Radiumhemmet, Karolinska Hospital and Department of Histology, Karolinska Institute, P.O. Box 60 500, 2-104 01 Stockholm, Sweden

The interaction between 5-fluoropyrimidines and DNA was investigated using an approach by which cells are lysed in dilute alkali to reveal drug-induced fragmentation of DNA. One mechanism used to induce DNA lesions is the incorporation of drug into DNA, resulting in the induction of alkali-sensitive DNA lesions. The presence of lesions results in fragmentation of DNA, which is visualized by agarose gel electrophoresis.

The second mechanism does not involve incorporation of drug into DNA. This mechanism is in all probability due to inefficient DNA repair of normally occurring defects in nucleotides.

In human colon adenocarcinoma cells treated with 5-FU one can detect both mechanisms. In cells treated with 5-FdU one can detect only the second mechanism.

Ref: Cancer Res. 44: 3414, 1984; Cancer Res., 46: 3866, 1986.

LOCALIZATION OF RADIOLABELLED MONOCLONAL ANTI-CEA ANTIBODIES AND FRAGMENTS IN COLON CARCINOMA, - DIAGNOSTIC AND THERAPEUTIC APPROACHES

J.-P.Mach, A.Bischof-Delaloye and B.Delaloye

Biochemistry Institute, University of Lausanne, CH-1066 Epalinges and Division of Nuclear Medicine, CHUV, CH-10aa Lausanne, Switzerland

Thirty-one patients with known colorectal carcinomas were injected with Fab and F(ab')2 fragments from the monoclonal anti-CEA antibody (MAb) 35 labelled with 3 to 6 mCI of [123]I and tested by emission computerized tomography (ECT) 6, 24 and sometimes 48 hr after injection. All 23 primary tumours and local recurrences except one were clearly visualized. Interestingly, 9 of these patients had almost normal circulating CEA levels and 3 of the visualized tumours weighed only 3 to 5 g. Among 19 known metastatic tumour involvements 14 were correctly localized by ECT (Delaloye et al., J.Clin.Invest., 77: 301-311, 1986). For therapeutic purposes, seven patients with liver metastases were injected in the hepatic artery with MAbs and anti-CEA labelled with 100 mCi of [131]I. None of the 7 patients showed any significant side effects for a period of observation of 5 to 10 months. We observed good localization of intact [131]I MAb in liver metastases, as documented by ECT obtained 4 days after injection, and an estimated radiation dose to the tumour of 1000 rads, but we have not yet obtained definite evidence of tumour regression.

VACCINATION AGAINST EPSTEIN BARR (EB) VIRUS ASSOCIATED MALIGNANCIES

M.Mackett and J.R.Arrand

Molecular Biology Department, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Withington, Manchester M20 9BX, U.K.

We have investigated the possibility of using vaccinia virus recombinants expressing EB virus genes as potential vaccines to prevent EBV associated tumours. EB virus membrane antigen gp340 was inserted into and expressed in several strains of vaccinia virus. The gp340 produced by the vaccinia recombinants was indistinguishable from authentic gp340 by the criteria examined. Rabbits vaccinated with one of the recombinants produced antibodies that neutralized EB virus. We have also inserted the EB virus glycoprotein gB into vaccinia virus either as the authentic protein or as an in frame fusion with Hepatitis B virus surface antigen. These two constructs should present the EBgB to the immune sytem of vaccinated animals in different ways. Vaccination of rabbits is underway and should tell us whether the EBgB gene product is capable of raising EB virus neutralising antibody and also give us an idea how best to present antigens to the immune system.

RFLP ANALYSES OF c-Ha-ras LOCUS IN HUMAN TUMOURS

M.Mackiewicz, B.Choina, A.Kadlof, E.Rudska(1), J.Hołowiecki(1) and S.Szala

Department of Tumour Biology, Institute of Oncoloy, Gliwice, Poland and (1)Clinic of Haematology, Silesian Medical School, Katowice, Poland

A restriction fragment length polymorphism (RFLP) of the human c-Ha-ras locus has been analysed in DNA from breast tumours, leukaemic cells and lymphocytes from normal individuals by agarose gel electrophoresis and Southern blot hybridization. Examination of the allele frequencies showed three "common" alleles (6.8, 7.5, 8.3 kb) occurring with frequencies 38.2%, 36.5% and 19.2%, respectively, and several "rare" alleles (about 6%). More Ha-ras homozygotes were noted in DNA isolated from tumours than from white blood cells in normal individuals. No

differences were seen in structure of c-Ha-ras proto-oncogene in the tumour and in the lymphocyte DNA isolated from the same person. However, some differences in several loci were detected when DNA containing multilocus repeated sequences (minisatellite DNA) was used as a probe. These results indicate that loss or redistribution of some DNA sequences occurs in neoplastic tissue.

THE SENSITIVITY TO CYTOSTATICS OF THE HUMAN MELANOMA XENOGRAFTS IN IMMUNE-DEPRIVED MICE

B.Malenica, J.Rogan-Grgas and R.Vasari

Central Institute for Tumours, Zagreb, Yugoslavia

We established two human malignant melanoma xenografts, MEL-1 and MEL-2, subcutaneously into immune-deprived mice by melanoma cells derived from the primary monolayer culture. The xenografts grew progressively till the animals death. Mel-1 was the faster growing tumour. Both xenografts spontaneously metastasized to the lung. The histological appearance of the xenografts and their metastases were similar to that of original tumours. Both melanoma xenografts were sensitive to DTIC and Cis-platinum in all parameters tested. It is concluded that the lung colony assay provides the best possibility to assess the antitumour activity of the cytostatics used.

ADDUCT FORMATION OF DIETHELSTILBESTROL AND STEROIDAL ESTROGENS WITH AMINO ACIDS IN VITRO

M.Metzler and B.Epe

Institute of Toxicology, University of Würzburg, D-8700 Würzburg, F.R.G.

Recent evidence suggests that the induction of changes in chromosome number, i.e. aneuploidy, is a critical event in the process of neoplastic cell transformation induced by stilbene estrogens such as diethylstilbestrol (DES) and also by natural estrogens and their metabolites. We postulate that the biochemical mechanism underlying aneuploidy induction by estrogens involves covalent binding of metabolically activated estrogens to proteins of the spindle apparatus. In support of this proposition, we have recently demonstrated that DES and 2-hydroxy estradiol (2-HO-E2), the major metabolite of estradiol-17β, are able to bind covalently to a specific binding site in the C-terminal region of tubulin upon activation with peroxidase/hydrogen peroxide. In order to identify the binding amino acid(s), we have now studied the reactivity of perioxidative metabolites of DES and 2-HO-E_2 towards various amino acids in vitro. Using [14]C-labelled estrogens and high performance liquid chromatography, we found the greatest extent of adduct formation with cysteine and tyrosine, while other amino acids gave only small amounts of adducts or did not react at all. This preferential binding of reactive estrogen metabolites to certain amino acids may help to explain the observed specificity in the covalent binding to tubulin.

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MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS AND THE EFFECT ON SOME MARKER ENZYMES BY PEROXISOME PROLIFERATORS

Svein-Ole Mikalsen, Tore Sanner, Rolf K.Berge(1) and Niels Aarsaether(1)

Laboratory of Environmental and Occupational Cancer, Institute of Cancer Research, N-0310, Oslo 3; and (1)Laboratory of Clinical Biochemistry, University of Bergen, N-5016 Haukeland Sykehus, Norway

Many hypolipidemic drugs and industrial plasticizers cause peroxisome proliferation in rat liver, and induce hepatic neoplasms in rats and mice. The peroxisome proliferators (PPs) show little or no evidence of direct interaction with DNA. The effects of the PPs clofibrate (CLO) and diethylhexyl phthalate (DEHP) on different marker enzymes and of morphological transformation are studied in Syrian hamster embryo (SHE) cells. Preliminary results indicate that both chemicals induce morphological transformation of SHE cells. They induced an increase in catalase activity (peroxisomes), while no increase was found for glucose-6-phosphatase (endoplasmic reticulum) and acid phosphatase (lysosomes). Malate dehydrogenase (mitochondria) showed more inconsistent results. We were not able to detect peroxisomal beta-oxidation in either PP-exposed or control cells. Electron microscopical studies of SHE cell peroxisomes are in progress.

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COMPARISON OF THE ABILITY OF GLASS FIBERS AND ASBESTOS TO INDUCE MORPHOLOGICAL TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS

Svein-Ole Mikalsen, Edgar Rivedal and Tore Sanner