SOMATIC CODE AND CANCER

**J.-C.Salomon**

C.N.R.S., B.P. 8, 94802 Villejuif, France

More and more informational molecules (IM) are known, but most of them are apparently plurifunctional (hormones, factors, mediators, receptors ...). Interactions are complex beyond description.

We propose the following hypothesis:

(1) a language of functions does exist, of which the IM are only the words. In order to get a synthetic view it is necessary to decipher specific functional messages, not at the level of IM, but at the level of their associations in meaningful sentences;

(2) between the genome and IM, interactions are not hierarchical: genes and their products are closely linked, since many gene products are IM which activate or repress other genes. The linear structure of the genome is completely functional because it is integrated into a network.

In cancers, the association of a tumour and of pareneoplastic phenomena could be a "Rosetta Stone" giving an insight into the somatic code.

LOCAL PROTEOLYSIS IN MALIGNANT TRANSFORMATION AND TISSUE DESTRUCTION

**E.-M.Salonen, J.Pöllänen, R.Stephens and A.Vaheri**

Department of Virology, University of Helsinki, 00290, Helsinki, Finland

We have shown previously that components of the proteolytic machinary, pro-PA and (Lys)-plasminogen, bind to immobilized fibronectin and laminin. This led us to introduce the concept "directional proteolysis" which implies that both normal and malignant cells direct pericellular proteolysis to specific sites. In actively growing HT-1080 sarcoma cells, we found using immunofluorescence and immunoelectromicroscopy, that u-PA was confined to cell-cell and focal contacts, colocalized with vinculin. PA-inhibitor (PAI-1) was deposited in cultures of both HT-1080 cells and normal human fibroblasts on the substratum except at focal contact sites. The different distributions of PA and PAI-1 may permit PA-mediated focal (directional) proteolysis in the presence of large amounts of PAI in the cell periphery.

The same mechanisms of proteolytic activation that operate in cancer also appear to act in other tissue destructive processes. We have identified plasmin in the tear fluid of patients with therapy-resistant corneal ulcers. Aprotinin inhibited this activity and was successfully used in patient treatment.

DETERMINATION OF ADDUCTS BETWEEN STYRENE OXIDE AND N-7 GUANINE WITH ACETYLATION METHOD IN VITRO

**K.Savela, A.Hesso and K.Hemminki**

Institute of Occupational Health, Topeliuksenkatu 41 aA, 00250 Helsinki, Finland

Styrene is an important monomer in the plastics and rubber industry. The natural metabolite of styrene oxide is styrene 7,8-oxide. The products of deoxynucleosides and DNA with styrene oxide are well characterized in vitro. Styrene oxide reacts through the $\alpha$ and $\beta$ carbon and four isomeric products are formed.

We have been developing a more sensitive method to detect the products of styrene oxide with N-7 guanine. The formation of two acetyl derivatives of N-7 alkylguanine were observed and isolated by HPLC analysis. The mass spectral and chemical investigations suggested the binding of acetyl group into N2-position of N-7 alkylguanine and into hydroxyl group of styrene oxide.

Furthermore we have incubated in small reaction vessels nanomolar amounts of tritiated acetic anhydride with N-7 alkylguanine. The bound [3]H-acetic anhydride can be estimated by using the internal standard.

ANTIGENOTOXIC EFFECT OF PHENOLIC ANTIOXIDANTS OF BENZO(a)PYRENE IN SOS CHROMOTEST IN THE PRESENCE OF S9 FRACTIONS OF MOUSE LIVER UNDER VARIOUS INDUCTIONS

**J.Sawicki and A.Bębenek**

Medical Academy, Department of Environmental Health Sciences, 02-097 Warsaw, Poland

The new bacterial test SOS Chromotest was used to study the in vitro effect of the antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on the genotoxicity of benzo(a)pyrene (BP). BP was activated by the S9 liver fraction of mice in the basal state and induced with 3-methylcholanthrene (MC) or Aroclor 1254. Results from our experiments demonstrated that genotoxicity of BP was decresed by BHA and BHT and this inhibitory effect depends on S9 fraction. The highest and lowest inhibition was observed when BP was

activated by S9 fraction from control and Aroclor-induced mice, respectively. There were also differences in the inhibitory potency between BHA and BHT. Our data suggest that type of S9 fraction (control or induced by different monooxygenase inducters) maya be critical for the evaluation of results obtained in SOS Chromotest, when the effects of chemopreventive agents on the genotoxicity of indirect acting compounds are analysed.

## REVERSION OF THE NEOPLASTRIC PHENOTYPE IN Ha-ras-TRANSFORMED RAT CELLS INDUCED BY TRANSFECTION WITH DNA FROM NORMAL HUMAN CELLS

**R.Schäfer, J.Iyer, E.Iten and A.Nirkko**

Ludwig Institute for Cancer Research, Inselspital, CH-3010 Bern, Switzerland

The neoplastic phenotype of FE-8 rat cells transformed by an activated human Ha-ras gene is suppressible upon fusion with normal cells (Griegel et al, Int. J. Cancer, 38: 697, 1986). The nature of the gene(s) involved in the suppression of neoplastic transformation is unknown. We attempted to revert the transformed phenotype in FE-8 cells by introduction via tranfection of DNA from normal human cells rather than by cell fusion. Six thousand transfectants harbouring the genetic information of normal human cells and of a cotransfected selectable marker (pY3) were isolated and subsequently selected for the normal phenotype based on the relative resistance of normal cells to treatment with ouabain. Primary and secondary transfectants were isolated in which the normal phenotype (dependence of serum and anchorage) appeared to be restored. The tumourigenicity in nude mice of these clones was also reduced. The expression of the mutant ras gene was not substantially reduced in revertants, nor was the biological activity of the oncogene impaired. From the presence of human repetitive DNA fragments in secondary transfectants we conclude that transfected DNA sequences are associated with the reversal of the neoplastic phenotype.

## AMPLIFICATION OF THE N-myc GENE IN PROGRESSION OF HUMAN NEUROBLASTOMA

**M.Schwab**

Deutsches Krebsforschungszentrum, Heidelberg, F.R.G.

Neuroblastoma is a childhood tumour, whose cells frequently show cytogenetic evidence for amplified DNA - "double minutes" (DMs) or "homogeneously staining chromosome regions" (HSRs). By serendipitous screening a DNA domain derived from the short arm of chromosome 2 was identified to be amplified in all tumours and cell lines derived from neuroblastomes and carrying DMs or HSRs. The core region of this DNA domain is characterized by the presence of a cellular gene N-myc that is related to c-myc in structure, sequence and the protein it encodes. N-myc is one member of a family of genes that have in common two highly conserved nucleotide boxes and are referred to as "myc-box" genes. Amplifications of another "myc-box" gene, L-myc, is frequently found in human small cell lung cancers.

Amplification of N-myc has been detected, with few exceptions, only in advanced stages of neuroblastoma. Early stages with amplification have extremely poor prognosis. The estimated progression free survival of patients with the most advanced form of neuroblastoma (stage IV) is roughly 50% in case there is a single copy of N-myc, 20% and 0% in case there are 3 to 10, or more than 10 copies respectively. These data suggest that amplification of N-myc may contribute to malignant progression of human neuroblastoma.

## THE INHIBITION OF POLYPLOIDIZATION OF CARCINOGEN-TREATED HEPATOCYTES PERSISTS IN PRIMARY CARCINOGENESIS AND AFTER TRANSPLANTATION

**P.E.Schwarze, G.Saeter and P.O.Seglen**

Department of Tissue Culture, Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo, Norway

During treatment of normal rats with 2-acetylaminofluorene (2-AAF) the liver normally increases in weight, protein and DNA content. However, polyploidization of hepatocytes is blocked, as indicated by the reduced percentage of bi-nuclear cells. After removal of 2-AAF polyploidization proceeds normally. In liver previously treated with the initiating agent diethylnitrosamine (DENA) and subsequently with 2-AAF, the hepatocytes never attained the degree of polyploidy of normal hepatocytes. In later appearing nodules and cancers most cells were diploid. Hepatocytes transplanted after the sequential treatment with the 2 agents appeared to be constitutively blocked in their ability to polyploidize, since nodules and cancers isolated from the host liver consisted predominantly of diploid cells. Treatment of the host with the polyploidization-promoting agent phenobarbital did not lead to more polyploid