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DECREASE IN ACTIN GENE EXPRESSION IN MELANOMA CELLS COMPARED TO MELANOCYTES IS PARTLY COUNTERACTED BY BrdU - INDUCED CELL ADHESION AND ANTAGONIZED BY L-TYROSINE INDUCTION OF TERMINAL DIFFERENTIATION

*Luis A. Gómez +, Mary Strasberg Rieber and Manuel Rieber **

I.V.I.C., Tumor Cell Biology Laboratory
Apdo. 21827, Caracas 1020 A, Venezuela

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Malignant transformation is frequently accompanied by changes in the cytoarchitecture of adherent cells, which may be influenced by fluctuations in actin gene expression. We now show that normal melanocytes express a 5 fold higher level of actin mRNA than their melanoma counterparts. Induction of terminal melanogenesis did not increase actin in melanoma cells. However, culture with the thymidine analog, Bromodeoxyuridine, increased actin expression in undifferentiated but not in differentiating melanoma. Cell detachment assays and cell shape comparisons revealed a direct correlation of actin mRNA with increased melanoma cell adhesion rather than with differentiation-mediated suppression of tumor growth.

Growth of highly invasive B16 melanoma BL6 cells with the thymidine analog, bromodeoxyuridine (BrdU) increases susceptibility to UV radiation (1), augments cell-substratum interactions in vitro and decreases extra-pulmonary metastasis in vivo (2). Also, human melanoma cultures respond to BrdU by increasing cell adhesion, lowering anchorage-independent growth in soft agar and decreasing tumorigenicity in nude mice (3). Since our laboratory is interested in identifying genes differentially expressed with melanocytic malignancy and suppression of melanoma cell growth, we now investigated whether the effects of BrdU on melanocytic cell shape and invasive behaviour involve changes in actin gene expression, since this gene is known to influence cytoarchitecture and cell adhesion (4). In a comparison of normal murine melanocytes and their malignant melanoma counterparts, we now show that the tumorigenic cells exhibit a 5 fold decrease in actin mRNA expression. This is partly counteracted by culturing melanoma cells with BrdU (1-3). However, actin transcription was not increased

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* Corresponding author; Fax: (582) 501 1382; email: mrieber@pasteur.ivic.ve; supported by CONICIT Grant S1-2667.

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by L-tyrosine which also suppresses melanoma growth by induction of terminal differentiation (5). Moreover, concurrent BrdU treatment and tyrosine treatment prevented the increase in actin expression observed in cells treated only with BrdU.

MATERIALS AND METHODS

a) Cells and tissue culture

Normal melanocytes Melan-A from C57/BL6 mice, dependent on phorbol esters and 10% serum to grow (6), were obtained from Dr. Dorothy C. Bennett, St. George's Hospital Medical School (London, England); BL6 melanoma cells also from C57/BL6 mice, responsive to induction of terminal differentiation and/or BrdU, were cultured in Dulbecco's medium plus 10% serum, including 2.5 μg/ml BrdU for 3 days (1), and for 2 mM L-tyrosine to induce differentiation, as previously described (5).

b) RNA preparation and Northern blots. Total RNA was prepared by the Trizol reagent protocol (BRL Life Technologies), fractionated under denaturing conditions in agarose containing formamide and MOPS buffer as described by Maniatis et al. (7) and blotted with alkaline conditions (8) onto Hybond N (Amersham) followed by UV fixation. Comparison of RNA loading in the various samples was assessed by UV analysis of Ethidium bromide-stained gels and detection of actin transcription was carried out with an actin probe (from ATCC), kindly provided by Dr. K. Brew, University of Miami, Biochemistry Department. Probe labelling with $dCTP^{32}$ was carried out with the Megaprime DNA labelling kit RPN 1607 batch 78 from Amersham, U.K. c) Densitometry was carried out with the NIH Image 1.54 software and a Macintosh computer.

RESULTS

BrdU restores cell adhesion in unpigmented but not in pigmented melanoma.

Cell adhesion measured by the relative detachment of cell cultures by 10 microgram/ml of trypsin, revealed that BrdU significantly increased melanoma cell adhesion, but this was essentially unaffected in the same cells treated with L-tyrosine (Fig 1, upper). Moreover, concurrent addition of L-tyrosine and BrdU prevented the BrdU effect on cell adhesion, but did not prevent pigmentation (Fig. 1). Relative detachment of normal melanocytes with trypsin was about 10 to 15%, resembling that of BrdU-treated Bl6 melanoma (not shown).

Decrease in actin expression in melanoma cells is partly counteracted by BrdU.

When comparable amounts of total RNA from normal melanocytes and malignant melanoma (Fig. 2, left, upper) were hybridized with actin cDNA, weak signals were detected in untreated melanoma compared to melanocytes (Fig. 2, lanes C and Mel). However, significant restoration of actin expression was detected in melanoma cells cultured for 3 days with BrdU. On the other hand, induction of terminal

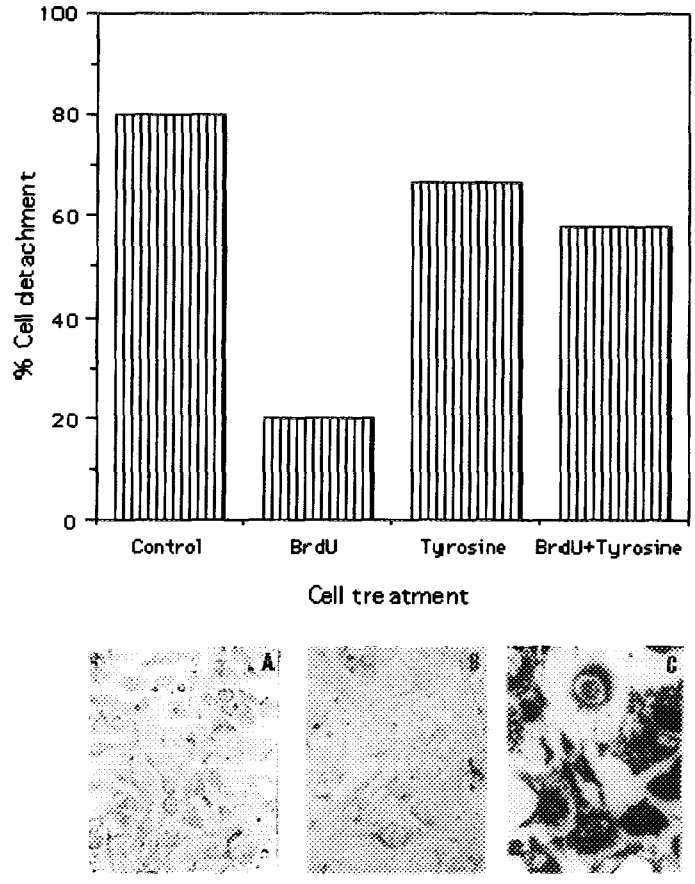

Figure 1. BrdU-induced decrease in B16 melanoma cell detachment is counteracted by L-tyrosine.

Upper, Cells were cultured for 3 days including 2.5 μg/ml BrdU or 2 mM L-tyrosine whenever indicated, to examine relative cell detachment in triplicate assays, using a 10 min treatment with 10 micrograms/ml of trypsin in isotonic saline .
Lower, Comparative cell adhesion of: A) B16 melanoma; B) B16 melanoma plus BrdU; C) B16 melanoma with BrdU plus tyrosine.
Note preferential cell spreading in B) .

differentiation leading to increased pigmentation, failed to increase actin expression by itself and counteracted the BrdU induced increase in actin RNA levels. A comparative quantitation of actin levels is shown in lower part of Fig. 2, indicating that BrdU increases actin expression by 2-2.5 fold in B16 melanoma ,effect counteracted by culture with L-tyrosine (lower Fig. 2). On the other hand, normal melanocytes showed about a 5 fold greater actin mRNA level.

Actin expression is susceptible to UV radiation in BrdU-sensitized cells.

Since the thymidine analog BrdU is also a radiation sensitizer (1), we investigated the effect of subsequent UV radiation on BrdU-treated B16 melanoma.

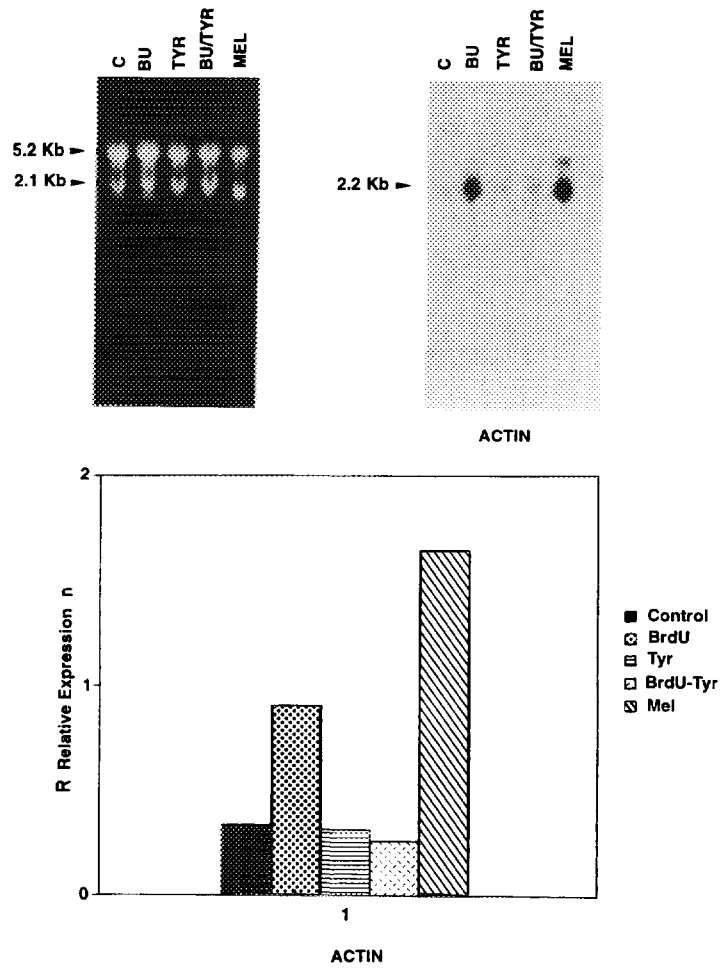

Figure 2. *Preferential Actin gene expression in melanocytes and BrdU-treated melanoma.*

RNA was extracted electrophoresed and blotted as indicated under Materials and Methods for hybridization with an actin probe.
Upper Left, EtBr profiles showing comparable loading in all samples.
Upper Right, preferential hybridization of actin probe to RNA from BrdU-treated cells (Bu) and from melanocytes (Mel).
Bottom, densitometric quantitation showing lower actin expression in RNA from pigmented cells treated with tyrosine (Tyr) or those treated with both tyrosine and BrdU (Bu + Tyr).

In agreement with Fig. 2, we confirmed the upregulating effect of BrdU on actin expression. However, the latter was found to be UV sensitive when BrdU-treated cells were exposed to 25 J/m², when compared to the expression of the GAPDH gene. This suggests that UV irradiation increases DNA damage and antagonizes the BrdU-induced increase in actin levels (Fig. 3).

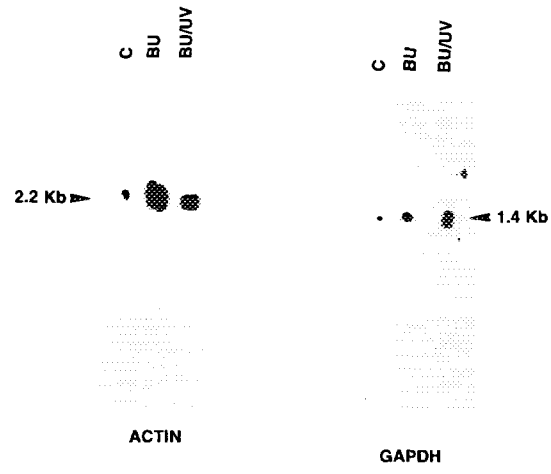

Figure 3. Actin mRNA is sensitive to UV radiation in BrdU-sensitized cells.

Blots containing comparable amounts of total RNA, as estimated in Fig. 2, were hybridized to actin cDNA or to GAPDH cDNA.
Note decreased actin signal in UV/BrdU treated cells compared to that of GAPDH.

DISCUSSION

In agreement with recent data in keloid fibroblasts showing that X-irradiation lowers actin mRNA (9), we have now shown that UV also decreases actin levels in BrdU-sensitized B16 melanoma. This may be due to the fact that the latter conditions promote significant DNA damage which may decrease the level of the thymidine analog, BrdU in the DNA of B16 melanoma. Also, concurrent exposure of these cells to BrdU which increased cell adhesion and L-tyrosine which promotes terminal differentiation, counteracted the effects of the pyrimidine analog in increasing both actin expression and cell adhesion. These results may be explained by the fact that terminal differentiation favours an early cell cycle withdrawal (5) which may lower the level of BrdU substitution in melanoma genomic DNA. This explanation is compatible with our control experiments in which we observed that a minimum of 2 cell doublings (3 day exposure) is required for an optimal effect of BrdU in increasing actin gene expression and cell adhesion. Our findings that actin gene expression is decreased in B16 melanoma compared to their parent melanocytes, and its up regulation by BrdU, identify actin as one of the genes targetted by BrdU to promote melanoma growth arrest both in vivo and in vitro (1-3). These studies showing a decrease in actin mRNA

in melanoma vs melanocytes and its upregulation in melanoma by BrdU coincide with the growth suppressing effects of this thymidine analog on human and mouse melanoma (1-3). These data implying actin as an important gene in melanoma growth, are also compatible with recent results indicating that actin expression is inversely correlated with melanoma metastatic potential (10).

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