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We have compared the proliferation and differentiation capacity in agar and liquid cultures of the crude conditioned media from the human urinary bladder carcinoma cell line- 5637 (BCM) and from the human placenta (HPCM). The colony stimulating factors (CSF) were added to bone marrow (BM) cultures from patients with acute myeloid leukaemia (AML) at diagnosis or in complete remission (CR) as well as to normal controls. Compared with HPCM, the BCM increased clonogenicity in 2/10 day 7 cultures from AML patients at diagnosis and in 3/15 of patients in CR. The corresponding figures for day 14 cultures were 2/6 and 1/13. When agar cultures were preceded by a liquid phase, the clonogenicity was not further increased. Observed increases were about 2-fold. The simultaneous use of both CSF had no additive effect. Cell yield was lower in 6/10 BCM stimulated liquid cultures from patients at diagnosis, and in 5/10 in CR, while the differentiation capacity was similar in BCM- or in HPCM-treated cultures. In normal controls the 2 CSF produced comparable results.

In conclusion, BCM - known as a pluripotent hemopoietic CSF - increases the clonogenicity in cultures of some AML patients when compared with HPCM.

## SENSITIVE ELISA METHOD DETECTS CISPLATIN-DNA ADDUCTS

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Highly sensitive immunoanalytical methods have recently become available for detecting structural DNA modifications caused by chemical carcinogens. With these methods it is possible to detect femtomole quantities of carcinogen-DNA adducts.

We have been developing sensitive ELISA methodologies to detect cisplatin-DNA adducts. Cisplatin (Cis-diamminedichloroplatinum(II)) is an antitumour drug, the main target of which is considered to be DNA. Polyclonal and monoclonal antibodies have been produced against cisplatin-DNA, cisplatin-polyG and cisplatin-GpG. The sensitivities of these antibodies are in the range of 50 to 100 fmol of cisplatin. They do not react with control DNA or cisplatin only. Cisplatin-GpG antibodies recognize enzymatically hydrolyzed cisplatin-DNA better than antibodies against cisplatin-DNA or cisplatin-polyG. Cisplatin-DNA antibodies react with DNA which has been purified from tissues of rats after intravenous injection of cisplatin. Recently, studies have been undertaken on cisplatin modifications of DNA in blood cells of cancer patients receiving cisplatin chemotherapy.

## PLASMINOGEN ACTIVATOR OF CLONOGENIC CELL POPULATIONS SEPARATED FROM FIBROSARCOMA

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Fibrosarcoma cells produce plasminogen activator (PA), a protease that converts the zymogen plasminogen into plasmin. Several studies indicate that tumour invasion is accompanied by proteolysis and that plasminogen activator, generated by highly malignant cells, is by far the most ubiquitous protease associated with malignant transformation. We have fractionated the fibrosarcoma cells on renographin 60 density gradients and compared the clonogenicity of these cells with their level of plasminogen activator production. Five populations of cells were separated in continuous gradients of renographin in the density range of 1.05 to 1.18 g/cm$^2$. PA activity of unseparated and five separated populations were determined using [125]I-fibrin as a substrate in a reaction between cell lysate and plasminogen. Cell populations collected at densities between 1.05 and 1.09,B1B2 were the most clonogenic. PA analysis demonstrate that PA activity is restricted mostly to B1 and B2. The differences in cell cycle parameters, determined by flow microfluorometry, between density separated bands are not striking. The results suggest that PA production is a characteristic of invasive cells.

## UTILIZATION OF N-ACETYL PUTRESCINE BY HUMAN MELANOMA CELLS IN VITRO FOR GROWTH IN THE PRESENCE OF DIFLUOROMETHYLORNITHINE (DFMO)

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The clinical response of human melanoma to DFMO has resulted in encouraging but limited benefit. A mechanism of resistance

was identified involving the deacetylation of circulatory N-acetylputrescine, an excretory product of polyamine metabolism. Three human melanoma cell lines (c8146c, c8161, and c832cy) were co-exposed to 1mM DFMO and a range of doses (1 to 50 μM) of putrescine or N-acetylputrescine. All three cell lines were able to overcome growth arrest by utilization of N-acetylputrescine in a clonogenic assay:

| Cell Line | Dose (μM) to 50% Recovery Put | Acput |
|---|---|---|
| c8146c | 7 | 68 |
| c8161 | 0-1 | 55 |
| c832cy | 0-1 | 20 |

Utilization of N-acetylputrescine to overcome DFMO growth inhibition was also demonstrated in cells grown as a monolayer. Potentiation of DFMO therapy in human melanoma might be achieved by use of an N-acetylputrescine deacetylase inhibitor.

## IMMUNOHISTOCHEMICAL PATTERN OF CEA AS A PROGNOSTIC FACTOR IN ADENOCARCINOMA OF THE HUMAN COLON

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The purpose of this study was the immunohistochemical localization of CEA in 198 cases of adenocarcinoma of the human colon. The immunohistochemical grading based on a localization pattern of CEA was used: I-apical, II-cytoplasmic/membranous/stromal, III-apical/stromal. The pattern and the intensity of immunoreactivity of CEA were analysed in relation to patients survival. The patients survival with II-cytoplasmic/membranous/stromal pattern of CEA expression was half that in those with I-apical and II-apical/stromal. Pattern II of CEA immunoreactivity correlated with patients survival in Dukes' stage C. This study shows that immunoperoxidase staining may identify sub-groups of patients with a poor prognosis. With strong positive tissue staining and II-cytoplasmic/membranous/stromal pattern of CEA expression.

## VIRAL TRANSDUCTION OF CELLULAR GENES IN NATURALLY-OCCURING FELINE LEUKAEMIAS

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De novo recombination leading to retroviral capture of cellular gene sequences appears to be an important oncogenic mechanism in the most common FeLV-associated neoplastic disease, T-cell lymphosarcoma. Multiple examples of myc gene transduction have been recorded (1) and more recently a naturally-occurring feline tumour has yielded the novel finding of a provirus containing the complete coding sequence of a β-chain T-cell antigen receptor gene (2). This potential new oncogene has been called v-tcr. Our current studies are aimed at establishing the possible role of v-tcr in T-cell leukaemogenesis by in vivo experiments with cats and mice, and in vitro by introducing v-tcr proviruses and gene constructs into T-cells. In these experiments we will also test possible synergistic interactions of v-tcr and v-myc and try to devise assay systems for oncogene interactions in T-cell leukaemogenesis.

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## SANDWICH HYBRIDISATION ON SEPHACRYL FOR THE DETECTION OF HPV STRAINS

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It is clear that the reliable detection and strain characterisation of HPV infection would be of great value in gynaecological practice. It is unrealistic to envisage the current methods of DNA detection (dot or slot blotting, followed by hybridisation to a radio-labelled probe and subsequent detection by auto-radiography) being used to screen the target number of patients per annum ($>10^5$ in the U.K.).

We have developed a sandwich hybridisation assay, using Sephacryl as the solid support, which is rapid, inexpensive and amenable to automation. Immobilisation of suitable restriction fragments, together with appropriate stringency of hybridisation, allows us to distinguish among the HPV strains.