Symposium no. 10

Gene Alterations in Human Cancer Cells

10.001

TUMOUR HETEROGENEITY OF RAS AND p53 ABNORMALITIES IN COLORECTAL CARCINOMA

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The dynamic heterogeneity of colorectal tumours was examined in terms of oncogene expression in 19 pts at different stages of disease. RAS and p53 oncogenes were stu-died in normal mucosa, adenomatous polyps, primary tumour and nodal and liver metastases. RAS mutations at codon 12 of KRAS were analysed using polymerase chain reaction followed by hybridization with oligonucleotide probes. Alterations in p53 gene were analysed by enzymatic digestion with two restriction enzymes, Hind III and Xbal, and hybridization with c DNA probes using the Southern Blot technique. 15 specimens from 6 pts showed point mutation at codon 12 of KRAS, 4/4 villous polyps, 0/2 tubular polyps, 4/18 primary cancers, 2 of 6 positive nodes and 3 of 4 hepatic metastasis. Nearly 50% of patients (9/19) showed alteration of p53 gene, either restriction fragment length polymorphism or allele loss in different tissue specimens of 2 polyps, 9 primary tumours and each of nodal and liver metastases. Four pts had both RAS and p53 mutations. While RAS and p53 oncogenes occured in 32% and 50% of pts respectively, the mutation is not stable throughout the disease process.

10.002

MOLECULAR GENETICS OF HUMAN GLIAL TUMORS : IU Ali*, A Saxena*, C Clark**, JT Robertson**, and EH Oldfield* *Surgical Neurology Branch, NINDS/NIH, Bethesda, MD. USA **Department of Neurosurgery, University of Tennessee, Memphis, TN. USA.

The development of human neoplasia generally involves genetic damage of very diverse nature occurring in a multistep process. Molecular analysis of these genetic lesions in many human tumors has identified a variety of mechanisms, which include both dominant and recessive chromosomal mutations. Such multiple genetic abnormalities have been identified in malignant gliomas, which account for the majority of primary tumors of the central nervous system. In our analysis of 40 primary and recurrent glioblastomas, loss of genetic information on chromosomes 10 and 17 was detected in 50-60% of the tumors. Furthermore, recurrent tumors, considered to be less well-differentiated and more aggressive, sometimes show allelic deletions on chromosome 10, which were not present in the primary tumors of the same patients. Deletion mapping of chromosome 17 in these tumors revealed a shared region of homozygosity that does not include the tumor suppressor gene, p53, whose functional inactivation is implicated in a wide variety of human cancers. Identification and characterization of these genes on chromosomes 10 and 17 as well as studies of other abnormalities of growth signalling pathways may provide biochemical explanations for neoplastic growth of glial cells.

10.003

CLONING OF THE GENE ENCODING TROP-2, A NOVEL CELL SURFACE MARKER OF HUMAN CARCINOMAS.

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We have previously identified a cell surface glycoprotein, gp50/Trop-2, which is expressed at high levels by trophoblast and by the majority of the human carcinomas, but is scarcely represented in the corresponding normal tissues. We speculate that this molecule may play a relevant role in tumor cell growth and invasion and may represent a target for novel diagnostic and immunotherapeutic strategies. To clone the gp50/Trop-2 we adopted the expression cloning procedure based on the CDM8 vector, modified by us to improve its efficiency. With the modified technique, we obtained transfection of 100% of the COS cells used, extremely efficient selection of positive cells by cell sorting in flow and an overall sensitivity of screening of cDNA libraries of 1 positive clone dispersed in 10^5 irrelevant ones. We constructed a cDNA library in the CDM8 vector using mRNA from the OVCA-432 cell line, which expresses gp50/Trop-2 at high levels. From this library we isolated a clone encoding gp50/Trop-2. This clone contains a 2.2 kb insert, which transfects gp50/Trop-2 and hybridizes with a single 2.3 kb mRNA species from positive sources, including genomic transfectants for gp50/Trop-2. Southern blot analysis indicates that Trop-2 is likely a single copy gene in human cells. Sequence analysis has determined homology between Trop-2 and GA-733-2/KSA-1/Trop-1, genes with similar tumor-specific expression.

10.004

H-ras and 11p deletion of ovarian cancer

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Several genetic lesions have been identified in ovarian cancer. Oncogenes are rarely activated, but the majority of ovarian cancers have evidence of loss of specific genetic material as indicated by cytogenetics and examination with polymorphic DNA probes. Among these is the loss of heterozygosity of the H-ras locus at 11p15.5 in 50% of cases. Using other polymorphic probes on 11p, we have found the deletion to include 11p13-11pter. On the other hand, we know from Northern Blot analysis of cultured ovarian cancer cells, that the H-ras gene is expressed. In order to examine whether the 11p deletion exerts its presumed biological effect through the H-ras gene, we have sequenced the remaining allele in 11 samples from 4 patients, using PCR. We have not found any mutation in and around codon 12, 13 or 61. This excludes a loss-of-competition-effect of mutated versus wild type H-ras as the biological important event in the deletion of 11p sequences in ovarian cancer.

10.005

EFFECT OF 4-HYDROXYNONENAL ON GENE EXPRESSION AND DIFFERENTIATION OF HL-60 HUMAN LEUKEMIC CELLS.

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4-Hydroxynonenal (HNE) is a product of lipid peroxidation with high biological activity. It has been demonstrated that HNE inhibits cell proliferation and DNA synthesis in different cell lines. The mechanism(s) by which HNE exerts its inhibitory effect is not yet completely known.
The promyelocytic leukemia cell line HL-60 is used extensively for studing proliferation and differentiation of myelo-monocitic cells. Moreover, HL-60 express several oncogenes,i.e. c-myc, c-fms, N- and H-ras.
In the days following the treatment with 1 uM HNE, we observed a block of HL-60 cell proliferation, accompanied by about 50% of differentiated cells. After aldehyde incubation, we analyzed c-myc and N-ras expression.

10.006

HUMAN PROGESTERONE RECEPTOR RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs) IN BREAST CANCER.

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Identification of at risk patients in breast cancer remains an unrealised goal. Our isolation of the cDNA (hPR-1) for the human Progesterone Receptor (PR) provided a molecular probe for detection of alterations in the PR gene. A 1.85kb fragment encoding the DNA-binding and hormone binding domain of the human PR was used to analyse RFLPs in blood and breast tissue. Genomic DNA was prepared from breast tumours and blood, digested by restriction enzyme Taq 1, and subjected to Southern blotting. In 8/42 blood samples and 11/47 breast tumours a distinct 1.90kb RFLP was detected. Blood from none of the 40 controls displayed this fragment, suggesting that this is a germ line anomaly in these patients not a mutation in tumour DNA.

PR DNA analysis can potentially identify at risk breast cancer patients.