in both HPC and HMEC. However, VEGF did not induce NF-κB inhibitors in either of these cell lines even though ...ation of NF-κB DNA binding by EMSA. Pretreatment of HPC ...d TNFα dependent degradation of IκBα and IκBε. Thus, VEGF ...mediated NF-κB activation in both HPC and HMEC by ...degradation of IκBα: VEGF alone can induce different subunits ...of degradation of IκBα. These data suggest that VEGF may ...signaling through NF-κB and alter the profile of genes activated ...the observed defects in DC maturation.

...RIIβ subunit of protein kinase A to CRE-containing ... Park, Y.G., Cho-Chung, Y.S. *National Cancer Institute,* ...

...(cAMP) regulates a variety of processes in mammalian cells by ...kinase A (PKA), which in turn regulates gene expression by ...transcription factors. There are two types of PKA, type I (PKA-I) ...which share an identical catalytic (C) subunit but contain ...subunits RI and RII respectively. It is well known that CAP ...(activator protein) senses cAMP levels in prokaryotic cells and ...regulation of several operons. The structural similarity of the ...CAP and RII regulatory subunit of PKA suggests the ...role for RII in eukaryotic gene regulation. We report that the ...is capable of physically interacting with the CRE (cAMP ...containing promoters of somatostatin, glycoprotein hormone ...phosphoenolpyruvate carboxykinase genes and can regulate the ...transcription. The RIIβ gene was fused with the glutathione S ...gene and the GST-RIIβ fusion protein was purified. Gel retar... revealed that the GST-RIIβ protein binds to the CRE-containing ...complex formation was concentration dependent and was pre... specific but not nonspecific competitor DNAs. Furthermore, in ...activation assays, induction of RIIβ overexpression in F9 embryonal ...enhanced the transcription of somatostatin-chloramphenicol ...(CAT) fusion gene. These results suggest that RIIβ subunit of ...binding to CRE-DNA regulates gene expression in cAMP-respon...

...depletion of estrogen receptor protein by emodin through pro... ...lytic pathway. Lau, Y.-K. and Hung, M.-C. *Department of Tumor ...University of Texas M. D. Anderson Cancer Center, Houston, Texas,* ...

...study the involvement of tyrosine kinase pathway in estrogen ...signaling pathway, we used emodin, a tyrosine kinase inhibitor, to treat ...breast cancer cell line which express high level of estrogen receptor ...We reported that emodin inhibited estrogen-induced expression of ...pS2 in MCF-7 breast cancer cells. Unexpectedly, treatment of the cells ...also depletes cellular levels of estrogen receptor protein in a dose-... dependent manner. The pulse chase experiment showed that the de... ...resulted from enhanced degradation of estrogen receptor protein, not ...from inhibition of synthesis. To examine the mechanism involved in the emodin-en... ...hanced degradation of estrogen receptor, inhibitors of the lysosomal (chloro... ...quine, leupeptin and MG115), and calpains pathways were used. We ...found that PSI and MG115, which specifically inhibit the chymotrypsin-like ...activity of proteasome, blocked emodin induced depletion of estrogen receptor ...protein. The results suggest that the proteasome proteolytic pathway may ...be involved in the emodin-induced decreases in estrogen receptor protein levels. ...We also examined the effect of emodin on the hsp90-estrogen receptor hetero... ...complex formation. We found that there was a marked increase in the ...amount of hsp90 in the data demonstrate that emodin may inhibit the dissocia... ...tion of hsp90 from estrogen receptor, resulting in the degradation of estrogen ...receptor protein. These findings indicate that proteasome-mediated protein deg... ...radation of estrogen receptor protein level, hsp90 can mediate the ...function of the estrogen receptor, and the possible use of emodin in the ...manipulation of this process.

...HER-2/neu dependent activation of NF-κB by Heregulin. Ka... ...Lee, E.K., Zhang, L., Shao, R.-P., Lin, S.-Y., Kumar, R., Chiao, P. ...*The University of Texas M. D. Anderson Cancer Center, Houston,* ...

...NF-κB can be activated by numerous agents including certain growth factors ...and insulin. Recently, activation of NF-κB has been shown to have ...effect. It has been reported that point-mutated oncogenic neu but ...not wild type can activate NF-κB. We investigated the possibility of activation ...of NF-κB by HER-2/neu mediated signaling pathway in a system where normal ...HER-2/neu is overexpressed and is activated indirectly by heterodimerization. ...Among transfectants which overexpress HER-2/neu, we found that upon ...stimulation, NF-κB is activated in these transfectants but not in the ...parental cells. This activation was dose-dependent at low concentrations of ...heregulin, but not detectable at high concentration, and this observation was ...correlated with increased cell survival.

#2785 **Transcriptional regulation of *connexin32* in hepatic cells.** Piechocki, M., Toti, R., and Ruch, R.J. *Medical College of Ohio, Toledo, OH 43614.*

Connexin32 (Cx32) is the major connexin expressed by hepatocytes and mediates gap junctional intercellular communication in the liver to maintain homeostasis and growth control. Expression of *Cx32* is often reduced in neoplastic and primary cultured hepatocytes and this is partly due to transcriptional inactivity. We investigated the molecular mechanism(s) governing *Cx32* transcription using a well-differentiated rat hepatoma cell line, MH1C1, that expressed *Cx32* and a rat liver epithelial cell line, WB-F344, that had no detectable expression. Deletion analyses of the rat *Cx32* promoter (−754 to −33) linked to the luciferase reporter gene in transient transfection assays revealed that the promoter was strongly active in MH1C1 cells, but had minimal activity in WB-F344 cells. The basal promoter element (BPE) was located within −134 to −33 and sequences further upstream conferred additional promoter activity. Several specific nuclear protein-DNA complexes within the transcriptionally active domains of the promoter were identified by gel shift assay and these complexes were different in the two cell types. Competition with an oligonucleotide for the YY1 consensus binding element and supershift assays using anti-YY1 antibody indicated that this transcription factor was a component of a complex that formed within −574 to −368. Southern analyses indicated that the gene was intact in both cell types, but was methylated in WB-F344 cells. Thus, the expression of *Cx32* appears to be regulated in a complex and cell-specific manner. Further identification of these mechanisms will provide insight into how *Cx32* expression is reduced during neoplastic transformation. (Supported by NIH/NCI CA57612).

#2786 **Interferon alpha induces association between JAK and p56*Lck* kinases in human cutaneous T cell lymphoma lines.** Sun, W.H. and Alsayed, Y.M. *Department of Pediatrics, Lurie Cancer Center, Northwestern University, Chicago, IL 60614.*

Interferon-alpha (IFNα) mediates its signaling through a pathway in which Janus tyrosine kinases (JAKs) and latent transcriptional factors (STATs) become activated. Src kinase-transformed cell lines exhibit activation of JAK-STAT, suggesting an interaction between these two signaling cascades. We sought to determine whether IFNα receptor-associated JAK kinases interact with p56*Lck*, a member of the Src family that is expressed predominantly in T cells. Studies using human T cell lymphoma lines (HUT78 and PB1) show that upon IFNα stimulation, tyrosine-phosphorylated Jak1 and Tyk2 kinases co-immunoprecipitate with p56*Lck* in a time-dependent manner, STAT3 also co-precipitates with p56*Lck* following IFNα treatment. *In vitro* binding studies using a p56*Lck*-GST fusion protein indicate that the physical association between JAKs and Lck is mediated through the SH2 and SH3 domains of p56*Lck*. Because both JAK-STAT and p56*Lck*-mediated signaling pathways are known to play important roles in the regulation of T cell growth, understanding interactions between them may provide insight into the mechanism of T cell neoplastic transformation and proliferation.

#2787 **Characterization of Ornithine Decarboxylase activities of human breast cancer cells (MCF-7) and spontaneously immortalized human mammary cells (MCF-10).** Zhu, H., Mathias, P.I., Lotz, W.G. and Savage, R.E. *National Institute for Occupational Safety and Health, Cincinnati, OH 45226*

Epidemiological studies suggest that increased risk of breast cancer is associated with exposures to electromagnetic fields (EMFs). EMFs have been demonstrated to increase Ornithine decarboxylase (ODC) activity in a variety of experimental systems. ODC, the rate limiting enzyme in polyamine synthesis, has been associated with chemical carcinogenesis. Very few if any studies have examined the effects of EMFs on breast cell ODC. Preliminary to our studies of EMF on ODC in human breast cancer cells (MCF-7) and spontaneously immortalized human mammary cells (MCF-10), we attempted to characterize the enzyme's response to a number of common ODC inducers. For the MCF-7 cells, time course studies suggested that ODC activity began to increase at 6 h, peaked at 12 h and then dropped sharply by 24 h. Treatment with 50 nM 12-O-tetradecanoylphorbol-13-acetate (TPA) for 12 h, ODC activity could be induced over 100 folds over the basal level. 9,10 Dimethyl 1,2 benzanthracene (DMBA) also induced ODC activity of MCF-7 cells in similar manner. In contrast, ODC activity was barely detectable, and was not induced by TPA or DMBA with concentration up to 400 nM in MCF-10A cells. The finding that melatonin, a cytostatic agent induced ODC activity was also peculiar. Melatonin induced ODC activity significantly in MCF-7 cells with 1 nM concentration at 12h. Studies are underway to determine the effects of EMF on the ODC activity of these and other breast cancer cell lines. This study supported in part by EMF RAPID Program funds provided by NIEHS under interagency agreement No. Y1-ES-0032.

#2788 **Potent induction of c-Fos phosphoprotein by substance P correlates with sustained phosphorylation of the cyclic AMP-response element binding protein at serine 133 in human astrocytoma cells.** Luo W, Sharif TR, and Sharif M. *Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38105.*

Receptor activation by growth factors or neuropeptides triggers signaling cascades transducing signals at the cell surface into nuclear activities that regulate gene expression. Substance P (SP) receptor is expressed in many astrocytic-derived cell lines. Previously, we have demonstrated that SP stimulates the mitogen-activated protein kinase pathway and induces the expression of c-Fos

PROCEEDINGS

EIGHTY-NINTH ANNUAL MEETING

American Association for Cancer Research

In Cooperation with the Center for Continuing Education at Tulane University Medical Center

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