CELL SURFACE CHANGES CAUSED BY GROWTH OF B16 MELANOMA CELLS IN BROMODEOXYURIDINE

Irene EVANS, Peter DiSTEFANO, Kenneth R. CASE and H. Bruce BOSMANN

Department of Pharmacology and Toxicology and *University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, USA*

Received 6 April 1977

1. Introduction

Most normal cells have cell surface properties different from their transformed or oncogenic counterparts (reviewed in [1]). While tumor cells will grow without being attached to a substratum, most untransformed cells require a surface to which they can adhere in order to grow [2,3]. Variations in cell adhesion mediated by changes in the cell surface could play a determinant role in the regulation of cell growth and development as well as in the mechanism of oncogenesis.

Bromodeoxyuridine (BrdUrd), a thymidine analogue, exerts a selective and reversible effect on specific cellular differentiative functions and oncogenic potential (reviewed in [4] and [5]). Although both DNA-linked and non-DNA-mediated effects of BrdUrd have been described [4], the mechanism of action of this drug is unknown. While much information has been gathered on the effects of BrdUrd on DNA structure, much less attention has been paid to BrdUrd's effects on the plasma membrane. In order to evaluate quantitatively the effect of BrdUrd on the plasma membrane, adhesivity of mouse melanoma cells was measured as well as their electrophoretic mobility and aqueous polymer partitioning behavior. The results show that BrdUrd treatment greatly increases the adhesivity of these cells without changing their electrophoretic mobility. Treatment with BrdUrd does, however, change the partition ratio of these cells in aqueous two-phase dextran–polyethylene glycol suggesting that basic alterations of the cell surface have occurred.

2. Materials and methods

Melanoma cells of the B16 line were grown in glass culture bottles as previously described [6]. Cultures were incubated at 37°C with 5% CO_2 and passaged by treatment with 0.025% trypsin solution. Bromodeoxyuridine and thymidine solutions were made up in 0.01 M phosphate buffered saline, pH 7 (PBS) and sterilized by passage through 0.22 μM Millipore filters. Solutions were stored at 0°C for no more than 1 month with drug concentrations monitored periodically as previously described [6].

Adhesivity studies were performed two days after BrdUrd or TdR (at 3 μg/ml media) was added. The media was removed, cells were washed twice with PBS and cultures incubated for varying time periods with 0.025% trypsin, or 0.20% EDTA in PBS. At specific time periods, duplicate cultures were gently rocked 5 times, and medium and detached cells collected, pipetted to disperse aggregates and counted using a Coulter model Z_B cell counter.

Determinations of the electrophoretic mobility of cells were made as described previously [7]. Cells, detached from the monolayer with 0.2% EDTA, were electrophoresed in a medium consisting of 0.0145 M NaCl, 4.5% sorbitol, 0.6 mM $NaHCO_3$, pH 7.2. Each determination consisted of 20–30 observations of mobility with a reversal of polarity between each observation.

Cells, removed from the monolayer as for electrophoretic mobility measurements, were partitioned in a

two-phase aqueous polymer system essentially as described by Gersten and Bosmann [8]. The system consisted of 5% (w/w) Dextran T 500 (Pharmacia, Lot #3936) and 4.0% (w/w) polyethylene glycol (Carbowax 6000, Union Carbide) dissolved in 0.1 M phosphate buffer (pH 6.98) containing 0.05 M NaCl. Cells were partitioned in total vol. 10 ml polymer solution which was allowed to stand for 1 h at 4°C. The number of cells in the total system before partition and the number in the top polymer phase after partition were determined using a Coulter Counter model Z_B equipped with 100 μm aperture. Partition ratios were expressed as the number of cells in the top phase divided by the number of cells in the total system.

3. Results and discussion

B16 cells treated with BrdUrd appear to flatten and adhere more tightly to the growth support [5,6]. In order to quantify their increased adhesivity, the detachability of the cells was measured after trypsin or EDTA solutions were added to remove them. These solutions are thought to detach cells by hydrolyzing cell-surface adhesive proteins or by complexing cations needed for attachment. When cells are treated with these solutions, a clear measurable difference in the number of BrdUrd treated cells detached in comparison to thymidine treated or control cells is found (fig.1). The control or thymidine treated cells show >60% detachment after 30 min treatment with trypsin or 50% detachment after EDTA incubation in contrast to <20% detachment respectively for the BrdUrd-treated cells during the same time period. These differences persisted when 10-fold higher concentrations of trypsin were utilized (data not shown). These results clearly show that the BrdUrd-treated cells are more difficult to release from their growth plate most likely due to an increased surface adhesivity equal to 2–3 times of control cells.

One property that is often altered between transformed and non-transformed cells is the charge on the cell surface. Transformed and highly oncogenic cells have often been found to have a higher surface net negative charge than their non-transformed or normal counterparts [9]. In order to determine whether BrdUrd treatment altered net surface charge the electrophoretic mobility of BrdUrd, TdR, or non-treated cells was determined by observing the electrophoretic mobility

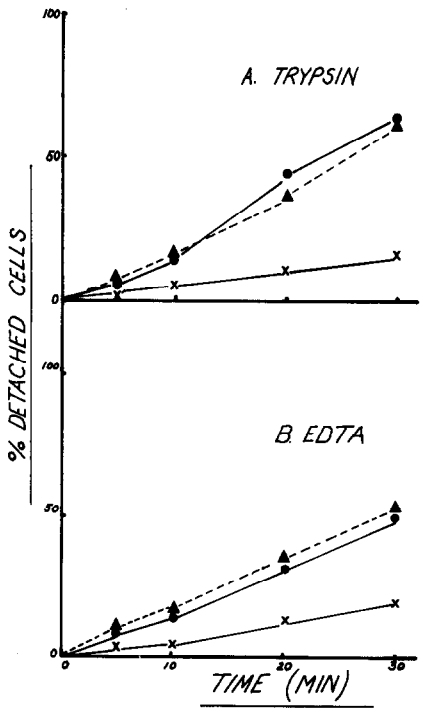

Fig.1. Kinetics of detachment of cells with trypsin and EDTA. (A) Cells previously treated with TdR or BrdUrd, 3 μg/ml media, for 2 days were removed from the monolayer using 0.025% trypsin for varying periods of time and detached cells counted in a Coulter Counter as described in Materials and methods. Total cell number in control cultures averaged 5×10^5 cells/55 cm^2 surface area culture bottle. Data represents mean of 2 experiments, each performed using duplicate bottles. (B) Detachment studies were performed as described above using 0.20% EDTA. Control cells (o-o-o), TdR-treated (△-△-△), BrdUrd-treated (x-x-x).

of cells in an electric field. The results, shown in table 1, show no appreciable change in electrophoretic mobility after treatment of cells with BrdUrd. Thus, no appreciable change in cell surface charge properties is detectable using this method.

Several studies have shown that increases in cell electrophoretic mobility are often accompanied by increasing cell partitioning suggesting that surface charge can be an important factor in both these separations. Several exceptions to this rule have been documented, however, such as the increasing partition, but not electrophoresis of stored human erythrocytes as compared to freshly drawn samples [10] and the

Table 1
Electrophoretic mobility of cells

Treatment	Electrophoretic mobility ($\mu m^{-1} . V^{-1} . s^{-1} . cm$)
None	2.14 ± 0.11
TdR (3 μg/ml)	2.01 ± 0.07
BrdUrd (3 μg/ml)	2.07 ± 0.10

Experiments were performed as described in the Materials and methods section. Values are means ± SEM for cells treated for 2 days with BrdUrd or TdR. Each mean is a value determined from 60 observations in 3 independent experiments.

separation of beef erythrocytes having low, intermediate or high partition ratios, but identical electrophoretic properties [11]. Since these latter classes of cells release differing amounts of sialic acid, the authors conclude that the partition method measures membrane associated charges buried deeper in the membrane than the cell-mobility method which can only detect charges present at the point of shear. Other evidence also suggests that cell partitioning, but not electrophoretic mobility can be affected by changes in fatty acid membrane components [12].

In order to determine whether surface alterations could be detected by this technique, BrdUrd-treated, TdR-treated and control cells, washed twice with PBS, were partitioned in the aqueous system described in Materials and methods. The data shown in table 2 indicates that much fewer (about 3/4 as many cells) of the BrdUrd-treated cells are able to move into the polyethylene-enriched top phase than the TdR treated or control cells. When partitioning was carried out in higher salt concentrations, (0.07 M) consistent differences in partition cells was also found (data not shown). These data make clear the BrdUrd does alter the surface properties of cells and suggests that the surface of BrdUrd-treated cells may be physically and/or chemically distinct from control cell populations due to an altered topology and possible alterations in charged surface groups.

Table 2
Partitioning of cells in dextran–polyethylene glycol system

Cells	Partition ratios	% Control
Untreated	0.358 ± .08	100%
TdR	0.374 ± .07	104%
BrdUrd	0.277 ± .06	77.4%

Cells were partitioned as described in Materials and methods. Cells were treated with BrdUrd or TdR at 3 μg/ml for 2 days. The partition ratio is expressed as top phase cell number divided by the total system cell number. Each mean (± SE) is a value determined from 70 observations in 7 independent experiments.

The mechanism by which BrdUrd blocks both differentiation and oncogenicity of melanoma and other cells is unknown. It has been shown in several systems that BrdUrds block of the induction of differentiation and the drug's suppression of tumorigenicity require incorporation of BrdUrd into the cellular DNA [4,5]. Therefore, if BrdUrd exerted its effects on differentiation and oncogenicity at some step via the outer cell membrane, one likely possibility is that incorporation of BrdUrd prevents the synthesis of specific classes of membrane proteins required for cellular differentiation and/or oncogenicity. These surface modifications could alter cellular function in at least two ways. First, BrdUrd treatment may alter the cell surface so that expression of neoantigens (embryonic, viral, or cellular) is allowed leading to changes in the treated cells immunogenicity. These changes may allow the host's immune system to more effectively attack and destroy BrdUrd-treated cells. Secondly, BrdUrd treatment may alter membrane properties so that growth of the cells in the host and the expression of the differentiated state is prevented. Recent work have centered on the possibility that membrane alterations may be involved in the control of cellular differentiation and malignant transformation [13,14]. Previous work has shown that BrdUrd treatment alters the synthesis of a putative membrane glycoprotein in neuroblastoma cells [15] and alters the biochemistry of the surface mucopolysaccharide coat of melanoma cells [16]. The coordinated suppres-

sion of melanogenesis and tumorigenicity by BrdUrd should be useful in determining what role surface macromolecules play in regulating cellular growth, differentiation and oncogenicity.

Acknowledgements

This work was supported in part by grants from USNCI and USNIGMS. Dr Bosmann is a Scholar of the Leukemia Society of America.

References

[1] Wallach, D. (1975) in: Membrane Molecular Biology of Neoplastic Cells, Elsevier, Amsterdam.
[2] Stoker, M., O'Neill, C., Berryman, S. and Waxman, V. (1968) Int. J. Cancer 3, 683–693.
[3] Freedman, V. H. and Shin, S. (1974) Cell 3, 355–359.
[4] Rutter, W. J., Pictet, R. L. and Morris, P. W. (1973) Annu. Rev. Biochem. 42, 614–621.
[5] Silagi, S. (1976) Int. Rev. Cytol. 45, 65–111.
[6] Evans, I. and Bosmann, H. B. (1977) Exp. Cell Res. in the press.
[7] McLaughlin, J., Case, K. R. and Bosmann, H. B. (1973) Biochem. J. 136, 919–926.
[8] Gersten, D. M. and Bosmann, H. B. (1974) Exp. Cell. Res. 87, 73–78.
[9] Ambrose, E. J., James, A. M. and Lowick, J. H. B. (1956) Nature 177, 576–577.
[10] Brooks, D. E., Seaman, G. V. F. and Walter, H. (1971) Nature 234,61–62.
[11] Walter, H., Tung, R., Jackson, L. J. and Seaman, G. V. F. (1972) Biochem. Biophys. Res. Commun. 48, 565–576.
[12] Walter, H., Krob, E. J. and Brooks, D. E. (1976) Biochem. 15, 2959–2963.
[13] Lyman, G. H., Preisler, H. D. and Papahadjopoulos, H. D. (1976) Nature 262, 360–363.
[14] Pollack, R. E. and Hough, P. V. C. (1974) Ann. Rev. Med. 431–446.
[15] Brown, J. C. (1971) Exp. Cell Res. 69, 440–442.
[16] Satoh, C., Kreider, J. W., Banks, J., Garlick, S. and Davidson, E. (1975) Cancer Res. 35, 2532–2539.