60 PHOSPHATIDATE AND LYSOPHOSPHATIDATE ARE SUBSTRATES FOR HUMAN ALKALINE PHOSPHATASES


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 [3H]PA to [3H]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_M = 0.13 mM), and PA was a competitive inhibitor of its p-NPase activity (K_M = 0.85 mM). LPA was also a competitive inhibitor of the p-NPase activity (K_M = 0.15 mM). Both Ca^2+ and Mg^2+ inhibited the PA phospho-hydrolase activity of placental ALP.

Conclusions a. Human ALP's 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.

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 immunoluminescent assay. Other analyses consisted of the determination of levels of estrogen (ER) and progesterone (PGR) receptors, epithelial growth factor receptor (EGFR), HER-2/new, 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 (p<0.01), S-phase fraction (p<0.01) and EGFR (p<0.01) differed between p53-negative and -positive tumors. Levels of p53, however, differed only between groups defined by PGR (p<0.01) or ploidy (p<0.04). Contingency tables showed 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/new (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 QUANTIFIED P53 PROTEIN 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 To investigate whether ovarian tumors elicit antibodies against p53, which can be detected in the sera. b. To investigate if the presence of this antibody is predictive of outcome.

Methods Serum samples from 206 women with cancer of the ovary were analyzed for the presence of p53 antibodies using a recently developed immunoblot assay based on time-resolved immunoluminescence. To examine the expression of p53, the 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 increase 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.