(HT-1080) were used as a model to study the effects of TGF-β on the cell-secreted proteolytic activity and deposition of extracellular proteins to the growth substratum. The secretion of plasminogen activators (u-PA and t-PA) and the endothelial type plasminogen activator inhibitor (PAI-1) were quantitated using caseinolysis assays, zymography and reverse zymography. TFG-β caused a significant decrease in the amounts of secreted u-PA and t-PA in WI-38 and CCL-137 cell lines. Concomitantly, the enhanced secretion and deposition of PAI-1 was observed both in WI-38 and HT-1080 cell lines. The deposition of PAI-1 was a primary effect of TGF-β and occurred rapidly within 8 hr. The accumulation of PAI-1 to the medium was more slowly as shown by metabolic labelings and pulse-chase experiments. The deposited PAI-1 was sensitive to removal by u-PA. Subsequently, complexes of higher molecular weight were detected in the medium. Our results suggest that a rapid and sensitive effect of TGF-β on both normal fibroblasts and malignant cells is the reduction of proteolytic activity which may be associated with the growth inhibitory properties of TGF-β.

ACTIVATION OF PROTEIN KINASE C IN INTACT HUMAN PLATELETS BY ANTHRACYCLINE-IRON COMPLEXES

C.Lanzi, P.Banfi, L.Gianni, L.Vigano' and R.A.Gambetta

Istituto Nazionale Tumori, Via Venezian 1, 20133 Milano, Italy

Doxorubicin activates human platelets while daunorubicin inhibits both serotonin release and protein kinase C (PKC) activation in thrombin. Complexation with Fe (III) decreased the concentration of Doxorubicin necessary to induce platelet activation and reversed the effect of daunorubicin from inhibition to activation of PKC. N-acetyl-doxorubicin remained ineffective even in the presence of Fe. Addition of catalase or superoxide dismutase had no effect on the activation; nevertheless the determination of malondialdehyde by the thiobarbituric acid method showed an increse of lipid peroxidation in platelets treated with the iron complexes that followed the same pattern of the activation of PKC. These results suggest that PKC activation in doxorubicin treated platelets could be mediated by free radical formation and lipid peroxidation.

Supported in part by grant 85.02561.44 from CNR, Rome

PHENOTYPE OF METASTATIC CELLS AS TARGET FOR ANTI-METASTATIC INTERVENTIONS

K.Lapis, J.Timár, K.Pál, L.Kopper and A.Jeney

1st Institute of Pathology and Experimental Cancer Research, SOTE, Budapest, Hungary

The surface of highly metastatic Lewis tumour was characterised by: (1)increased GAG biosynthesis, (2)increased heparan sulphate/chondroitin sulphate ratio, (3)increased sialylation of gal/galNac terminated glycoproteins. As a consequence of membrane properties, the highly metastatic cells expressed high affinity to ECM components (GAG), fibronectin, collagen I-III, and showed immunoresistance against NK cells and macrophages. Meanwhile there was no change in cell proliferation kinetics. Targets for anti-metastatic interventions were as follows: (1)proliferation (CY.13324, tiazofurin), (2)cell membrane (KL-1o3; anti-GAG agent), (3)heterotypic interactions (PGI_2) (4)immunoresistance (KL-1o3, lentinan, macrophage infusion). The anti-proliferative agents were equally effective against tumour lines. The anti-GAG agent - immunotherapy - was able to inhibit the highly metastatic tumour, probably altering the heterotypic interactions and turning the immunoresistant tumour immunosensitive again. The PGI_2 and macrophage infusion proved to be effective only against immunosensitive tumours.

GROWTH FRACTION/DNA ANALYSIS USING Ki-67 ANTIBODY IN FLOW CYTOMETRY

J.K.Larsen, B.T.Mortensen and J.Christiansen

Finsen Laboratory and Department of Internal Medicine, Rigshospitalet, Copenhagen, Denmark

The nuclear antigen Ki-67 present in proliferating cells (Gerdes et al, J. Immunol., 133: 1710, 1984) was determined flow cytometrically in PHA stimulated lymphocytes and in HL-60 human promyelocyte leukaemia cells. In stimulated lymphocytes 81% of cells were found to be Ki-67 positive in comparison to 85% positive with anti-bromodeoxyuridine and 80% and 77% positive with the antibody independent staining methods using Hoechst 33342/ethidium bromide and mithramycin, respectiely. In HL-60 cells induced to differentiate by DMSO, the Ki-67 negative fraction, as well as the G0/G1 DNA fraction, was increased in comparison to an undifferentiated control.