Symposium no. 10: Gene Alterations in Human Cancer Cells

10.025

HEPATOCYTE STIMULATORY FACTOR III(HSF III) HUMAN INTERLEUKIN FOR DA-1a CELLS(HILDA)mRNA EXPRESSION IN HUMAN HEPATOMA(HEP G2)CELLS.

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The Hepatocyte Stimulatory Factor III(HSF III)stimulates acute phase protein production(fibrinogen, 2 microglobulin and thiostatin)in hepatoma cells.Structural analysis and biological assays demonstrated that HSF III is identical to other factors with different growth, differentiation and activation properties i.e. HILDA,LIF,DIA,OAF .The gene encoding for HSF III/HILDA is located on chromosome 22q12.We studied mRNA expression for HSF III in HEP-G2 cell line.Total RNA was extracted from RPMI 1640 supplemented with 10% fetal calf serum cultured HEP-G2 cells at exponential phase of growth.Northern Blot analysis was done with a 32-P labelled cDNA probe and a 0.7 Kb fragment extracted from pC10-6R clone using Xho I restriction enzyme.The characteristic 3.8 Kb and a minor 1.8 Kb mRNA forms were detected.So cells of HEP-G2 cell line express mRNA of the gene encoding for HSF III/HILDA. No structural gene abnormality was observed.This study shows that the HEP-G2 cell line constitutively expresses mRNA for HSF III in the absence of structural gene abnormality.Future research will delineate the role of HSF III on proliferation of hepatoma cells.

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10.026

LOCALIZATION OF INTEGRATION SITES OF HTLV-1 PROVIRAL SEQUENCES IN HUMAN GENOMES.

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The integration of retroviral sequences into host genome has been investigated by fractionation of DNA-segments according to base composition and localization of HTLV-1-specific sequences established in the fractions by PCR. The compositional compartments of human genome in which integrated HTLV-1 sequences have been found were characterized by gene concentration, trancriptional and recombinogenic activities.

10.027

ACTIVATION OF c-Ki-*Ras* BY POINT MUTATION IN BOP-INDUCED PRECANCEROUS LESIONS AND ADENOCARCINOMA OF SYRIAN HAMSTER PANCREAS

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We have investigated the activation of *ras* genes by point mutation at codons 12, 13 or 61 in experimentally induced pancreas carcinogenesis in Syrian hamsters by allel-specific oligomer hybridization of *ras* sequences amplified from tissue lysates by the polymerase chain reaction. In 19 out of 20 adenocarcinomas codon 12 (85%) or 13 (10%) of c-Ki-*ras* was mutated; this accordance with the *ras* activation in human pancreatic cancer underlines the relevance of the hamster model for studying pancreatic carcinogenesis. All mutations were G→A transitions (gly→asp) of the second base in the codons involved, suggesting that point mutation of c-Ki-ras is induced by the methylating carcinogen *N*-nitrosobis(2-oxopropyl)amine (BOP). We test this hypothesis by investigating c-K-*ras* activation in precancerous lesions. First results indicate that the *ras* gene is frequently activated in ductal carcinoma-in-situ. Results from current work on focal cystic, ductular and ductal lesions as well as on hyperplastic acinar tissue and areas of pancreatitis will be presented.

10.028

Temperature Gradient Gel Electrophoresis (TGGE): Screening for Point Mutations in the Human p53-Gene

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The tumour-suppressor gene p53 is point mutated in many human carcinomas. The p53 protein is a nuclear phosphoprotein involved in cell-cycle regulation, while the mutated protein acts as an oncoprotein. Point mutations in genes can be detected by combining PCR and TGGE. This method allows to screen for point mutations. In our study we investigated gynecological malignancies such as cervical and endometrial carcinomas. After PCR, the amplified DNA-fragments can be separated by means of TGGE, even if they differ only in one single base position. Principle of this method is the difference in mobility between partially melted and fully denatured forms of short DNA molecules melting in two or three melting domains. Fragments are generated during PCR with a 40bp-stretch consisting only of G and C bases. These PCR products are suitable for analysis in TGGE, because they melt in two domains, the stable one being the "GC-clamp". Point mutations in the genomic template for PCR create heteroduplex-molecules, consisting of wild-type and mutated strands. Such molecules have reduced thermal stability and therefore reduced mobility in TGGE. System optimization is supported by computation of melting maps (Lerman, 1987) providing helpful informations about melting behaviour. Experimental parameters were obtained by performing Perpendicular TGGE (DIAGEN-TGGE-System, Germany). Our data on point mutation in exons 5-8 of the p53 gene in human cervical and endometrial carcinomas will be presented.

10.029

INVESTIGATION OF ALLELE LOSS AT 17p13 LOCI YNZ22 AND P53 GENE IN PRIMARY BREAST CANCER BY PCR-RFLP TECHNIQUES AND ITS RELATION TO P53 OVEREXPRESSION AND DISEASE OUTCOME.

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In addition to conventional southern blot studies, we have developed and applied improved PCR based non radioactive methods of monitoring intergenic loss of heterozygosity (LOH) in YNZ22 and P53 gene. The primers flanking the polymorphic restriction sites were employed and the PCR products generated from tumor and blood DNA were visualized on gels as such or after their restriction with appropriate enzyme and LOH scored. Using these techniques in a sample 114 breast tumors 64% LOH in P53 gene and 43% LOH in YNZ22 locus was found. Among 19 tumors informative for both loci, LOH at 17p13 never occured without P53 involvement. No homozygous deletions or rearrangement were found for these loci. Overexpression of P53 protein with MAbs detecting point mutations in 50% of the 50 cases showed no correlation to LOH. Both of these parameter show no significance in the short term disease free survival (3 years). The results of this study argue for an important role of already heterozygous intergenic P53 mutations in human breast cancer

10.030

ACTIVATION OF p185HER2 IN THE HUMAN LUNG ADENOCARCINOMA CELL LINE CALU3.

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The HER2/neu gene encodes a 185 KDa protein (p185HER2) with homology to epidermal growth factor receptor and intrinsic tyrosine kinase activity. In a human lung adenocarcinoma cell line, Calu3, the p185HER2 was found to be phosphorylated even when the cells were grown in the absence of growth factors. To understand this phenomenon in detail we have studied the effect of the concentrated supernatant of Calu3 cells on the expression of p185HER2. Furthermore, the possible spontaneous dimerization of this receptor was investigated by using monovalent antibodies directed against the extracellular domain of the oncoprotein.