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

J.Skouv, B.Christensen, J.Kieler and H.Autrup

The Fibiger Institute, Ndr. Frihavnsgade 70, 2100 Copenhagen Ø, Denmark

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

G.T.Snoek, A.Feijen, W.J.Hage, W.van Rotterdam and S.W.de Laat

Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

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

Jasna Sorić(1) and Julius A.Gordon(2)

(1)Central Institute for Tumours, Zagreb, Yugoslavia; and (2) University of Colorado, Health Sciences Center, Denver, Colorado, U.S.A.

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

S.N.Stacey, C.Hansen, J.Skouv and H.Autrup

Laboratory of Environmental Carcinogenesis, The Fibiger Institute, Danish Cancer Society, Ndr. Frihavnsgade 70, DK-2100, Copenhagen Ø, Denmark

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