The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, U.S.A.

Sequence analysis of the breakpoints involved in the translocations of non-Burkitt B-cell tumours has also provided evidence that in most cases the chromosome translocations occur at the pre-B-cell stage of differentiation during the process of VDJ joining and that the VDJ recombinase is responsible for the translocation by catalyzing the joining of the involved chromosomes. Three observations indicate that this is the case: (1) in the great majority of non-Burkitt lymphomas, the translocation breakpoints involve the 5' region of a J segment; (2) extra nucleotides (N regions) are detected at joining sites in both the t(11;14) and the t(14;18) chromosome translocations; and (3) heptamer and nonamer signal sequences, separated by a spacer of 12 nucleotides, that closely resemble those involved in physiologic VDJ joining, occur on chromosomes 11 and 18 near breakpoints. Thus one can speculate that in a rare B-cell, the recombinase mistakenly joins a heavy chain J segment to a cellular proto-oncogene instead of the proper immunoglobulin gene segment, leading to oncogene deregulation.

INHIBITION OF TUMOUR ANGIOGENESIS AND TUMOUR METASTASIS IN MICE DEFICIENT IN MAST CELLS

P.Crowle(1) and J.Starkey

Departments of Biology(1) and Microbiology, Montana State University, Bozeman, Montana 59717, U.S.A.

Conflicting reports exist on the role of mast cells in neoplastic disease. We examined the growth, angiogenic response and spontaneous metastasis of B16BL6 melanoma cells, insensitive to *in vitro* killing by murine mast cells, in mast cell deficient W/Wv and control litter-mate mice. We inoculated 10^5 tumour cells subcutaneously into the external ears of 25 W/Wv and 25 control mice. Tumour latent periods, incidence, growth rates and the incidence of spread to draining lymph nodes were the same for both groups of mice. In contrast, the rate of neovascularization was slower, and the incidence and number of spontaneous pulmonary metastases was lower in W/Wv mice than in controls (26.5% and 1.9 *vs* 60.0% and 10). We conclude that host mast cells may facilitate early tumour angiogenesis and haematogenous metastasis. Experiments are in progress to confirm these interpretations using mast cell reconstituted W/Wv mice.

NIH-CA 39611, NIH-5506RR08218-03.

ELEVATED EXPRESSION OF c-*myc* IN A HUMAN COLON CARCINOMA CELL LINE IS NEITHER ACCOMPANIED BY AMPLIFICATION NOR REARRANGEMENT OF THE GENE

F.Csaikl, U.Csaikl(1) and M.Vetterlein

Institute of Tumour Biology, and (1)Institute of Applied and Experimental Oncology, University of Vienna, Vienna, Austria

Over-expression of oncogenes is thought to be correlated to malignant progression of certain types of human tumours. In normal cells, c-*myc* expression is under stringent control, while regulation seems to be altered in a variety of tumours. Investigating a human cell line originating from a colon carcinoma, we found a four-fold elevated c-*myc* expression compared with β-actin. In spite of this, in a sarcoma derived cell line, the expression of both was equal. The over-expression was as high as in the preleukaemic cell line HL60, which is known to over-express c-*myc*. Northern blotting experiments showed in all samples a size of 2.0kb and 4.4kb for mRNA and pre-mRNA, respectively. DNA analysis revealed the absence of gene amplification and rearrangement of the c-*myc* locus. Since the colon cell line contains only one chromosomal translocation we are attempting to correlate the c-*myc* activation with this chromosomal abberration.

CARCINOEMBRYONIC ANTIGEN (CEA) DETERMINATIONS IN COLORECTAL CANCER

J.H.Czalbert(1) and L.Hornok(2)

Outpatient Clinic, Dunakeszi(1), 2nd Department of Surgery and (2)Postgraduate Medical University, Budapest, Hungary

In a programme of screening and follow-up for colorectal cancer (CC) in the city of Dunakeszi, Hungary, in 1983-86, the authors analysed the significance of serial CEA determinations in 68 patients and the findings were compared with those in the scientific literature. CEA levels of more than 30 µg/ml prior to surgery proved to indicate poor prognosis; in these cases, operation revealed advanced stage of disease. During follow-up, the CEA values increased following surgery and reached a level of more than 60 µg/ml in two patients. These patients died within a short time in spite of appropriate treatment. An increasing trend was observed in four patients; on the basis of additional investigations, suitable treatment was performed. In one case recurrence was

suspected despite a CEA-value that fell within the normal range. Differing conclusions in the literature as to the accuracy of the test have also been reported.

It is concluded that out of the currently available markers periodically-repeated CEA-determinations supplemented with appropriate clinical investigations may be used for controlling patients who have undergone surgery for CC.

PROSTAGLANDIN H SYNTHASE CATALYZED METABOLISM OF HETEROCYCLIC AROMATIC AMINES OF THE IQ-TYPE AND THEIR ACTIVATION TO MUTAGENS

G.H.Degen and D.Wild

Institute of Toxicology, University of Würzburg, Versbacher Str. 9, D-8700 Würzburg, F.R.G.

IQ (2-amino-3-methylimidazo[4,5-f]quinoline) and related compounds occurring in heat-processed, protein-rich food are known to be mutagenic upon activation by mixed function oxidases and cause hepatic and extrahepatic tumours in rodents. IQ and 3 analogs (Kaiser et al, Chem.-Biol. Interact. 57: 97, 1986) were recently studied in a modified Ames-test and displayed prostaglandin H synthase (PHS)-dependent activation to mutagens in the order: iso IQ > IQ > NI>> demethyl-IQ (Wild and Degen, Carcinogenesis, 1987, in press). Metabolism of IQ and analogs incubated in vitro with PHS from ram seminal vesicle microsomes supplemented with arachidonic acid or hydrogen peroxide has now been studied by HPLC and TLC: NI, demethyl-IQ, IQ and iso-IQ were oxidized by PHS-peroxidase (80, 68, 54 and 18% respectively) and yield coloured products with different efficiency. Co-oxidation and/or co-oxygenation of IQ-type compounds may be responsible for their PHS-dependent activation to mutagens. Horseradish peroxidase under comparable conditions scarcely metabolized IQ, and interestingly, it did not activate IQ to a mutagen.

The data point to PHS as an activating system for food-borne arylamines of the IQ-type. This may be relevant for their extra-hepatic tumourigenic action.

Supported by the Deutsche Forschungsgemeinschaft (SFB 172).

CHANGES IN LIVER CELL PLOIDY EMERGING DURING RAT HEPATOCARCINOGENESIS

A.Deleesner and K.J.Bell

Laboratorium Antropogenetika, V.U.B., Brussels, Belgium, and Joyce Loebl, Newcastle, U.K.

Nuclear DNA content of hepatocytes was quantified during the early steps of rat hepatocarcinogenesis by TV based densitometry using an image analysis device Magiscan 2A (Joyce Loebl, G.B.). Putative preneoplastic lesions as foci and nodules were induced by the triphasic "Gerlans protocol" (Initiator=DEN, Selection=2-AAF+CCl_4 or pH, Promotor=Phenobarbital). The amount of DNA in the hepatocellular nuclei was determined densitometrically on Feulgen-stained sections. The animals were sacrificed at 1 day before and 5, 8, 12, 15, 18 days after CCl_4 treatment and furthermore after 1, 2, 3, 5 months Phenobarbital (PB) treatment. Comparison is also made between the use of CCl_4 or partial hepatectomy during the selection procedure. This study reveals a shift towards a diploid hepatocellular population after the end of the selection phase and later on the emergence of preneoplastic lesions with a high frequency of diploid nuclei. The observed diploidisation might be a relevant parameter for tracing of early lesions during hepatocarcinogenesis; the analysis of the early lesions is finally improved by our TV-based image analysis system.

DETECTION OF HYALURONIDASE IN HEPATOMA CELL CULTURE MEDIUM WITH A SENSITIVE INDIRECT ENZYMO - IMMUNOLOGICAL ASSAY

B.Delpech, C.Chauzy, P.Bertrand, A.Olivier and C.Maingonnat

Centre Henri - Becquerel, rue d'Amiens, 76000 Rouen, France

Hyaluronic acid was adsorbed onto plastic microtest plates. It was measured with an indirect enzymo-immunological technique taking advantage of its capacity to bind a proteoglycan (hyaluronectin, HN) supplemented with alkaline phosphatase conjugated rabbit anti-HN antibodies. The presence of active hyaluronidase was detected by the destruction of insolubilized hyaluronic acid in proportion to the hyaluronidase concentration of samples. Human hepatoma cell lines HepG2 and PFC/PRF/5 were cultivated with,then without foetal calf serum. Cell culture media as well as cell extracts could digest adsorbed hyaluronic acid. Soluble hyaluronic acid was degraded into smaller molecules as shown by liquid chromatography. The secretion in culture medium was estimated at 2×10^{-11} NFU/cell/min. The activity was suppressed by heating at 50° C for 5 minutes or by protease digestion. The optimum pH was 3.5.