Symposium no. 10: Gene Alterations in Human Cancer Cells

10.007

Insulin/IGF-1 modulation of estrogen-responsive gene expression in human breast cancer cells. C.M.W. Chan, D.L.Manning & C.D. Green, Dept. of Biochemistry, Liverpool University, UK.

pLIV-1 and pS2 are two estrogen-responsive genes expressed in the human breast cancer cell line, MCF-7. The level of expression of the two genes is not affected by insulin in the absence of estradiol. However, the presence of both insulin and estradiol induces pLIV-1 and pS2 mRNAs to levels at least twice those seen with estradiol alone. Insulin (10^{-7}M) may be replaced by 10^{-10}M insulin-like growth factor (IGF-1). Both pLIV-1 and pS2 are also induced by the antiestrogen tamoxifen, to levels 50-60% of those seen with an optimal concentration of estradiol. Interestingly, there is no synergism seen between tamoxifen and insulin or IGF-1.

Induction of pLIV-1 and pS2 mRNAs by estradiol alone is not affected by cycloheximide (protein synthesis inhibitor). However the stimulation of the estradiol response by insulin/IGF-1 is abolished by cycloheximide. Evidently the increases in mRNA levels caused by the peptide factors occur by a mechanism that is different from, but dependent upon, the action of estradiol and apparently requires continued protein synthesis. (Supported by the Cancer Research Campaign)

10.008

K-RAS MUTATIONS IN COLON ADENOCARCINOMAS
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In K-ras oncogene, the codon 12 mutation is the most frequent and early one in colon adenocarcinomas. We have analyzed colon neoplasms compared to paired adjacent normal mucosa samples. High molecular weight DNA was obtained with salting-out method and amplified (PCR). 32P labelled oligonucleotide probes specific for normal and 6 mutated sequences at codon 12 of K-ras gene were examined. Preliminary results of our multicentric study show that mutations in this codon is present in 28.6% of tumors. The K-ras oncogene determination in colon carcinoma may have the clinical rilevance, but valutation of others oncogenes (p53,c-myc, c-fos and so forth) is necessary for contribute to more accurate staging, malignance and prognosis.

10.009

Human neuroblastoma cells induced to differentiate with γ-IFN show high levels of 2',5' oligoadenylate synthetase.

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The involvement of the 2',5' oligoadenylate synthetase (2,5OAS) in mediating the differentiative effect of γ-Interferon (γ-IFN) was evaluated in the human neuroblastoma cell line LAN-5 treated with recombinant γ-IFN. Northern blot analysis of total and poly(A) RNA clearly indicated that 2,5 OAS mRNA levels increased in a dose and time dependent manner, being detectable before any maturative change was observed. Maximum increase was observed after 48 hrs when a dose of 500-1000 U/ml was used. Since 48 hrs of treatment 2,5 OAS mRNA levels slowly decreased, being faintly detectable after 96 hrs. Inhibition of transcription with Actinomycin D indicated that 2,5 OAS mRNA was quite stable, having an half life of 3.5 hrs. A parallel increase in the 2,5OAS enzymatic activity, evaluated by means of a competitive RIA test, was observed. Namely, the activity, undetectable in untreated LAN-5 cells, increased up to 78 pmol olygoadenilate/mg total protein after 48 hrs, and afterwards slowly decreased. After 5-7 days of treatment with γ-IFN LAN-5 cells were morphologically differentiated and cell proliferation was completely suppressed. The apparence of biochemical markers of neuronal differentiation confirmed the ability of γ-IFN to induce neuroblast maturation. These results support a direct involvement of 2,5 OAS in γ-IFN-induced differentiation of neuroblastoma cells.

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10.010

PROTO-ONCOGENE MUTATIONS IN HUMAN BREAST CANCER (HBC).
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This is a preliminary report of mutations in HBC. The genetic organization of c-myc, L-myc and neu genes was examined in tumors from ten patients with poorly differentiated infiltrating ductal cell carcinomas with epsilateral axillary lymph node metastasis. We observed genetic abnormalities in six cases. Two tumors had only neu sequences amplified, one had L-myc and three had both. The amplification of neu gene was accompanied by increased RNA expression. C-myc was not altered in any samples. Amplification of neu and L-myc was seen in two pregnant patients; one presented with bone metastasis. Neu was amplified in a patient who developed contralateral breast cancer and bone metastasis within one year from diagnosis. This study suggests that L-myc and neu mutations may be useful prognostic indicators. The alteration of neu and L-myc genes in tumors from pregnant patients warrants further investigation. Additional studies are in progress.

10.011

Effect of cAMP treatment on IRBP mRNA expression of Y-79 retinoblastoma cells.

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Interphotoreceptor-retinoid binding protein (IRBP) is a large 140 Kd glycoprotein found in the interphotoreceptor matrix and is conserved among vertebrates. It is highly tissue-specific, being limited in expression to retinal photoreceptor cells and a subset of pinealocytes. IRBP is also expressed in retinoblastoma cells (Y-79). Previously we have obtained two clones for human IRBP containing the entire translated region and 7Kb of 5' flanking sequence in order to study the regulation of gene expression. A restriction fragment of one of the clones was used to probe total RNA from Y-79 cells. A single mRNA of 4.8 Kb was recognized on northern blots. Y-79 cells grown on a Poly-D-lysine-coated substratum assume a photoreceptor-like morphology and express IRBP mRNA in abundance, while cells in suspension culture exhibit a much lower message level. After treatment with the differentiating agent cyclic AMP monolayer cultures of Y-79 cells show a partial morphological and biochemical differentiation towards a glial-like phenotype. This is accompanied by a decrease of IRBP mRNA with respect to the amount of IRBP in Y-79 grown on PN. Our data indicate that cAMP is able to induce a differentiative modulation of Y-79 cell phenotype.

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10.012

Nm23 expression correlates with malignancy of human melanoman and nevi. V.A. Flørenes, G.M. Mælandsmo, Ø. Bruland, O. Myklebost, Ø. Fodstad. Dept. of Tumor Biology, The Norwegian Radium Hospital, Oslo, Norway.

The nm23 gene was originally cloned from a variant of the K-1735 murine melanoma cell line, and is supposed to function as a suppressor gene, at least in some rodent tumors and in human breast cancer. Here we have examined 15 benign nevi and 35 biopsies from human melanomas of different metastatic potential for expression of nm23. The gene was differentially expressed in the melanomas (8-fold difference in hybridization intensity), whereas the expression in benign nevi was generally low. Unexpectedly, in all cases where we could compare the transcription level in benign and malignant tumor material from the same patient, the expression was higher in the melanomas (9/9). Six human melanoma cell lines tested expressed the nm23 gene, but no relationship between expression and the metastatic potential was observed.

Our data suggest that low nm23 expression is not assosiated with malignancy and metastatic competence of melanocytic human tumors.