In the present study, P-Tyr antibodies were used to investigate the existence of human tumours expressing abnormal levels of tyrosine phosphoproteins and tyrosine kinases. Among eighteen cell lines examined, the antibodies identified a number of tumours with a detectable level of proteins phosphorylated on tyrosine. Among these were a major protein with an approximate Mr of 150,000 in a gastric carcinoma; two proteins, with Mr of 130,000 and 100,000 in a colon carcinoma; a major protein with Mr of 170,000, tyrosine phosphorylated in both a urinary bladder and an epidermoid carcinoma. Among the haemopoietic malignancies screened, in two Philadelphia-positive chronic myelogeous leukaemias, P-Tyr antibodies recognized the chymeric bcr-abl 210,000 Mr protein and its substrates. These phosphoproteins were not found in samples harvested from normal gastro-intestinal or urinary bladder epithelium, nor in control fibroblasts and lymphocytes. Two of the above proteins have associated tyrosine kinase activity. These data support the idea that a number of human malignancies contain an abnormal level of proteins phosphorylated on tyrosine and that the latter is an exploitable tumour marker.

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GROWTH SUPPORT, TOXICITY AND SOME METABOLIC EFFECTS OF HOMOCYSTEINE IN NON-TRANSFORMED AND CHEMICALLY TRANSFORMED C3H/10T1/2 CELLS

R.Djurhuus, A.M.Svardal, P.M.Ueland, J.R.Lillehaug and R.Male

Clinical Pharmacology Unit, Department of Pharmacology and Department of Biochemistry, University of Bergen, Bergen, Norway.

Inability to grow in a medium where methionine is replaced by homocysteine has been demonstrated for several malignant cell lines and postulated as a characteristic feature of the neoplastic phenotype as most normal cells thrive under these conditions.

To investigate this hypothesis in a well defined cell culture system we examined the effects of homocysteine on non-transformed (Cl 8) and two malignant clones (Cl 16 and Cl T422) of the C3H/10T1/2 mouse embryo fibroblasts, with regard to toxicity, ability to support growth and effects on methionine metabolism and glutathione level. Homocysteine was toxic to all cell lines and showed a drastic effect on cell morphology. These effects were not seen with homocysteine thiolactone.

Homocysteine thiolactone supported growth of the normal Cl 8 cells almost to the same extent as methionine, the malignant Cl 16 cells showed moderate growth reduction whereas Cl T422 grew slowly when methionine was replaced with homocysteine thiolactone.

The ability of homocysteine to support growth correlated well with alteration of methionine metabolism as the intracellular level of S-adenosylhomocysteine increased in all three cell lines in homocysteine thiolactone supplemented medium, while the S-adenosylhomocysteine content increased in Cl 8 cells, was constant in Cl 16 cells decreased in Cl T422 cells under the same conditions.

The glutathione content showed small variations between normal cells and Cl 16 cells during exponential growth, Cl T422 showed a distinct lower level of glutathione in methionine supplemented medium, and, in contast to Cl 8 and Cl 16 cells, showed 3-4 fold increase in glutathione when methionine was replaced by homocysteine.

EFFECTS OF BUTYLATED HYDROXYANISOLE OF THE MONOXYGENASE SYSTEM AND THE ACTIVATION OF BENZO(A)PYRENE BY 3-METHYLCHOLANTHRENE-INDUCED NUCLEAR FRACTION

K.Dobrzański(1), E.Henning(1), M.Rojas(2) and K.Alexandrov(2)

(1)Medical Academy Warsaw, Poland; (2)IRSC, Villejuif, France.

The effect of dietary administration of butylated hydroxyanisole (BHA) on the 3-methylcholanthrene (MC)-induced hepatic monooxygenase system (MFO) of nuclear fractions was investigated in male mice. The experiment has indicated similar qualitative effects of BHA on components of MC-induced and control MFO. BHA did not change the amount of cytochrome b5 and activity of NADH- and NADPH-cytochrome c reductases, but lowered the content of cytochrome P-450 and aryl hydrocarbon hydroxylase activity. These effects of BHA resulted in similar significant differences in benzo(a)pyrene (BP) metabolism after incubation of BP with both control and MC-induced nuclear fractions. BHA feeding reduced the BP metabolism and the binding of BP metabolites to DNA in control and MC groups. These experiments have indicated the greater effect of BHA on MC-induced nuclear fraction compared with the control. This effect is opposite to our previous findings with microsomal fractions.

SELECTION OF HUMAN MELANOMA METASTATIC VARIANTS

J.-F.Doré, S.Bertrand and M.Bailly

INSERM U 218, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon Cedex 08, France

Human melanoma variants and clones with increased metastatic abilities were obtained from melanoma cell lines in nude mice and in immunosuppressed new-born rats. Subcutaneous transplantation in a nude mouse of a human melanoma metastatic nodule resulted in a subcutaneous tumour (NTT) and in spontaneous lung (NTP) and lymph node (NTG) metastases (Neulat-Duga et al., Invasion and Metastasis, 4: 209-224, 1984) which were first maintained in vivo by subcutaneous passages in nude mice and then cultured in vitro as cell lines. Cytogenetic studies showed that all three tumour lines have a common origin and that metastases resulted from a population selection. After 15 in vitro passages, NTP cells were injected s.c. in nude mice : serial transplantation was accompanied by an increase in metastatic abilities of tumour cells. Melanoma cell lines, tumourigenic but non metastatic in nude mice, were xenografted to ATS-treated new-born rats. 3 weeks after s.c. injection of 10^6 cells, nearly all rats developed tumours and a proportion of them had lung and lymph node metastases. Agar cloning of M4Beu line showed that it is heterogeneous and contains poorly tumourigenic, but highly metastatic cells.

DETECTION OF AFLATOXIN-LIKE SUBSTANCES IN THE GENERAL DANISH POPULATION

L.O.Dragsted and H.Autrup

Fibiger Institute, Laboratory of Environmental Carcinogenesis, Danish Cancer Society, Copenhagen, Denmark

A competitive ELISA assay for detection of Aflatoxin B1 (AFB) in urine has been developed using a monoclonal anti-AFB antibody. The assay has been characterized with respect to sensitivity towards a range of aflatoxins and derivatives. The aromatic structures of AFB with the anisole group as well as the lactone region are required for competitive action in the assay. AFB concentrations down to 0.1 ng/ml could be detected.

Most urine samples from 80 normal Danish volunteers were positive in this assay, containing 0.1 to 10 ng-eqv. AFB per ml. The structure of the urinary aflatoxin-like antigenic substance (ALAS) is presently unknown. ALAS is a true competitor with AFB for the antibody and can be concentrated by affinity chromatography. We are presently attempting to identify the chemical structure of ALAS.

LOSS OF HETEROZYGOSITY ON CHROMOSOME 22 IN PRIMARY TUMOUR MATERIAL FROM MENINGIOMA

J.P.Dumanski(1), E.Carlbom(2), V.P.Collins(1) and M.Nordenskjöld(3)

(1)Ludwig Institut for Cancer Research, (2) Department of Neurosurgery and (3)Clinical Genetics, Karolinska Institute, Stockholm, Sweden

Cytogenetic analyses have shown that monosomy 22 is common in primary cultures of meningioma. A small fraction of these tumours have also shown deletions on chromosome 22. We have analysed restriction fragment length alleles at eleven loci on chromosome 22 in primary tumour material and the corresponding constitutional tissue from 20 patients with meningiomas, using polymorphic DNA markers. Loss of constitutional alleles along the whole chromosome 22 were found in 6 cases, consistent with a non-disjunction event. In addition, 5 meningiomas showed loss of alleles on part of chromosome 22 while at least one other locus on chromosome 22 showed retained heterozygosity. Analysis of polymorphic loci on 15 other chromosomes revealed only a few tumours with single losses. A more extensive analysis of cases with deletions may help to localise a recessive meningioma gene regionally on chromosome 22.

DNA DAMAGE AND THIOL DEPLETION CAUSED BY FECAPENTAENE-12 IN HUMAN FIBROBLASTS

J.M.Dypbukt, C.C.Edman, K.Sundqvist, S.M.Plummer(1), C.C.Harris(1) and R.C.Grafström

Department of Toxicology, Karolinska Institutet, Stockholm, Sweden; and (1)Laboratory Human Carcinogenesis, National Cancer Institute, Bethesda, Maryland, U.S.A.

Fecapentaene-12 (fec-12) is a fecal mutagen that also is genotoxic in cultured human fibroblasts (Plummer et al, Carcinogenesis 7, 1607-1609, 1986). Further studies indicate that survival of fibroblasts measured as colony forming efficiency or trypan blue exclusion was decreased to approximately 50% between 0.5 to 1.0 µm fec-12 after either 1, 3 or 24 hr exposure times. The cellular content of total thiols (mainly glutathione) was decreased in a dose dependent manner up to 1.0 µM fec-12 which decreased thiol content to 60% of control. Higher doses of fec-12 did not cause further thiol depletion. Because depletion of GSH was not coupled with formation of GSSG, these results indicate that fec-12 depletes cellular thiols by direct conjugation. As analyzed by alkaline elution, fec-12 also caused several types of DNA damage. Primarily DNA