The optimum NaCl concentration in formiate buffer was 0.25 ± 0.05 M. These features indicate that a lysosomal-like hyaluronidase is secreted by hepatoma cell lines.

Supported by the University Rouen, the Federation des Centres de Lutte contre le Cancer and l'Association de Recherche sur le Cancer.

RSV-INDUCED CELL TRANSFORMATION : EFFECT OF PROTEASE INHIBITORS ON FIBRONECTIN AND PLASMINOGEN ACTIVATORS

G.De Petro, D.Bellotti and S.Barlati

Division of Biology and Genetics, Department of Biomedical Sciences, University of Brescia, Italy

RSV-induced cell transformation is promoted by fibronectin fragments (FNdp), tissue-type plasminogen activator (t-PA) and by 12-0-tetradecanoylphorbol-13 acetate (TPA). It is known that high level of plasminogen activator (PA) activity is present in the conditioned medium (CM) of RSV-transformed cells which are also depleted of an organized extracellular matrix (ECM): this loss might be due to the direct catalytic action of PA on ECM proteins. In this study we report that: 1) RSV-transformed chicken embryo fibroblasts (CEF) release in the CM, in the absence of serum, FN peptides with MW between 230 and 110 kD and different molecular forms of PAs (MW ranging between 180 and 43 kD); 2) TPA induces an increased secretion of PAs and FN fragments: PAs and FN fragment release is suppressed by 2mM benzamidine but not by 100 IU/ml trasylol; 3) benzamidine is able to inhibit the transformed phenotype; some protease inhibitors exert a differential effect on the quantitative release of FN in RSV-transformed CEF and in uninfected CEF.

This work was supported by CNR, PF 'Oncologia' and by AIRC.

MODULATION OF P53 EXPRESSION DURING CELLULAR TRANSFORMATION WITH SV40

W.Deppert and M.Haug

University of Ulm, Department of Biochemistry, P.O. Box 4066, D-7900 Ulm, F.R.G.

We recently demonstrated that SV40 transformed cells harbour non-complexed p53 (free p53) which is metabolically stable in addition to p53 complexed with the large T antigen. These findings suggested that a mechanism for p53 stabilization independent from large T/p53 complex formation also operates in cellular transformation by SV40. To explore this hypothesis further, we have analyzed p53 epression in mouse BALB/c 3T3 cells abortively infected with SV40. These cells transiently express SV40 large T, but are not stably transformed. We have shown that in these cells neither p53 complexed to large T nor free p53 is metabolically stable. However, if stably transformed cells are selected from abortively infected cells by a focus assay and analyzed for p53 expression, both complexed and free p53 are metabolically stable. Our experiments demonstate (1) that complex formation of p53 with large T *per se* does not stabilize p53 and (2) that p53 stabilization is a transformation specific event which seems to be a second step in cellular transformation by SV40.

CHOLESTEROL ESTERS AND CELLULAR PROLIFERATION IN THE LIVER

S.Dessì, B.Batetta and P.Pani

Istituto di Farmacologia e Patologia Biochimica, Universita di Cagliari, Italy

Several investigators have attempted to correlate the induction of cholesterol synthesis with cellular proliferation. This has been repeatedly evaluated *in vitro* through the inhibition of HMGCoA reductase. *In vivo*, the inhibition of cholesterol synthesis is not easily achieved. We have studied cellular proliferation induced by an hepatic mitogen, lead nitrate, during fasting, a condition associated with very low levels of cholesterol synthesis. The accumulation and synthesis of cholesterol esters under such conditions has been investigated in relation to DNA synthesis.

This work was supported in part by CNR 86.00510.44, Rome, Assessorato Igiene e Sanita, Regione Autonoma della Sardegna, Italy; Dr.B.Batetta receives support through a "SARAS-Raffinerie Sarde" career development award.

PROTEINS PHOSPHORYLATED ON TYROSINE AS MARKERS OF HUMAN MALIGNANCIES

M.F.Di Renzo, S.Giordano, D.Cirillo, L.Naldini, L.Chiado Piat and P.M.Comoglio

Department of Biomedical Sciences and Oncology, University of Torino, Italy

Previous work has shown that proteins phosphorylated on tyrosine are selectively detectable by antibodies against phosphotyrosine (P-Tyr) in cells transformed by retroviral class 1 oncogene-encoded kinases endowed with non regulated activity (Di Renzo *et al*, Eur. J. Biochem., 1986).

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.

Work supported by the Italian C.N.R. and A.I.R.C.

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