epithelial non-transformed immortalized rat cells of the Clone 9-3 line (Vanhamme and Szpirer, Exp. Cell. Res., 169: 120, 1987). Here we report that several of the H-ras-1-transformed methionine-dependent clones can yield methionine-independent revertants at a high frequency. We analyzed these revertants for several of their properties, including cloning-efficiency in soft agar to determine whether reversion of the methionine-dependent character is associated with full reversion of the transformed phenotype. Methionine-independent revertant clones were found to retain their ability to grow in agar, indicating only partial reversion of the H-ras-1 induced transformed phenotype.

REACTIVITY OF ANTIBODIES TO DNA MODIFIED BY BENZO(A)PYRENE IS DEPENDENT ON THE LEVEL OF MODIFICATION - IMPLICATIONS FOR QUANTITATION OF BENZO(A)PYRENE-DNA ADDUCTS IN VIVO

F.J.Van Schooten, E.Kreik, M.J.X.Hillebrand and F.E.van Leeuwen

The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Antibodies specific for DNA modified by (±) trans-7,8-dihydroxy-anti-9,10-epoxy-7,8,9,10 tetra hydroxybenzo(a)pyrene (BPDE) have been used in an enzyme-linked immunosorbent assay (ELISA) to quantitate the products of BP covalently bound to DNA. The antibodies were made by immunizing rabbits and mice with BPDE-modified DNA (2% modified) complexed with methylated bovine serum albumin. The resulting polyclonal and monoclonal antisera showed a high reactivity towards single-stranded BPDE-DNA, but had a lower reactivity for double stranded BPDE-DNA. The free N2-deoxyguanosinyl adduct of BPDE was less well recognized and no affinity was detected for BPDE-tetrols or DNA modified with N-acetoxy-AAF. A high cross-reactivity was found with DNA modified with (±) trans-1,2-dihydroxy-anti-3,4-epoxy-1,2,3,4-tetrahydrochrysene (CDE).

The antibody-reactivity towards BPDE-DNA depended on the level of modification; in the competitive ELISA as little as 4 fmol BPDE-DNA (42 pmol/µg) was sufficient for 50% inhibition, whereas 17 fmol of adduct was requird when [3]H-BPDE of a low level of modification (1 to 10 fmol/µg) was used as inhibitor. Samples of [3]H-BP-DNA isolated from the livers of mice treated with various doses of [3]H-BP were examined by ELISA. The binding values, calculated from the immunoassay, were in good agreement with the values from radioactivity measurements. The combination of standards of a low level of modification and highly specific antisera in a competitive ELISA is a valuable tool in the detection and reliable quantitation of human exposure to PAHs.

This work is supported by grant NKI 85-11 from The Netherlands Cancer Foundation (KWF).

EXPRESSION OF c-sis IN HUMAN MALIGNANT MESOTHELIOMA CELL LINES

M.A.Versnel, H.C.Hoogstedon(1), M.J.Bouts, Th.H.van der Kwast(2) and A.Hagemeijer

Department of Cell Biology, Immunology and Genetics, (1)Department of Pulmonary Disease and (2) Department of Pathology, Erasmus University and Academic Hospital Dijkzigt, Rotterdam, The Netherlands

Malignant mesotheliomas are mesodermally derived tumours. Occasionally a reactive connective tissue growth occurs. The expression of mRNA of PDGF A and PDGF B (c-sis) was studied in malignant mesothelioma cell lines and normal mesothelial cells. From five patients with confirmed malignant mesotheliomas, seven malignant mesothelioma cell lines were isolated. All were found to have chromosomal aberrations. Normal mesothelial cells were derived from patients without a malignant mesothelioma and had a normal karyotype. All malignant mesothelioma cell lines were found to express the 4.2 kb c-sis mRNA abundantly while the normal mesothelial cells did not express this messenger. The PDGF A chain was expressed by normal as well as malignant mesothelial cells. These studies indicate that the c-sis oncogene may possibly play a role in this type of malignancy.

Supported by The Netherlands Cancer Foundation (Koningin Wilhelmina Fonds).

RECESSIVE MODE OF INHERITANCE OF MELANOMA FORMATION IN XIPHOPHORIN FISH

J.R.Vielkind(1) and D.C.Morizot(2)

(1)B.C.Cancer Research Centre, The University of British Columbia, Vancouver, B.C., Canada; and (2)University of Texas Science Park, Smithville, Texas, U.S.A.

Studies on oncogenes provide evidence that the transformed phenotype is conferred onto cells in a dominant fashion. Studies on retinoblastoma, Wilms tumour, etc. show contrary evidence, i.e. tumours are due to homozygosity or to a total loss of recessive genes. Some of this controversy in interpreting genetic mechanisms in tumourigenesis could best be resolved in an

animal model system with well-established tumour inheritance, such as the hereditary melanoma formation in small tropical fish, platyfish and swordtails. In this system, a 1:1 Mendelian segregation of benign and malignant melanomas is observed. As suggested by Vielkind (1976) and Ahuja, Schwab and Anders (1980), this is due to a single regulatory locus. Studies on the differentiated state of the pigment cells in the two melanoma types show almost completely differentiated cells in the benign and very poorly differentiated cells in the malignant type. We have now mapped this locus, and can show a recessive, perhaps deletogenic, mode of melanoma inheritance. Thus the regulatory locus, termed Diff, presumably codes for information necessary in differentiation; it does not influence the severity of the melanoma in a dominant fashion.

[99m]Tc AND [111]In LABELLING OF MONOCLONAL F(ab')$_2$-FRAGMENTS AGAINST PROSTATIC ACID PHOSPHATASE FOR RADIOIMAGING OF PROSTATIC CANCER

P.Vihko, M.Hartikka, M.Soderva11(1) and R.Vihko

Biocenter Oulu, Department of Clinical Chemistry, University of Oulu, Finland and (1) Farmos Group Ltd., Research Center, Oulu, Finland

Tumour detection by external imaging following administration of radiolabelled monoclonal antibodies specific for tumour-associated antigens has drawn considerable attention in the diagnosis of cancer. Although radioisotopes of iodine have been employed for labelling antibodies, these labels are not stable, and significant deiodination may take place rapidly in vivo. An alternate approach to attach [99m]Tc or [111]In to the antibody using the anhydride of DTPA as a bifunctional chelate.

Monoclonal F(ab')$_2$-fragments (1 to 10 mg/ml) against prostatic acid phosphatase were derivatized with cDTPAA (molar ratio of cDTPAA/F(ab')$_2$ = 1:1, 5:1, 10:1, 20:1). The best labelling efficiencies (90 to 95% or 70 to 80%) using [111]In or [99m]Tc were obtained with molar ratio of cDTPAA/F(ab')$_2$ of 5:1 and with protein concentrations of 10 mg/ml. Under these conditions the antibodies retained their immunoreactivity totally and had no aggregation formation when studied by SDS-PAGE. A successful purification process for [99m]Tc labelled antibodies was developed to increase specific activity of labelled antibody. The radioactive antibody derivatives synthesized revealed metastases of prostatic cancer when used in radioimaging studies.

URACIL-DNA GLYCOSYLASE IN NORMAL AND MALIGNANT HAEMATOPOIESIS

Juhani A.Vilpo and Pirjo Koistinen

Laboratory of Molecular Hematology, Department of Clinical Chemistry, University of Oulu, SF-90220 Oulu, Finland

The activity of uracil-DNA glycosylase, a repair enzyme for the excision of uracil from DNA, was studied systematically in different types of blood and bone marrow cells in normal individuals, in haematological malignancies, and in established leukaemia cell lines. The patients represented a wide range of acute and chronic leukaemias.

The highest uracil-DNA glycosylase activities were found in primitive cells of normal and malignant haematopoiesis, although considerable variation was noted in blastic leukaemias. The expression of uracil-DNA glycosylase gradually diminished towards the more mature cells. This was observed in normal bone marrow, in chronic granulocytic leukaemia, and in TPA-induced malignant histiocytes. Blood lymphocytes in healthy individuals and in chronic lymphoproliferative disorders had stronger uracil-DNA glycosylase expression than the other mature cells, such as erythrocytes, granulocytes, and platelets.

INVESTIGATION OF CALCITONIN (CT) BINDING TO ITS RECEPTORS (CTR) IN MCF-7 HUMAN BREAST CANCER CELL LINE

B.Vincze, I.Lőrincz, I.Pályi, S.Kerpel-Fronius, I.Számel, I.Szántó, E.Svastics and J.Sugár

National Institute of Oncology, Budapest, Hungary

The CTR content of 10 breast cancer tissues was measured by a single-point inhibition technfique using human [125]-I-CT as labelled ligand and salmon CT (sCT) as inhibitor. Six cases out of 10 proved to be CTR-positive, the CTR content (mean ± SD) was 620±298 femtomol/mg protein and all the normal samples were CTR-negative. In the CTR-positive cancer tissue the cytosol estradiol receptor (ER) level was 154±90 femtomol/mg protein. On the basis of our human study the CT binding to MCF-7 human ER-positive breast cancer cell line was investigated. The CTR content of the intact cells was 5.3 femtomol/10^6 cells and the Kd value was 0.413×10^9 M, indicating that the binding was very specific to the CTR. According to an exchange assay, the CTR binding sites were already occupied by the sCT within 10 min. A 3 hr exposure of the