60 PHOSPHATIDATE AND LYSOPHOSPHATIDATE ARE SUBSTRATES FOR HUMAN ALKALINE PHOSPHATASES

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There is considerable evidence that phosphatidate (PA) and lysophosphatidate (LPA) are important second messengers in signal transduction processes in a variety of cell types.

Objectives a. To determine the ability of PA and LPA to serve as substrates for the placental, intestinal and tissue nonspecific isoenzymes of alkaline phosphatase (ALP). b. To assess the importance of ALP in the catabolism of PA and LPA.

Methods PA hydrolysis was measured by the conversion of [^{3}H]PA to [^{3}H]diacylglycerol and by the ability of PA to serve as a competitive inhibitor of the hydrolysis of p-nitrophenyl-phosphate (p-NPP). LPA hydrolysis was measured by the ability of LPA to serve as a competitive inhibitor of the hydrolysis of p-NPP.

Results The placental isoenzyme hydrolyzed PA at the rate of 7 nmol/min/unit of ALP activity, and the tissue nonspecific isoenzyme at 10% of this. p-NPP was a competitive inhibitor of the PA phospho-hydrolase activity of placental ALP (K_i = 0.13 mM), and PA was a competitive inhibitor of its p-NPPase activity (K_i = 0.85 mM). LPA was also a competitive inhibitor of the p-NPPase activity (K_i = 0.15 mmol/L). Both Ca^{2+} and Mg^{2+} inhibited the PA phosphohydrolase activity of placental ALP.

Conclusions a. Human ALPs will hydrolyze PA and LPA. b. The monovalent salt form of PA is the substrate. c. PA is an unlikely physiological substrate for ALP, but LPA is a better candidate.

61 SERUM p53 AUTOANTIBODIES: INCIDENCE IN OVARIAN CARCINOMA

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p53 tumor suppressor protein has been found to become immunogenic in certain types of cancer (breast, colon, lung), and antibodies against p53 have been detected in the sera of cancer patients.

Objectives a. To investigate whether ovarian tumors elicit antibodies against p53 which can be detected in the serum. b. To investigate if the factor that makes this protein immunogenic is its overexpression by the tumor.

Methods Serum samples from 206 women with cancer of the ovary were analyzed for the presence of p53 antibodies using a recently developed immunoassay based on time-resolved immunofluorometry. To examine the expression of p53 by the tumor, immunohistochemical analysis of tumor sections was used.

Results Circulating antibodies against p53 were found in 22.3% (46/206) of the patients. Some sera contained extremely high levels of p53 antibodies (up to 9,000,000 arbitrary Units/L). Sequential analysis of positive sera demonstrated that p53 antibody levels change during the course of the disease, reflecting disease progression or regression. Immunohistochemical analysis of tumor sections showed that ovarian tumors which elicit p53 antibodies are those which overexpress the p53.

Conclusions These data demonstrate that antibody generation against p53 is a common event in ovarian cancer, and that serological analysis of p53 antibodies may be a promising, non-invasive test for disease monitoring and possibly diagnosis.

62 RELATIONSHIPS BETWEEN MUTANT P53 PROTEIN OVEREXPRESSION AND OTHER PROGNOSTIC INDICATORS IN BREAST CANCER

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Mutation and overexpression of p53 protein occurs in 20-50% of breast cancers and has been shown to be an independent prognostic indicator.

Objective To relate the levels of p53 protein in breast tumor extracts with other biochemical and flow cytometric findings of potential or demonstrated prognostic value.

Methods p53 protein was measured in extracts by a time resolved immunofluorometric assay. Other analyses consisted of the determination of levels of estrogen (ER) and progesterone (PGR) receptors, epidermal growth factor receptor (EGFR), HER-2/ neu, cathepsin D, prostate specific antigen (PSA), S-phase fraction, and DNA ploidy.

Results Weak negative correlations were found between p53 and both ER ($p=0.01$) and PGR ($p=0.04$) and a positive correlation between p53 and S-phase fraction ($p=0.02$). Wilcoxon Rank Sum analyses showed that levels of ER ($p<0.01$), PGR (pc0.01), S-phase fraction ($p<0.01$) and EGFR ($p<0.01$) differed between p53-negative and -positive tumors. Levels of p53, however, differed only across groups defined by PGR ($p<0.01$) or ploidy ($p=0.04$). Contingency tables found negative associations between p53 and ER ($p<0.01$) and PGR ($p<0.01$) and positive associations between p53 and EGFR ($p=0.02$), HER-2/neu ($p<0.01$) and aneuploidy ($p<0.01$).

Conclusions The presence of p53 in breast tumors relates to several other variables suspected to predict aggressive tumor phenotypes.

63 PROGNOSTIC VALUE OF IMMUNOFLUOROMETRICALLY QUANTIFED P53 PROTEIN IN OVARIAN CARCINOMA

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The prognostic significance of p53 accumulation in ovarian cancer has not yet been firmly established.

Objective To evaluate the relationships between immunofluorometrically measured p53 with patient survival and clinicopathologic factors associated with prognosis.

Methods Statistical analyses consisted of Chi-square tests for associations between variables, Kaplan-Meier survival curves for p53-negative and -positive groups and for subgroups defined by different stage, grade, or residual tumor presence, and the Cox regression model at both univariate and multivariate levels.

Results Advanced stage (III-IV) and grade (G3), serous histotype, and presence of residual tumor were associated with cancer relapse and death. Patients with p53-negative tumors had longer disease-free survival than patients with p53-positive tumors ($p=0.03$). A similar tendency was seen for overall survival ($p=0.06$). p53-positive patients had twice the risk of both relapse and death compared to p53-negative patients. Multivariate analysis revealed that only presence of residual tumor significantly predicted patient outcome. However, patients with well (GI) or moderately (G2) differentiated cancers had higher risk of recurrence when p53 was present compared to when p53 was absent ($p<0.01$).