Symposium no. 10: Gene Alterations in Human Cancer Cells

10.019

EFFECT OF Cu(II) AND OF EPI-DOXORUBICIN-Cu(II) COMPLEX ON PROTEIN KINASE-C.

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Protein Kinase-C (PKC) modulates the function and expression of specific proto-oncogenes and oncogenes. PKC activity increases when the enzyme is associated with the cell membranes. In this investigation we studied the effect of the epidoxorubicin-Cu complex, which acts likely at the membrane level. Cu(II) inhibits PKC (IC_{50}=100μM), Cu(epiDXR)2 develops a significantly higher activity (IC_{50}=25μM), while epiDXR shows a weak activity. The inhibition occurs both at the regulatory and at the catalitic domains of PKC.
ESR studies show that divalent Cu is responsible for the inhibition of the enzymatic activity; the complexation to epiDXR and/or PKC increases the stability of the electronic configuration of Cu(II), thus increasing the inhibitory activity of the metal on the enzyme.

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10.020

STRUCTURAL PECULIARITIES OF HA-RAS 1 PROTO-ONCOGENE IN HUMAN STOMACH CARCINOMAS

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20 patients with stomach carcinomas and 25 intact donors were examined.Amplification and rearrangement of HA-RAS alleles were observed in single carcinomas,but point mutation of the 12-th codon or any other HA-RAS alterations were not shown.Patients with stomach carcinomas have the following HA-RAS allelic frequency:A1-0.63;A2- and A3-0.13;A4-0.088;A1.2-0.022.Rearrangement of HA-RAS 5'-flank and deletion of one allele of this gene were established in some DNA samples of Leukocytes and sperma of intact donors. 5'-flank sequence of HA-RAS has individual genetic specific features with Mendelian type inheritance.It has not changes in stomach carcinomas.Thus,HA-RAS structural alterations are not frequent in stomach carcinomas.

10.021

ARG, A 145 KD PROTEIN WITH TYROSINE KINASE ACTIVITY BELONGS TO THE ABELSON SUB-FAMILY OF NON RECEPTOR TYROSINE KINASES

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The Abelson subfamily of non receptors tyrosine kinases is defined by the products of c-abl and arg (Science 1986,234,1545; Proc Natl Acad Sci USA 1990,87,5802) genes. Human arg is expressed, like human c-abl, as two transcripts that encode two proteins different only in their amino termini. The arg proteins are structurally closely related to the c-abl proteins. The arg kinase domain is 94% identical to that of c-abl and the SH2 and SH3 domains are 90% identical. Arg and c-abl have approximately the same degree of similarity with Drosophila abl, suggesting that Drosophila abl is the homologue of both human genes. Segments of the arg coding sequence, were cloned into a bacterial expression vector (Methods in Enz.1990,185,60) in order to express recombinant peptides to use in the production of specific antibodies. The same bacterial expression system was used for expressing the full length arg coding sequence. The full length arg protein expressed in bacteria is an active protein of 145 Kd with kinase activity phosphorylating tyrosine residues. The cellular arg protein, as well, was identified, in the cellular lysate of human fibroblasts, using the arg antibodies and an in vitro autokinase assay. The cellular protein is about 145 Kd, the same size of the bacterially expressed protein.
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10.022

ACTIVATION OF C-SIS AND C-FOS GENE EXPRESSION IN HUMAN MENINGIOMAS

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Studies of allele loss in meningioma localized a putative tumor-suppressor gene in chromosome 22q12.3-qter. The protooncogene c-sis maps to 22q12.3, the upper limit of the meningioma chromosome region. As allele loss was found at this polymorphic locus, we investigated whether its transcriptional activity is modified in meningioma. Northern analysis of 13 meningiomas showed c-sis mRNAs in most tumors, including those shown to be hemizygous for c-sis DNA. Production of PDGF-B by meningeal- derived cells, which do not express c-sis mRNA, but possess PDGF, may activate an autocrine pathway possibly involved in the growth and maintenance of meningioma. Attempts at identifying other protooncogenes activated in meningioma showed epression of c-fos in many tumors. Data on induction of c-fos by PDGF and a c-sis responsive element in the c-fos promoter suggest a link between c-sis and c-fos activation in this benign tumor. Supported by A.I.R.C.

10.023

EFFECT OF THE RIBOSOME-INACTIVATING PROTEIN SAPORIN 6 ON GENE EXPRESSION AND KINASE ACTIVITY IN LEUKEMIC CELLS IN VITRO.

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The effect of Saporin 6, a protein from plant origin, has been tested :
(i) on gene expression (c-myc,ODC,H3) through Northern blotting of the mRNA from treated cells;
(ii) on kinase activity, evaluated after immunoprecipitation with different Abs and immunoblotting of treated cells.
Results indicate both a decrease of mRNA expression of c-myc and of cdc2-cyclinB activity (43%) after 6 h of treatment with various concentrations of Saporin 6. Such time is very short in respect to that at which the inhibition of protein synthesis is evident. The effect on cycle G_1 phase is discussed.

10.024

DISSECTION OF C-FOS INDUCTION PATHWAYS IN A T-LYMPHOMA CELL-LINE LOW-RESPONSIVE TO SERUM INDUCTION.

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The c-fos gene was shown to be poorly inducible by serum in the murine T-lymphoma cell line Eb, as compared to other cell lines. In order to establish which of the multiple pathways of c-fos induction is responsible for the low levels of c-fos mRNA 15' after serum stimulation of growth arrested Eb cells, inducers of specific pathways were tested. Treatment of Eb cells with TPA, (50 ng/ml) an activator of the protein kinase C and the calcium ionophore A23187 (0.2 μM), gave rise to a full c-fos induction. Both mRNA levels and time course kinetics were found to be as reported for standard cell types. By contrast addition of the adenylate cyclase activator forskolin (10 μM), lead to mRNA levels which were as low those observed after serum induction. These findings suggest that protein kinase C and calcium mobilization pathways of c-fos induction are working in Eb cells, but are not activated by serum induction. The adenylate cyclase pathway is likely the only one active when c-fos is induced by serum. We are testing this hypotesis by means of inhibitors of different c-fos induction pathways.