EIGHT DIFFERENT HUMAN BREAST CANCER CELL LINES

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Cytochromes P-450 are a family of haemoproteins involved in the metabolism of both endogenous and exogenous compounds, i.e. carcinogens. P-450c is inducible by TCDD. Induction of this gene measured by slot blot analysis of cellular RNA, and its effect on the metabolism of BP7,8-diol was studied in eight human breast cancer cell lines. The cells were grown under well defined conditions and treated with different concentrations of TCDD for 24 hr prior to addition of tritium labelled BP7,8-diol (675 nM). The basal level of P-450c mRNA was the same in all cell lines. The TCDD induced levels of P-450c mRNA followed the order: MCF-7>T47-D>ZR-75-1>3909>3522 in a dose-dependent manner. Three lines, AL-1, BT-20 and CAMA-1 did not respond at all to TCDD. Pretreatment of the cells with TCDD changed the BP7,8-diol metabolite profile. An unidentified compound with the retention between that of 9,10- and 7,8-diol was the major metabolite in TCDD treated cells. These results demonstrate that human breast cancer cell lines differ greatly from each other with respect to inducibility of P-450c by TCDD and that the induction influence the BP7,8-diol metabolite profile.

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DORMANCY AND PROGRESSION OF B LEUKAEMIC CELLS IN AKR MICE

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The high incidence of spontaneous T-cell lymphoma in AKR mice (arising predominantly in the thymus) can be abolished almost completely by thymus removal at the age of 1 to 3 months. Only 10 to 15% extrathymic lymphoid tumours occur late in life following thymectomy, nevertheless each of the thymectomized AKR mice is a carrier of dormant potential lymphoma cells (PLC). Transplantation of lymphoid cells from 8 to 14 months old AKR mice (thymectomized at the age of 40 to 60 days) into the appropriate intact or thymectomized recipients caused B cell leukaemia development of AKR origin in 100% of the recipients. Immunosuppressive treatment involving preferentially T-cell function like ATS, corticoids, X-rays and retroviruses isolated from AKR (DTV) were found to stimulate the progression of dormant PLC present in thymectomized AKR mice towards B-cell lymphoma development. Splenectomy of 8 to 12 months old thymecytomized mice and intravenous reinjection of their own splenocytes back resulted in breakdown of dormancy in 50 to 60% of the mice, suggesting the possible restrictive spleen microenvironment role on PLC dormancy.

A SHORT SYNTHETIC PEPTIDE FRAGMENT OF HUMAN INTERLEUKIN 1 β (hIL-1β) INCREASES HUMAN NATURAL KILLER (NK) ACTIVITY

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We previously characterized a synthetic peptide of IL-1β (fragment 163-171), which has immunostimulatory but not inflammatory activity. In this study we examined the effects of this peptide on natural cytotoxicity of human cells. Peripheral blood mononuclear cells (PBMC), preincubated in medium containing interleukin 2 (IL-2), exhibited a dose-dependent augmentation of NK activity against K562 leukaemia cells. In contrast, both IL-1β and the synthetic peptide were unable to stimulate the cytotoxicity of these cells. However, when PBMC were further depleted of monocytes by adherence to plastic, a marked increase of NK activity occurred in the presence of the peptide, but not in the presence of hIL-1α or β . Significant augmentation in cytotoxicity was obtained by preincubating lymphocytes for 18 hr with 10 to 100 µg per ml of the peptide. This effect is likely to be the result of the induction of IL-2 by the peptide, which, in contrast to the entire IL-1 molecule, does not stimulate the synthesis of prostaglandin E2, a potent inhibitor of NK activity.

GROWING CELL CULTURES EXERT DISTINCTIVE COLONY MORPHOGENESIS

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Morphogenesis of colonies of transformed or malignant cells <u>in vitro</u> requires feeder cells or enriched medium and

reveals growth regulation and cell density dependent reactions. The culture system described for growing L929 cells in feeder layers promotes colonies of homologous cells, cultured on the surface agar layer. A cell density of $0.5x10^6$ cells per ml reach confluency at 24 hr and these stationary cultures cause a complete inhibition of colony formation. This inhibition is extended not only to homologous cells but to prokaryotic cells as well. Streptococcus grows slowly and forms tiny colonies on stationary cultures, on the contrary a growing cell culture promotes development of greater diffused colonies. Such model systems of colony morphogenesis on stationary and growing feeder cells may prove more sensitive estimating regulatory actions of pharmacological and biological substances or cell to cell interactions.

OCHRATOXIN A IN HUMAN BLOOD IN RELATION TO BALKAN ENDEMIC NEPHROPATHY AND URINARY SYSTEM TUMOURS IN BULGARIA

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In an effort to provide further evidence for the hypothesis that a mycotoxin is involved in the aetiology of Balkan endemic nephropathy(BEN) and that the later is associated with the occurrence of urinary system tumours (UST), a survey was made for the occurrence of ochratoxin A(OA) in human blood samples collected from people living in an area with BEN and high incidences of UST compared with those from another non-endemic area in Bulgaria. In all, 312 people were analysed and OA was found in the serum of people from both endemic and non-endemic areas. But a much greater proportion of samples containing OA (26.3%) was found in the serum of patients with UST and/or BEN whereas the proportion of OA in the serum of people from the non-endemic area approximately to 7.7%. The highest concentration found was 35 ng ochratoxin A /g serum.

A TRANSFORMING GROWTH FACTOR PRODUCED BY SV40-3T3 CELLS

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A growth promoting activity was purified from serum-free medium conditioned by SV40-transformed 3T3 cells seeded at high density (CM). The purification steps consisted of gel permeation chromatography of the acid-soluble CM fraction followed by cation exchange and reverse-phase high pressure liquid chromatography. A partially purified preparation of growth factor was found capable of stimulating both thymidine incorporation as well as the proliferation rate of quiescent 3T3 cells. This fraction also induced anchorage-dependent non-transformed cells (NRK) to form colonies in soft agar. The presumptive transforming properties associated with the growth promoting activity as well as its possible relationship with known TGFs or PDGF-like factors are currently under investigation.

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IGF1 RECEPTORS (IGF1-R) IN 72 PRIMARY HUMAN BREAST CANCER. RELATION WITH ESTRADIOL AND PROGESTERONE RECEPTORS (ER, PgR)

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IGF1 (Insulin-Like Growth Factor (1) stimulates the proliferation of human breast cancer cells. We have characterized the R-IGF1 in four breast cancer cell lines in long-term tissue culture: we followed this work determining the R-IGF1 concentration in 76 primary breast cancers. The labeled IGF1, 200 uCi/ug (Pr Humbel-Zurich and Amersham-France) was incubated for 5 hr at 4°C with 400 ug of breast cancer membrane proteins, in the presence or absence of a partially purified IGF1 preparation. Only 6.6% of the tumours bound less than 1% of the total radioactivity (IGF1-R-); 18.4% bound 1 to 2% (IGFR+), 75% of the tumours bound more than 2% (IGF-R+). The range was 0 to 16.4%. There is a relation between IGF1-R+ and RPg+ (χ^2=8.6,p=0.003) and between IGF1-R+ and the menopause (χ^2=6.8, p=0.009). The concentration of IGF-R is correlated (Spearman test) to RE (p=0.0018) and to RPg (p=0.0011). There is a linear positive correlation between log IGF-R and log RE (n=59, p=0.025) and between IGF-R and log RPg (N=54, p=0.0025). These results suggest that (1) as breast cancers contain IGF-1-R they could be sensitive to this growth factor, and (2) an IGF-R lowering drug could be a beneficial treatment for these patients.

MODULATION OF THE HUMAN Ha-ras-1 ONCOGENE EXPRESSION BY DNA METHYLATION