INDUCTION OF c-myc TRANSCRIPTION IN HUMAN UROTHELIAL CELLS BY TPA IS INFLUENCED BY THE STATE OF GROWTH

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The effect of the skin tumour promoter TPA (12-0-tetradecaboylphorbol-13-acetate) has been examined in cell lines derived from human urothelium. When mortal cells were treated with a single dose of TPA a transient increase in the intracellular levels of c-myc and c-fos level was affected. The mortal urothelial cell lines grow relatively slowly with a population doubling time of 1 to 4 weeks compared to 1 to 2 days for the immortalized cell lines. Continous labelling experiments furthermore showed that 99 to 100% of the immortalized cells were in the growth fraction, in contrast to only 59 to 80% of the mortal cell lines, suggesting that a relatively large fraction of the mortal cells were in a non-dividing state of growth. When the immortalized HCV 29 cell lines was serum-starved for 3 days, we found that a single dose of TPA transiently induced high levels of both c-fos and c-myc. These results indicate that the state of growth is important for the cell response to TPA.

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THE ROLE OF HYDROPHOBIC INTERATIONS IN THE PHOSPHOLIPID DEPENDENT ACTIVATION OF PROTEIN KINASE C

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The putative cellular receptor for tumour promoting phorbol esters, Ca++, phospholipid dependent protein kinase (PKC) is activated by interaction with negatively charged phospholipids. Endogenous (diacylglycerol, DAG) and exogenous (phorbol-12-myristate 13-acetate, PMA) activators of PKC in themselves do not activate the enzyme but enhance the phospholipid dependent activation. We studied the effects of hydrophobic interactions on the activation of PKC by phosphatidyl serine (PS). We have demonstrated an inverse relationship between the unsaturation index of PS and the ability to activate PKC. In saturated PS dispersions, no additional activation of PKC by DAG or PMA was found; by contrast in unsaturated PS dispersions DAG/PMA increased PKC activity by a factor 2 to 3. Upon addition of PC to the PS dispersions, the vesicular character of the lipid bilayer was maintained and the activating effects of DAG and PMA increased.

These results indicate that the fatty acid composition of activating phospholipids and the composition of biological membranes could regulate the activation of PKC in vivo during differentiation processes and tumour promotion.

CALCIUM-DEPENDENT ISOLATION OF THE 36 kD SUBSTRATE OF pp60src KINASE: FRACTIONATION OF THE PHOSPHORYLATED AND UNPHOSPHORYLATED SPECIES

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We have developed a new simple purification of the 36 kD protein, a major substrate of both viral and growth factor-receptor associated tyrosine protein kinase, and its complex from normal and SR-RSV-transformed CEF. This procedure employs a DEAE-Sephacel column and introduces the calcium-dependent adsorption of 36 kD protein. The use of EGTA step gradients differentially elutes the 36 kD molecule from the DEAE-Sephacel column, - 2 mM EGTA elutes poorly phosphorylated molecules while heavily phosphorylated 36 kD protein requires 4 or 6 M EGTA. Tyrosine phosphorylation of the 36 kD protein is increased 2 to 3 fold following a short term incubation of whole cells with micromolar vanadate. The elution pattern of the 36 kD protein obtained from lysates of vanadate treated cells was identical to untreated cell lysates. We conclude that the function of the 36 kD protein may be calcium ion dependent and may be influenced by the phosphorylation state of the protein.

ONCOGENE STRUCTURE AND EXPRESSION IN HUMAN UROTHELIAL CELL LINES

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The objective of this study has been to compare the structure and expression of cellular oncogenes in immortalised, non-tumourigenic cell lines with

tumourigenic and relatively normal counterparts. The immortal but non-tumourigenic human bladder urothelial cell lines HCV29 and Hu609 were compared with the immortal, tumourigenic line T24, and the mortal, non-tumourigenic line Hu1752. Slot Blot analysis of basal levels of oncogene mRNAs demonstrated that the immortal, non-tumourigenic lines overexpressed 20-fold either c-myc or c-sis, while T24 overexpressed both of these oncogenes 20-fold. TPA induced c-fos but not c-myc RNA in HCV29, Hu609 and T24 while both these oncogenes were induced in Hu1752 (Skouv et al, J. Cell. Biochem., in press). Southern blotting revealed no rearrangement or amplification of the c-myc gene which might account for its overexpression or lack of TPA inducibility. Several other oncogenes also appeared structurally intact and unamplified. Only T24 demonstrated a mutation at codon 12 of the H-ras gene. The mechanisms of the c-myc and c-sis overexpression are under further investigation.

## HEPARAN SULPHATE ISOLATED FROM BASEMENT MEMBRANE SOURCES PROMOTES TUMOUR CELL INVASION OF 3-DIMENTIONAL MATRICES

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In order to evaluate the roles of individual matrix components during tumour cell invasion, we examined the behaviour of several different metastatic murine tumour cells seeded onto collagen I gels containing a second defined matrix component. After 10 days of culture, the numbers of cells which had invaded the complex gels and total cell numbers were determined. Heparan sulphates isolated from basement membrane sources gave a singular promotion of invasion of the complex gels for all tumour types tested, with more highly metstatic variants exhibiting further enhanced invasion. Depending on the cell type, hyaluronic acid gave either a marginal promotion or a slight suppression of invasion. Laminin and chondroitin sulphate gave no effect or a slight decrease, while addition of collagen IV led to a decrease in all cases. Comparison of the effects of dextran and dextran sulphate suggested tha negative charge per se was not important. Heparan sulphate appears to have a particular importance in tumour cell matrix invasion.

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## CHEMOTHERAPY AND COLLAGEN MATRIX IN TUMOUR TRANSPLANTS

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Chemotherapeutic drugs affect not only the tumour cell itself but also the collagen matrix and its components. The subrenal capsule assay method offers a new model for studying the effect of different drugs on matrix structure, distribution and synthesis and their mode of action.

In this study, mammary tumours were transplanted into the renal capsule of immunocompetent mice and rats and subjected to chemotherapy. The results showed a high transplant success rate with primary tumour structure being retained. Drugs having limited effects, showed preserved tumour basement membranes, moderate drug effects were reflected in thinning or thickening of the collagen I and III positive fibres. As shown in this study chemotherapeutic drugs cause alterations in amount and structure of collagen matrix depending upon the efficacy of the drug affecting the survival and growth of the transplanted tumour.

## CHARACTERIZATION OF A TUMOUR-ASSOCIATED SERINE PROTEASE

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Cancer patients have elevated serum and urine levels of a 6 kD trypsin inhibitor called tumour associated trypsin inhibitor, TATI (Stenman et al, Int. J. Cancer, 30: 53, 1982; Huhtala et al, J. Biol. Chem. 257: 13713, 1982). Expression of protease inhibitors in cancer is associated with increased protease activity. Of earlier known proteases only trypsin and acrosin are readily inhibited by TATI, but these proteases are not known to be expressed by tumours. We have therefore searched for other proteases reacting with TATI and now identified and characterized such a protease in fluid from mucinous ovarian cysts. The substate specificity of this protease is similar to that of trypsin. In contrast to trypsin it has a pH optimum of 10 and an isoelectric point of 4. These characteristics suggest that we have identified a new tumour-associated protease, which could play a role in the elevation of TATI in cancer patients.