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Novel missense mutation in the phosphatase PP2A catalytic subunit characterised in a solid tumour cell line

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Introduction: The protein serine/threonine phosphatase type 2A (pp2a) is a heterotrimer complex composed of a catalytic subunit (pp2aC), the scaffolding A subunit (pp2aA), and the variable B regulatory subunit. The pp2a complex attenuates the phosphorylation of various signals involved in cell survival and proliferation. Missense mutations in the coding sequence of the PPP2CA catalytic subunit were previously described, and studies of the pathological effects require further investigation. Of interest low pp2a activity was described as a potential mechanism of Chronic Myeloid Leukaemia blast crisis, hence the use of the CML cell line, K562, as a model of pp2a studies. Increasing evidence show that decreased pp2a activity is implicated in the progression of multiple tumour types and provides a potential therapeutic target.

Aim: To characterise mutations in the PPP2CA coding sequence using a panel of solid tumour cell lines and to measure the activity of the mutant subunit.

Methodology: Primers were designed to amplify the coding sequence of the PPP2CA transcript using cDNA derived from cell lines. The amplicon was ligated in a vector (TA cloning kit) followed by bacteria transformation. DNA was extracted from a number of colonies and sent for sequencing. *In vitro* transcription, translation system will be used to synthesis the mutant pp2aC protein. As a control, K562 cells were cultured in the presence or absence of the pp2a activator, FTY720. Cells were harvested at different time points and cell lysates were prepared in low detergent lysis buffer. The phosphatase activity was measured using a fluorescent-based enzyme-substrate reaction.

Results: Screening of the cell line panel characterised a novel mutation in the PPP2CA coding sequence of PC3 (prostate carcinoma) at codon 227. This nucleotide substitution A679G results in an amino acid change from threonine to alanine. This mutation was not found in 50 random normal samples. The protocol to assess phosphatase activity has been optimized. The K562 pp2a activity increased from 70U/mL to 4400U/mL upon addition of 2.5µM FTY720. The activity of the mutant pp2a is currently being investigated.

Conclusions: The presence of the novel missense mutation in the catalytic subunit of pp2a, supports the potential deregulation of pp2a *in vivo*. This provides the basis to classify specific group of tumour patients that might benefit from targeted therapeutic drugs. Further studies shall study the functional properties of the mutants and investigate the incidence of these mutations in solid tumour biopsies.