

## LAMININ AND FIBRONECTIN INTERACTIONS WITH HUMAN MELANOMA CELLS: EFFECT OF GLUCOCORTICOIDS

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The mechanism of tumor cell invasion appears to be a highly complex and multistage phenomenon. One necessary step in the invasive process is the interaction of tumor cells with the extracellular matrix, mediated by specific cell receptors (L. A. Liotta, 1984, *Am. J. Pathol.*, 117: 339-348).

Receptors for extracellular matrix proteins called integrins, have been described as transmembrane glycoproteins consisting of two associated subunits ( $\alpha$  and  $\beta$ ), presenting a Mr of 110,000 —

200,000 and low affinity (dissociation constant  $K_D = 10^{-8} - 10^{-7}M$ ). Laminin (LM) and fibronectin (FN) integrin receptors belong either to the subfamily of very late antigens (VLAs) ( $\beta_1$  subunit) or to the cytoadhesin subfamily ( $\beta_3$  subunit) (R. O. Hynes, 1987, *Cell*, 48: 549-554).

Previous studies have reported that integrins appear to be important in melanoma cell lines metastatic capacity (R. H. Kramer, 1989, *J. Biol. Chem.*, 264: 15642-15649). The melanomas'

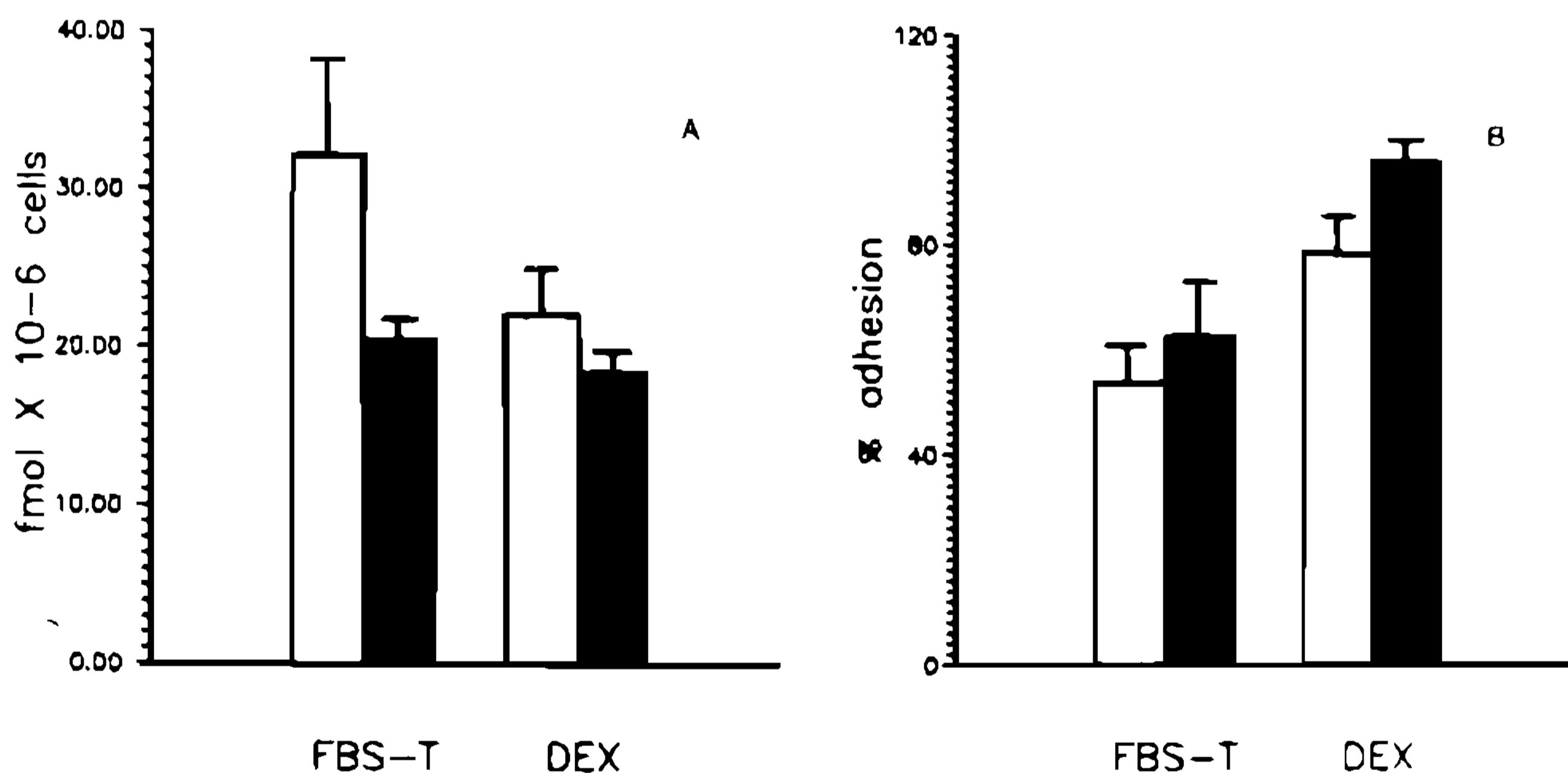


Fig. 1: effect of dexamethasone on LM and FN receptor levels and adhesion capacity in MEL-85 cells. A: receptors were determined by direct binding of <sup>125</sup>I-LM (3.0 nM) (□) and <sup>125</sup>I - 120 fragment of FN (8.0 nM) (■) to cells treated with FBS-T (FBS minus endogenous steroids) or 10<sup>-7</sup>M dexamethasone for 3 days. B: cells cultivated for 3 days in the presence of FBS-T or 10<sup>-7</sup>M dexamethasone were labelled with <sup>3</sup>H Leucine and then plated in 500  $\mu$ l aliquots (5 x 10<sup>4</sup> cells) on LM (□) or FN (■) coated wells (24 multiwell plates, Costar) (10  $\mu$ g/ml) and incubated for 1 h at 37°C in 3% CO<sub>2</sub>. Bars are mean  $\pm$  SD of 5 experiments.

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progression has been described as steroid hormones dependent, although the effect of these hormones seems sometimes contradictory. Several investigators have demonstrated a growth inhibition of human melanoma cell lines by glucocorticoids (M. J. Walker, 1988, *Semin. Oncol.*, 15: 512-523). On the other hand, glucocorticoids used in melanoma therapy seems to be associated with exacerbation of metastatic melanoma (A. D. Varella et al., 1981, *Cancer*, 48: 1353-1362).

In view of these data, the human melanoma cell line MEL-85 was used to verify the effects of dexamethasone on the expression of LM and FN receptors and their relationship with cell adhesion capacity on LM and FN.

The characterization of LM and FN receptors in MEL-85 cells cultivated with RPMI-1640 medium and 5% FBS (fetal bovine serum) was demonstrated by direct binding of cells to iodinated LM (0.2 — 20.0 nM) or to the 120 kDa fragment of FN (0.2 — 40.0 nM) in the presence or absence of a 100-fold excess of unlabeled LM or 120 kDa fragment respectively. The sigmoidal saturation curves produced, suggests the existence of at least two LM and FN binding components. Hill analysis showed a biphasic pattern and Hill coefficients of  $n_{H1} = 0.4$  for the first and  $n_{H2} = 1.8$  for the second component to LM binding suggesting that the latter displayed positive cooperativity. The estimated  $K_D$ s from the Hill plot were 23.0 nM and 65.0 nM for the first and second components respectively. Hill analysis of the FN binding data indicated also a two components system similar to that observed in LM binding. The second component showed positive cooperativity ( $n_{H2} = 1.6$ ), and a  $K_D = 161.0$  nM, but presented a lower binding capacity ( $73.0 \pm 4.0$  fMol  $\times 10^{-6}$  cells) than that observed with LM ( $412.0 \pm 42.0$  fMol  $\times 10^{-6}$  cells).

Preliminary results in the molecular weight LM receptor determination were obtained by subjecting octylthioglucoside extracts of MEL-85 cell lysates to affinity chromatography on LM-Sepharose. The eluted material analyzed by SDS-PAGE and transferred onto nitrocellulose, showed a band with apparent Mr of 116,000 — 130,000 recognized by a rabbit polyclonal antibody raised against the human FN receptor  $\beta_1$  chain (kindly provided by Dr K. Yamada, NIH, Bethesda).

The fact that an antibody to the  $\beta_1$  component of integrins reacts with 116 — 130 kDa band and the low affinity displayed by direct binding of labeled LM with apparent  $k_D$  values in the same order of magnitude as that reported for integrins, sup-

ports the evidence that MEL-85 cells LM receptor is a  $\beta_1$  integrin.

In order to verify the effects of glucocorticoid on the cell-extracellular matrix proteins interaction, LM and FN receptor levels were determined by direct binding using a single labeled LM (3.0 nM) or FN (8.0 nM) concentration in MEL-85 cells previously treated with  $10^{-7}$ M dexamethasone. Cells cultivated in 5% FBS-T (steroid hormones depleted with dextran-charcoal) (control) presented a LM receptor capacity of  $31.8 \pm 7.3$  fMol  $\times 10^{-6}$  cells. The values of these receptor levels in cells treated with dexamethasone were not statistically different from the control. In the same way, dexamethasone did not alter the FN receptor levels presented in control ( $20.5 \pm 1.0$  fMol  $\times 10^{-6}$  cells) (Fig. 1A).

However, MEL-85 cells labeled with  $^3$ H leucine ( $5 \times 10^6$  cpm  $\times 2 \times 10^{-6}$  cells) previously treated with glucocorticoid showed different adhesion capacity on tissue culture plastic dishes coated with  $10 \mu\text{g/ml}$  of LM or FN after incubation for 1 h at  $37^\circ\text{C}$  in 3%  $\text{CO}_2$ . Cells cultivated in the presence of  $10^{-7}$ M dexamethasone showed a significant increased attachment on LM or FN when compared to cells cultivated in 5% FBS-T (Fig. 1B) ( $p < 0.002$  Mann Whitney test). Alterations in LM and FN distribution pattern determined by immunofluorescence using rabbit polyclonal anti-LM or FN antibodies was also presented after dexamethasone treatment. In the control cells (cultivated with 5% FBS-T) LM or FN were organized in extracellular bundles, whereas after dexamethasone-treatment an intracellular granular pattern was observed.

The increased adhesion promoted by exposure of MEL-85 cells to dexamethasone might be a consequence of enhanced LM and FN receptors expression levels, but our results showed that these parameters have not been altered. A possible explanation for this increased adhesion is that glucocorticoid could have induced a structural change in receptors influencing its organization in the plasma membrane or causing receptor internalization after binding (U.M. Wewer et al., 1987, *Cancer Res.*, 47: 5691-5698), as suggested by LM and FN distributions examined by immunofluorescence. Other possibility would be changes of cytoskeleton proteins (actin and talin) organization, since dexamethasone treatment alters morphological pattern of MEL-85 cells showing marked pleomorphism characterized by development of cytoplasmic expansions resembling dendritic process.