cell line (1).

We analyzed the present results statistically with computer-generated curves fit to the points which represent the percentages of metaphases labeled at various times after the [^{3}H]thymidine pulse. For cell cycle analysis, several methods have been used such as the computer analysis by Takahashi et al. (19) or manual determinations (13,14,20). Regardless of model used, the second wave of the PLM curve was always attenuated and therefore difficult to interpret. This could be due either to a loss of synchrony of the labeled cell population, or to low labeling intensity in the 2nd wave (21). Although all phases of the cell cycle of the 10-1 astrocytoma cells were prolonged after exposure to gangliosides, our most significant result was that the cells were delayed in the postmitotic phase since G_1 was extended by 2.2 hrs or 40%. Indeed, we have reported that gangliosides decreased the rates of cell proliferation and thymidine incorporation in human neural cells (9).

In conclusion, the lengths of human brain tumor cell cycle phases can be determined in culture and in cells obtained from a gemistocytic astrocytoma biopsy, the phases of the cell cycle could be manipulated by the addition of gangliosides to the growth medium. More studies need to be done to determine the functional significance of our observations concerning the effects of membrane components (gangliosides) on lengthening of the cell cycle phases of human neural cells as gangliosides have been determined to have membrane receptor activity (22).

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