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

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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-fluorosaliclylate which forms long-lived fluorescent complexes with Tb³⁺; 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.