## IN VITRO RESPONSE OF CPA SYNCHRONIZED HUMAN BREAST CANCER CELLS TO CHEMOTHERAPY

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This laboratory has been engaged to exploit hormonal modifications of cell growth kinetics to potentiate the effectiveness of antitumour drugs. In the present study, EVSA-T breast cancer cells, endowed with receptor proteins for androgens, have been exposed in vitro to cyproterone acetate (CPA). The Thymidine Labelling Index (TLI), measuring the percentage of DNA synthetizing cells (S-phase) was compared to the growth fraction (GF) evaluations by Primer dependent $\alpha$ DNA Polymerase Index (PDP-LI) and by an immunoperoxidase assay that exploited a monoclonal antibody against DNA polymerase. As a consequence of a 24 hr exposure to CPA, cells were synchronized in the G1 phase, since kinetic determinations scored 12 by the TLI assay (% S phase) and 61 by the PDP-LI (% GF). By cell counting and colony survival assay we then investigated the cytotoxicity of doxorubicin and methotrexate on the synchronized EVSA-T cell line. It has been found that CPA treated cells exhibited increased sensitivity to methotrexate and not to doxorubicin in comparison with CPA untreated control cells.

## IMMUNOLOGICAL CHANGES IN LUNG CANCER PATIENTS

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Lymphocyte subsets of 74 lung cancer patients were studied using monoclonal antibodies at diagnosis, to evaluate the presence of immune imbalance and its possible relation with prognosis. Statistical analysis of the data was performed and significant results are reported below:

| | Cancer Patients | Controls | P |
|---|---|---|---|
| OKT4 | 772 + 291 | 989 + 376 | <0.01 |
| OKT4% | 41 + 6.8 | 45.24 + 4.16 | <0.05 |
| OKT8% | 33.3 + 6.39 | 29.34 + 3.84 | <0.05 |
| T4/T8 | 1.27 + 0.37 | 1.54 + 0.23 | <0.01 |

In our data histotype, PS and tumour extension did not influence the degree of immunological change. The comparison of survival curves of patients with similar PS, histotype and tumour extension but normal or decreased ratio showed no significant difference.

## A CYTOGENETIC STUDY OF A CONSECUTIVE SERIES OF 35 OVARIAN TUMOURS

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In order to study the cytogenetic patterns in borderline tumours (N=6), malignant neoplasms (N=16) and metastatic neoplasms (N=13) of the ovary a consecutive series of patients operated on in the Department of Gynaecology and Obstetrics, Odense University Hospital during a two year period (1.1.84 to 31.12.86) were investigated cytogenetically.

For each tumour, all material received was investigated using short-term culture conditions and G-banding technique. The number of metaphases obtained from each tumour varied between 0 and 800.

The study demonstrated a very large intra- as well as inter-tumour chromosome variation. Although no specific chromosome abberation was demonstrated in this series of ovarian tumours, an increasing cytogenetic complexity was seen when going from borderline tumours through primary tumours to metastatic tumours. The importance of consecutive series of tumour material in cytogenetic studies is emphasised by this investigation.

## THE POTENTIAL CARCINOGENIC ACTIVITY OF ESTRADIOL AND CATECHOLESTRADIOLS

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Estradiol is metabolically activated by microsomes to the catecholestradiol, 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2). The biphasic activities of these compounds were detected using the Chromotest Assay. At low concentrations, these steriods stimulated synthesis of beta-galactosidase, whilst at high concentrations, after the peak value, bacteria were killed and beta-galactosidase

was not produced. Thus, these compounds may possibly be toxic carcinogens. To test this hypothesis estradiol and catecholestradiols were administered to Sprague-Dawley rats. An increase in prostate mass was observed. In human hyperplasia of the prostate we found that the catecholestrogen concentration was three times higher in malignant tumours than in benign growths.

## INOSITOL PHOSPHATES AND PHOSPHOINOSITIDES IN RAT LIVER NODULES

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We have investigated the inositol phosphate turnover system in liver nodules from rats. These nodules, considered to be preneoplastic lesions, have many histological and biochemical alterations, e.g. a larger growth fraction and an altered response to growth factors.

The total amounts of phosphoinositides and inositol phosphates were measured in normal and nodular liver, as well as the turnover rate of the different compounds after stimulation with vasopressin.

Consistent with earlier findings, the basal level of phosphatidyl inositol was roughly doubled in the nodules, though neither the polyphosphoinositides nor the inositol phosphates showed any marked differences.

The nodular cells responded to vasopressin with a quicker than normal elevation of the inositol trisphosphate amount, but to the same level as the normal liver. The normal cells showed a six-fold increase of inositol tetrakisphosphate, which we have not been able to show in nodular cells.

## INDUCED DIFFERENTIATION IN HUMAN LEUKAEMIA/LYMPHOMA CELL LINES. A SHORT OVERVIEW

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In recent years cell lines representative of most types of human leukaemia and lymphoma have been established in vitro. Such lines have been found to be instrumental in studies aimed at understanding (1) whether the differentiation block, typical of leukaemia/lymphoma in vivo, is reversible in vitro and if so, whether the induced differentiation will be terminal, i.e. associated with a G1/G0 cell cycle block, and (2) the deranged genetic control of proliferation/differentiation in leukaemia/lymphoma. These studies clearly show that at least for non-lymphoid cell lines (HL-60, U-937, K-562, MEL, Ku 812, M 1-2, THP-1) induction of terminal differentiation is indeed possible by e.g. phorbol ester, vitamin D3, retinoic acid, interferon, and that several protooncogenes are regulated during this process.

## ANTI-MELANOMA PROPERTIES OF CHEMICAL INDUCERS OF DIFFERENTIATION: IN VITRO AND IN VIVO STUDIES

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The effects of three chemical inducers of cell differentiation, sodium butyrate, dimethylthiourea (DMTU) and tetramethylurea (TMU), were studied on mouse and human melanoma cell lines and B16 melanoma tumours. Sodium butyrate, dimethylthiourea (DMTU) and tetramethylurea (TMU) were found to inhibit melanoma cell growth, clonogenicity in soft agar and tumourigenicity in syngeneic mice. Sodium butyrate, DMTU or TMU also induced morphological and biochemical changes in melanoma cell lines. These changes include cell enlargement, development of endoplasmic reticulum and golgi complexes, and enhancement of NADPH cytochrome c reductase and $\gamma$-glutamyl transpeptidase activities. These phenotypic alterations are in part compatible with a more differentiated phenotype. Systemic administration of sodium butyrate, DMTU or TMU to mice inoculated with B16 melanoma cells resulted in delayed tumour appearance and prolonged survival of the mice. These studies form a basis for further evaluation of the potential therapeutic use of chemical inducers of differentiation in solid tumours.

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## DOWN REGULATION OF NK CELL ACTIVITY IN MoLV LEUKAEMOGENESIS: EVIDENCE FOR TUMOUR CELL MEDIATED SUPPRESSION

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