Symposium no. 9

Transgenic Mice as Tools for the Analysis of Multistage Carcinogenesis

9.001

PKC ACTIVITY AND EARLY GROWTH RESPONSE GENE EXPRESSION DURING DIETHYLNITROSAMINE RAT HEPATOCARCINOGENESIS

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We measured the PKC activity in the early stages of hepatocarcinogenesis induced by DENA+partial hepatectomy (PH) (1). Since the PKC pathway can activate the expression of different early growth response (EGR) genes, the correlation between PKC activation and EGR expression was also examined. PKC was activated, with accumulation of diacylglycerol, 1 h after PH with simultaneous rapid induction of c-jun and c-fos mRNAs. 5 h after PH, PKC activity returned to normal and c-jun mRNA levels slowed down. 7 days after PH, PKC activity and c-jun expression were found higher in respect to controls. Present data suggest a relationship between PKC activation and EGR gene expression in the early stages of DENA hepatocarcinogenesis in the rat.

(1) Solt D.B., Farber E. (1976) Nature 263, 702-703.

9.002

PRODUCTION OF ANTIBODIES AGAINST THE NEW PROTEIN PH34.

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The cDNA PH34 has been isolated from the embryonal carcinoma (EC) cell line Nulli-SCC1. The level of the corresponding mRNA is down regulated during differentiation of the cells induced by hexamethylenebisacetamide (HMBA) and, although at a lower level, by retinoic acid (RA). The regulation of the mRNA is post-transcriptional and the analysis of the aminoacid and nucleotidic sequences revealed no homology to anything previously studied. Based on the predicted aminoacid sequence a synthetic polypeptide corresponding to a good immunogenic epitope was synthesized. The 17 aa. polypeptide conjugated with KLH was then used to produce monoclonal antibodies. P3U1 mieloma cells were fused with splenocytes from immunized mice and two positive clones were identified by EIA analysis. The supernatant of one of the clones recognizes a cytoplasmic antigen by immunofluorescence. A rabbit antiserum has also been developed.

9.003

RESCUING OF TWO KIRSTEN SARCOMA VIRUS STRAINS WHICH CAUSE DIFFERENT TRANSFORMING EFFECTS.

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Our previous studies on the occurrence of tumours in rodents suggested the rescuing of 2 Kirsten murine sarcoma virus (KiMSV) strains which cause different transforming effects. This possibility agrees with reported data that the LK2 pool causes thyroid carcinomas in Fischer rats, while the MRT-1196 pool is without carcinogenic effects in this model system. Preliminary molecular biology approaches seem to confirm that the cited pools belong to 2 different KiMSV strains. Moreover, they have divergent effects in cultured NIH3T3, in that the MRT-1196 pool greatly increases cell proliferation (7730 cells/mm^2) compared to the LK2 pool (1450 cells/mm^2). The characterization of these KiMSV strains may help to understand the mechanisms of retroviral carcinogenesis.

9.004

MODEL SYSTEMS FOR STUDYING THE POTENTIAL USE OF THE BREAST-ASSOCIATED MUCIN, PEM, IN CANCER THERAPY.

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Mucins are expressed in a tissue specific manner by many epithelial cells and the tumours derived from them. The mucin produced by the lactating breast, PEM, is coded for by the gene MUC1, and in tumour cells the carbohydrate side chains of this mucin appear to be shorter than their normal counterparts. This results in the exposure of epitopes which are generally masked in normal cells and so may be thought of as 'non-self'. Some of these epitopes are recognised by cytotoxic T cells and thus, the MUC1 gene and its products may be useful in the immunotherapy of cancer. To have a model where PEM is expressed as a self antigen, we have developed a transgenic mouse strain which expressed PEM in a tissue specific manner and glycosylated in the same way as in humans. These mouse models will allow for preclinial studies on the use of PEM based antigens and PEM directed antibodies in tumour rejection.

9.005

PHENOTYPIC PATTERNS OF PRENEOPLASTIC AND NEOPLASTIC HEPATIC LESIONS IN HEPATITIS B VIRUS TRANSGENIC MICE

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Overproduction of Hepatitis B virus large envelope polypeptide by transgenic mice is regularly associated with the development of hepatocellular adenomas and carcinomas. The cytomorphological and cytochemical analysis of livers from such mice at different ages revealed the early appearence of diffuse hepatocellular alterations (ground glass appearance and increase in cell size) followed by the emergence of focal hepatic lesions. The cells in foci differed from those in the extrafocal parenchyma by their size, their tinctorial properties, glycogen content, enzymatic profile and expression of HBsAg and AFP. Transitions from the focal hepatic lesions to hepatocellular adenomas and / or carcinomas were frequently observed. The cellular changes preceding the hepatic neoplasia in transgenic mice were in principle similar to those well known from preneoplastic foci found during chemical hepatocarcinogenesis. They apparently represent a general biological response of the liver parenchyma to oncogenic agents and are closely related to the mechanism of neoplastic transformation of the hepatocytes.

9.006

p53 AND KIRSTEN RAS IN SMOKING-, AND RADON-ASSOCIATED LUNG CANCER.

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Exposure to radon decay products increases risk of lung cancer in both smoking and non-smoking underground miners. Both p53, and ras mutations are frequent in human lung cancers, but the relation of these mutations to the known etiological factors in lung cancer is unknown. We have analyzed exons 4-9 of p53, and codons 12 and 61 of K-ras by dideoxynucleotide sequencing after amplifying tumor tissue dissected from formalin-fixed, paraffin-embedded preparations. Five confirmed mutations and one deletion was found in p53, while no mutations could be detected in K-ras. There was positive correlation between the immunohistochemistry using a polyclonal antibody towards the wild type p53 and the mutational analysis of p53.