Activation of the Ha-ras oncogene in rat and human cells is associated with a point mutation at a particular position, usually the 12th codon of the first exon. Although it has been postulated that the point mutation (G→A) observed in rat mammary tumours induced by the methylating carcinogen N-nitrosomethylurea is the result of misreplication of DNA containing the premutagenic lesion O^6-methylguanine, neither this nor any other relationship between DNA damage and oncogene mutagenesis/activation have been examined directly. Furthermore, since it has been suggested that the pattern of oncogene activation may be related to preceeding carcinogen-induced DNA damage and, hence, be carcinogen-specific, examination of the pattern of mutagenesis and activation induced in the human Ha-ras oncogene and its relationship to particular carcinogen-DNA adducts would be of particular interest in studies of the etiology of human cancer. Our current studies have involved research on DNA modification, mutagenesis and activation in the human Ha-ras oncogene (involving the construction in vitro of alkylated forms of the protooncogene, transfection into procaryotic or DNA sequqence modifications).

MODIFICATION OF THE INTRACELLULAR pH OCCURS DURING THE DIFFERENTIATION OF LEUKAEMIC CELL LINES (HL-60 AND U937) TOWARDS MONOCYTE LIKE CELLS

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We have measured the intracellular pH (pHi) during the monocytic differentiation of HL-60 cells induced by human recombinant interferon gamma (rHu-IFN-γ, RU42369), and of U937 cells induced by retinoic acid (RA).

pHi was monitored either by the fluorescence of intracellularly-trapped bis-carboxyethylcarboxyfluorescien, or by the distribution of [14]C benzoic acid. In both cases there is an increase in the pHi from 7.00 ± 0.03 to 7.13 ± 0.01 for rHu-IFN-γ treated cells, and from 7.02 ± 0.02 to 7.23 ± 0.03 for RA treated U937 cells.

In both cell lines the pHi is regulated by two mechanisms: a Na+/H+ exchange system and a Na+ dependent HCO_3^-/Cl^- exchange system which both catalyze an influx of [22]Na^+. Their pharmacological and biochemical properties have been defined. During the differentiation process, the activity of the Na+/H+ exchange system is increased at all the pHi values comprised between 6.20 and 7.60. The activation of this system is not a rapid phenomenon as observed with growth factors on quiescent cells. No activation could be detected during the first three hours of culture with the drug. The maximal effect is obtained two days after rHu-IFN-γ addition and three days after RA addition.

EVALUATION OF THE REACTIVE PRINCIPLES RESPONSIBLE FOR GENOTOXICITY AS A PREREQUISITE FOR CARCINOGENIC EXPOSURE MONITORING OF HALOGENATED ETHYLENES AND BUTADIENE

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For halogenated ethylenes and other alkenes, several reactive metabolites could be relevant for exposure monitoring. For vinyl chloride (VC) and vinyl bromide (VBr) the detection of 7-N-(2-oxoethyl)-guanine (a reaction product of the corresponding epoxide and guanidine) in liver DNA of rats after exposure of the animals to VC or VBr supported the central role of the epoxides as carcinogenic principle in metabolism of these compounds. A possible role of the reactive VC-metabolite chloroacetaldehyde (CAA) in formation of DNA-adducts and in genotoxicity of VC could be excluded on the basis of experiments with bischloroethylether, a CAA forming agent. Three different epoxides, epoxybutene (EB), epoxybutanediol and diepoxybutane have been suggested as reactive metabolic intermediates in butadiene metabolism. After exposure of mice to butadiene, 7-(1-hydroxy-3-buten-2-yl)guanine (a product of reaction of EB with guanine) could be identified in liver DNA of mice. This supports a central role of EB in BD induced carcinogenesis.

TRANSFORMING GROWTH FACTOR-BETA REGULATES THE PROTEOLYTIC ACTIVITY OF CULTURED NORMAL AND MALIGNANT CELLS

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Cultured embryonic fibroblasts (WI-38, CCL-137) and a fibrosarcoma cell line

(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

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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.

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PHENOTYPE OF METASTATIC CELLS AS TARGET FOR ANTI-METASTATIC INTERVENTIONS

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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

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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.