reacts with normal and tumour cells of several human epithelia including breast and lung. It recognizes a saccharidic epitope heterogeneously carried by different kinds of glycoconjugates, i.e. mucins, glycoproteins and a glycolipid. Soluble and glycolipid extracts from surgical specimens of normal breast and lung tissues, mammary carcinomas and lung carcinomas of different histotypes were analyzed by SDS-PAGE and immunoblotting or immunoreaction on HPTLC. Although glycoproteins of various molecular weight were present on almost all the tissues examined, the expression of the glycolipid molecule seemed to be limited to neoplastic conditions. These results suggest that, as for other MAB-defined structures, it is more likely the type of antigenic glycoconjugate rather than the presence of the defined determinant that is specific for the differentiation and/or transformation of epithelial cells.

Partially supported by CNR no. 86.00643.44.

LATENCY, ANTIGENICITY AND ras GENE ACTIVATION OF METHYL-CHOLANTHRENE- INDUCED MURINE FIBROSARCOMAS

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To see whether the short latency period of chemically-induced, highly immunogenic murine fibrosarcomas is associated with the presence of activated ras-oncogenes, high molecular weight DNA from 6 antigenic and 4 non-antigenic methyl-cholanthrene (MCA)-induced BALB/c fibrosarcomas were used to transfect NIH/3T3 recipient cells. DNAs from 5 of 6 antigenic but only 1 of 4 non-antigenic tumours, contained transforming genes as shown by the foci observed after 14 to 21 days of culture. Multiple copies of λ-phage sequences, used as a marker, were present in DNAs isolated from the transfectants. Preliminary results of a Southern blotting analysis of the first cycle transfectants using Ha, Ki, and N-ras probes indicate the activation of ras-family genes in 6 transfectants derived from 3 different antigenic fibrosarcomas. Thus, the transforming activity of BALB/c fibrosarcomas DNAs, mediated by activated ras oncogenes in transfection assay, seems to be associated with their degree of antigenicity.

INDUCTION OF HEAT SHOCK GENE EXPRESSION IN RAT LIVER DURING GROWTH AND NEOPLASIA

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We investigated the expression of rat hepatic heat shock protein (HSP) gene expression under the influence of growth and hepatocarcinogenicity, because of the potential of heat shock as a therapeutic modality and the importance of the heat shock response induced by cellular stress. We found a 5-fold increase over baseline in both HSP 83 and HSP 70 (Mr 83,000 and 70,000) transcripts by 24 hr after partial hepatectomy which normalized by 5 days. A 42° C rat heat shock for 3 min induced a 4.5 and 20-fold increase in HSP 83 and HSP 70 mRNAs. Acute administration of diethylnitrosamine induced a time and dose (50 to 200mg/kg) dependent increase in HSP 80 mRNA; 4 weeks of dietary 2-acetylaminofluorene also did likewise. Primary hepatocellular carcinomas (HCC) had constitutively elevated HSP gene transcripts compared to age-matched controls, which increased further on rat heat shock. This elevated constitutive HSP gene expression was also found in several heptoma cell lines and 4/8 human hepatomas. HSP gene expression is thus increased transiently during normal liver growth, by acute and chronic carcinogens, and in a stable manner in rat and human primary hepatomas.

IN VITRO CHEMOSENSITIVITY TESTING OF HUMAN LUNG CARCINOMA CELLS

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In vitro evaluation of drugs against human cancers is of clinical interest because the procedure may predict the chemotherapeutic response in patients. Drug sensitivity of lung carcinoma cells from freshly explanted tumours was determined using two tests: the Clonogenic Assay and the Dye Exclusion Test. Three drugs, Cis-platinum, Adriamycin and Vincristine were tested in a group of patients with small cell carcinoma and adenocarcinoma. The data obtained from this study reveal that the clinical activity of these standard drugs is confirmed by the findings that a significant number of tumour specimens were also sensitive to these drugs in vitro. The rates of drug activity in adenocarcinoma were much lower corresponding to the clinically recognized resistance of ths tumour type. Detailed prospective in vivo-in vitro correlations have not as

yet been accomplished.

Supported by grant Special Project CNR "Oncology", contract no. 85.855.44.

IDENTIFICATION OF HUMAN UROTHELIAL CELLS PROPAGATED IN CULTURE

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Cell lines derived from non-malignant and malignant human urothelium have been established. These cell lines have a human karyotype, express keratin and react with monoclonal antibodies against either normal urothelium or bladder tumour associated antigens. The human and epithelial origin of these cell lines was further confirmed by studies of species specific isozymes, by electron microscopy and by tumour histology. Based on life-span in culture, tumourigenicity in nude mice, the cell lines have been classified into three diffeent categories of transformed cells. Ongoing research aims at characterizing the cell lines with respect to isozyme phenotype and karyotype. These characteristics will be compared with the tissue-type of the cell lines in order to evaluate the value of isozyme phenotyping and chromosomal characteristics as methods for the identification of established cell lines.

ACTIVATION OF THE ras-GENE: TRUNCATION INSTEAD OF POINT MUTATION

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Identically to the transforming cellular ras genes detected by gene transfer assay in 3T3 cells, Harvey-ras in Harvey sarcoma virus and Balb sarcoma virus is thought to be activated by point mutations at codon 12 (Balb SV), or 12 and 59 (HaSV) of the transforming protein p21. To test this hypothesis, we have exchanged parts of viral ras containing codons 12, or codons 12 and 59, or the complete coding region with the corresponding regions of normal rat proto-ras 2 or proto-ras 1 respectively. Viruses generated from the proviral clones all showed efficient transforming function in vitro and in vivo. Sequence comparison between normal and viral Harvey-ras genes revealed a previously undetected 5' exon termed exon (-1) that is contained in normal proto-ras but is always truncated in transforming viral ras genes. Because of the uncertainty of the 5' ends of ras, transforming cellular ras genes are also possibly truncated. We conclude that point mutations are not necessary for the transforming function of ras and propose that truncation of the normal gene activates the Harvey-ras gene. We have reisolated a few of the recombinant viruses and have obtained sequence data. The transforming function of new recombinant viruses containing upstream sequences of proto-ras which are truncated in the wild type virus has been investigated.

INDUCTION OF THE AROMATIC HYDROCARBON (AH) RECEPTOR AND OF DRUG METABOLIZING ENZYMES BY VARIOUS AROMATIC AMINES IN RAT LIVER

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The aromatic amines 2-acetylaminofluorene (AAF), transacetylaminostilbene (AAS) and 2-acetylaminophenanthrene (AAP) have different carcinogenic properties in rat liver. Only AAF is a complete carcinogen and exerts promoting effects. These have not yet been defined on a molecular basis. We have now studied interactions with the Ah receptor which has been suggested to play a role in promotion. The amines (100 µmol/kg, AAS 20 µmol/kg) and 3-methylcholanthrene (MC) as a control were administered by intraperitoneal injection into female Wistar rats daily for 5 days, for induction of ornithine decarboxylase (ODC) for one day. In addition to ODC activity and the hepatic Ah receptor level, the activity of Ah receptor controlled drug metabolizing enzymes and of microsomal epoxide hydrolase (EHm) were determined. All amines tested induced ODC in rat liver. The time course of this induction differed. AAs increased both the hepatic Ah receptor level and EHm 2-fold. AAF and AAP stimulated ethoxyresorufin-O-deethylase activity maximally by 4.2-fold, which is a small effect in comparison to the 80-fold increase by MC. The results indicate that AAF and AAP, but not AAS, may interact with the Ah receptor in vivo. AAS, however leads to other specific biological responses.

A TUMOUR-SPECIFIC INHIBITING FACTOR: MS-TIF

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