15 The p53 suppressor gene product quantified in cell lines, tumour tissue and biological fluids using an ultra-sensitive time-resolved fluorescence immunoassay

Hassapoglidou, S., and Diamandis, E.P.
Department of Clinical Biochemistry, Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, M5T 2S8, Canada.

Mutations of the p53 tumour supressor gene are the most common genetic alterations in human cancers. Currently, it is believed that the wild-type p53 nuclear phosphoprotein is a negative regulator of cell growth. The mutated form not only loses its negative regulating activity but gains a function which may contribute to malignant cell transformation. Recently, monoclonal and polyclonal antibodies against mutant and wild-type p53 have been developed and used to study the protein by Western blot analysis and immunohistochemistry. Here we describe a non-competitive quantitative immunoassay for p53 protein (mutant or wild-type) which is simple to perform, fast and ultrasensitive. We coated solid-phases (microtiter wells) with a goat anti-mouse IgG (GAM-Ig). In the assay, p53 reacts with an anti-p53 monoclonal antibody which is simultaneously captured on the solid-phase GAM-Ig. The anti-p53 monoclonal is either PAB 240 which recognizes only mutant p53, or PAB 421 which recognizes both wild-type and mutant p53. After washing, we add a polyclonal anti-p53 detection-antibody followed by a goat anti-rabbit antibody labelled with alkaline phosphatase (ALP). The activity of ALP is determined by using the new substrate 5-fluorosalicylphosphate. ALP releases 5-fluorosalicylate which forms long-lived fluorescent complexes with Tb^{3+}; these are quantified by time-resolved fluorometry. This assay was used to quantify mutant p53 in cell lines that have mutated p53 genes, in tumour tissue homogenates and other biological fluids.