The p53 Protein Detected by Immunohistochemical Staining Is Not Always Mutant

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Abstract In human mammary carcinoma, positive immunohistochemical staining for p53 protein is not always indicative of mutation in the p53 gene. Although positive staining is seen in excess of 50% of tumours, mutations have been found in only some 20% of cases. In this presentation, positive p53 staining in mammary carcinomas will be related to the presence and absence of mutation and other possible underlying mechanisms.

In some positively stained tumours a mutation has been found. In others, no mutation has been demonstrated and apart from possible stabilisation by a protein such as MDM2, there are alternative underlying mechanisms for this discrepancy. Wild type p53 is elevated in response to DNA damage. This effect can be seen in patients given pre-operative chemotherapy and in cell lines irradiated with UV light and with x-rays. Strong positive staining in scattered nuclei has been found in cell lines with activated *ras* and *myc* genes. We postulate that this may also be the reason for similar patterns observed in human tumours.

Comparable mechanisms may be active in inherited cancers. Although positive p53 staining in some Li-Fraumeni syndrome patients is associated with mutation, in other Li-Fraumeni-like families, no mutation has been found despite positive staining in tumour **and** normal tissues.

Whatever the mechanism underlying the stabilisation of the protein, increased expression of p53 protein in the majority of tumour cells appears to be associated with poor prognosis in breast carcinoma.

Biomarker for Breast Cancer Chemoprevention: Antimalignin Antibody

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Abstract Recognin M has been isolated from MCF-7 malignant mammary cells. It is a 10 kD cancer polypeptide antigen rich in glutamic and aspartic acids related to malignin isolated from glial brain tumors (Glu13, Asp9, His2). An IgM auto-antibody against Recognin M, antimalignin, has been isolated from human serum, produced both as a mouse monoclonal antibody and in human form by challenge of human lymphocytes with the antigen *in vitro*. It has been isolated from malignant cells obtained at surgery and autopsy by elution and immunoabsorption to its immobilized purified antigen. Antimalig-

nin antibody is cytotoxic to malignant cells *in vitro* in concentrations of picograms/cell. In a 20-year study involving several hundred physicians and three independent laboratories in the U.S., and three hospitals and one laboratory in the U.K., we have found that the concentration of antimalignin in serum, in μg/ml, (1) of normal healthy non-tumor-bearing humans increases moderately each decade between the third and the seventh, as the risk of cancer increases ($p < 0.001$; $N = 1972$), (2) increases earlier and more markedly in as yet apparently unaffected members of high-risk cancer families ($p < 0.001$; $N = 1106$), and (3) is markedly increased in concentration in human serum within weeks of the occurrence of malignant transformation and returns to normal within 3 months of successful treatment ($p < 0.001$; false positives and false negatives <5% on first determination, <1% on repeat determination; $N = 600$).

Quantitative determination of serum antimalignin antibody is therefore of interest for use as a non-invasive biomarker to indicate successful results in breast cancer chemoprevention trials.

In addition, purified antimalignin antibody (MTAG), due to its demonstrated specificity in fluorescent and other chromogen staining of cell membranes with exposed malignin epitopes, is applicable for use alone or as part of a battery of pre-dysplasia or dysplasia-based surrogate endpoint biomarkers in both individual and computerized cytometry.

Validation of the Use of C-2/C-16α Estrogen Metabolites as Markers for the Action of Chemopreventive Agents in the Prevention of Breast Cancer

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Abstract Studies from this laboratory have demonstrated that negative modulation of the C-2/C-16α ratio of estradiol metabolites serves as a marker of the action of oncogenes and carcinogens which increase tumorigenicity, while positive modulation of this ratio measures the preventive effects of chemotherapeutic and chemopreventive agents on tumors and tumor cells. In order to facilitate human studies on chemopreventive agents and facilitate the measurement of this ratio, we have validated an ELISA assay using monoclonal antibodies developed by Immunocare, Inc., coated to 96-well plates.

Urine samples (10λ) were diluted in buffer and hydrolyzed with mixed glucuronidase and sulfatase to cleave the conjugates. Aliquots of the hydrolysate were added to ELISA plates coated with the C-2 and C-16α antibodies respectively and the appropriate labeled antigens were added. After incubation the plates were washed, the color reagent added, and the plates read kinetically to determine the amount of compound present. A standard curve is run on each plate along with high and low standards. All samples were run in triplicate and the mean values determined. The ratios were computed automatically by the reader.

Blind comparisons of duplicate urine samples showed a mean r value of 97%. Mean intra-assay variability was under 8% and inter-assay variability was under 10%.

Studies involving diet modification and the differences between breast cancer patients and controls are underway.
