Symposium no. 2

Biology of Melanomas

2.001

FUNCTIONAL ROLE OF CELL ADHESION MOLECULES IN CELL-MEDIATED CYTOTOXICITY OF HUMAN MELANOMA CELLS.

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The cell adhesion molecules (CAMs) ICAM-1 and LFA-3 and their counter-receptors LFA-1 and CD2, are differentially distributed on metastatic melanoma lesions. In fact, 34 and 35 out of 38 melanoma specimens investigated were reactive, althought to a different extent, with the anti-ICAM-1 mAb CL203.4 and with the anti-LFA-3 mAb TS2/9, respectively. None of the samples investigated expressed LFA-1 or CD2. Similar results were obtained analyzing the expression of CAMs on long term cultures of human melanoma cells. mAb blocking experiments demonstrated that ICAM-1 and LFA-3 are involved in melanoma cell lysis by allogeneic natural killer (NK) cells and lymphokine activated killer (LAK) cells. The extent of inhibition of melanoma cell lysis ranged between 10% and 30% depending on the target melanoma cells used. mAb blocking experiments performed using freshly explanted melanoma cells as targets and autologous tumor infiltrating lymphocytes (TILs) as effector cells gave similar results but with a more consistent reduction (60% to 80%) in the extent of lysis. These data suggest that CAMs might be involved in the clinical course of malignant melanoma and that tumor cells with weak or absent expression of CAMs might be able to escape the immune cell surveillance of cytotoxic cells.

2.002

HIGH-PLOIDY TUMOR CELLS MAY BEHAVE AS A RESERVE SUBPOPULATION

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Tumors often show cells with an abnormal DNA content (aneuploid cells) and cells which DNA content differs from one to another (heteroploid cells). Even incipient tumors or neoplastic cell lines, quite homogeneous in their DNA content, have a certain number of cells with a DNA content greater than that of the modal tumor population. The biological significance of these high-ploidy tumor cells is at present unknown. We have studied these cells in two murine tumor lines by means of autorradiography, time-lapse microcinematography and fluorescence-activated cell sorting, assessing that they have a longer life than the modal tumor population and that they can productively divide in vitro. We have also showed that these high-ploidy cells are especially resistant to high-dosages of the chemotherapeutic agent methotrexate. These results lead us to suggest that high-ploidy tumor cells may behave as a reserve subpopulation.

2.003

CYTOKINES EXPRESSION IN HUMAN MELANOMA CLONES: INTRATUMOR HETEROGENEITY.

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By reverse PCR we have examined the expression of cytokines such as IL-1 alpha/beta, TNF alpha/beta, IL-2, IL-4, IL-6, IL-7 and INF-gamma in 3 human metastatic melanoma cell lines 665/1, 665/2 and 665/R derived from 2 different metastatic lesions and from a local recurrence of the same patient respectively. In addition, 15 clones obtained from 665/2 melanoma were also analysed. A marked inter- and intra-tumor heterogeneity for the cytokine RNA expression was found. TNF alpha/beta were expressed in all 3 melanomas studied, while IL-1 alpha/beta, and IL-6 were restricted to 665/2 and 665/1. The analysis of 665/2 clones showed that they could be grouped in 2 distinct classes. Five clones (2/4, 2/14, 2/51, 2/60, 2/17) expressed IL-1 alpha/beta, TNF alpha/beta and IL-6, while other clones as 2/21, 2/41, 2/39, 2/56, revealed no detectable RNA for these cytokines. Previous data obtained in our laboratory indicated that the same clones producing cytokine RNA were highly susceptible to lysis mediated either by autologous T and by allogeneic LAK cells and showed a lower melanin content. Additional 662/2 clones will be analysed to further reinforce these correlations.

2.004

ESTROGEN RECEPTORS AND METABOLISM BY MELANOMA CELLS IN RELATION TO IFNs-MODULATED GROWTH "IN VITRO"

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The aim of the present study was to investigate interferons (IFNs) effects on growth and metabolism of A2058, M14, Colo38 and ME10538 human melanoma cell lines. To this end we evaluated: a) 3H-thymidine incorporation and DNA content; b) site I and II estrogen (E) receptors (R), in both soluble (S) and nuclear (N) fractions; c) E conversion rates using RP-HPLC analysis with UV and "on line" radiometric detection. In some but not all the cell lines studied, both α and β-IFN heavily but differently affect site I and II ER. Moreover, the same cells show to possess many steroid enzyme activities. Thus, they exhibit a hormone-sensitive status; possibility to combine IFNs and anti-estrogens at least for treatment of hormone-sensitive human melanomas is considered.

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2.005

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Summary

The expression and modulation of Progelatinase by cytokines in a series of four human cutaneous melanoma cell lines, A375, DX3, LT5.1 and SK23 and four human ocular melanoma cell lines, MEL47, MEL52, MEL56 and MEL57 was investigated in vitro. All expressed a Progelatinase with an apparent molecular weight of 72 kDa, whilst five of the cell lines, DX3, LT5.1, MEL47, MEL52 and MEL57 constitutively expressed an additional Progelatinase with an apparent molecular weight of 92 kDa as demonstrated by gelatin zymography. TNF α induced the expression of a 92 kDa gelatinase in A375 cells in the 72 kDa enzyme which occurred at 4 hours, and following 24 hour TNF stimulation expression continued for up to 72 hours following the removal of unbound TNF α.

Furthermore, co-incubation of tumour cells with TNF and TGF β 2 induced the expression of the 92 kDA Progelatinase in SK23 and MEL 56 cells and caused an upregulation of this molecular weight species in A375 cells.

2.006

EXPOSURE TO LEUKOCYTE INTERFERON (IFN-a) REDUCES THE NK CELL-MEDIATED LYSIS OF HUMAN MELANOMA CELLS.

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The HLA class I antigens negative melanoma cell line FO-1 is highly susceptible to NK cell-lysis compared to its HLA class I positive variant FO-1H. The latter was obtained following transfection of FO-1 cells with β2m gene. Treatment of FO-1 melanoma cells with IFN-α (2000U/ml) for 48 hrs neither induced the expression of HLA antigens on the cell surface nor modulated their susceptibility to lysis by natural killer (NK) cells. On the other hand, a consistent upregulation (over 30 channels of relative fluorescence intensity, analyzed by flow cytometry) of HLA class I antigens expression and a 30% reduction in their lysis by NK cells was observed following treatment of FO-1H cells under the same experimental conditions. These data are in keeping with our previous observation demonstrating an inverse relationship between HLA class I antigens expression and susceptibility of melanoma cells to NK cell lysis. In addition, since IFN-α is currently used for the treatment of metastatic malignant melanoma, it could be suggested that the antigenic profile of tumor cells might influence the clinical course of the disease and the outcome of therapeutical approaches using high doses of IFN-α.