presence of specific anti-p53 antibodies. Furthermore, Western blot analysis demonstrated that the antibodies present in human sera reacted specifically with the mutant p53 protein extracted from the COLO 320 HSR (+) tumor cell line. These data demonstrate that anti-p53 antibody generation is a common event in colorectal carcinoma and that these antibodies may be used for disease monitoring and possibly diagnosis.

10 COLORECTAL CARCINOMA ELICITS A HUMORAL IMMUNE RESPONSE AGAINST P53 MUTANT PROTEIN

Angelopoulou, K., Diamandis, E.P., and Bunting, P.S., Department of Clinical Biochemistry, The Toronto Hospital, and Sunnybrook Medical Centre, Toronto, Ontario, Canada

p53 mutations are the most frequent genetic alterations in human cancers. Very high frequencies of p53 gene aberrations has been found in colorectal carcinoma (75-80%). Because p53 gene mutations typically result in increased p53 protein levels in tumor cells, this cellular protein might become immunogenic during tumor development. To test for this hypothesis, we analyzed sera from 268 patients with colorectal cancer for the presence of anti-p53 antibodies. For this purpose we used a recently developed assay which involves solid phase immobilization of a monoclonal anti-p53 antibody, p53 antigen derived from the colorectal carcinoma cell line COLO 320 HSR(+), serum sample and an alkaline phosphatase-labeled goat anti-human antibody. Circulating antibodies against p53 were found in 20% (53/268) of the patients. Because our assay technique was quantitative, we were also able to measure antibody titers in all positive sera. Some sera contained extremely high levels of anti-p53 antibodies (up to 500,000 artibrary units/L). Sequential analysis of positive sera demonstrated that antibody titers were correlated with the progression or regression of the disease. These antibody changes were also closely related with serum levels of CEA. Protein A affinity chromatography purification and HPLC analysis were performed for some positive sera. The results clearly confirmed the