Investigating the interaction of RBBP6, p53 and MDM2 in normal and cancerous celllines

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Introduction: Worldwide, cancer is a major health issue, causing millions of deaths every year. Retinoblastoma binding protein 6 (RBBP6) has been found to be over-expressed in several cancers. Due to studies in mice, RBBP6 is thought to facilitate the interaction between p53 and its major negative regulator, MDM2, causing increased degradation of p53. It is also suspected that RBBP6 may promote the ubiquitination and degradation of p53 protein through its own E3 ubiquitin ligase activity. This relationship makes RBBP6 a potential drug target.

Methodology: Immunocytochemistry was used to investigate the localisation of RBBP6 and p53 in cancerous and normal cell lines and colocalisation in the cancer cell lines, MCF7 and A549. Colocalisation results were analysed using the JACoP plugin from ImageJ. Western blot was used to visualise co-immunoprecipitation assays investigating the interaction between endogenous p53, RBBP6 and MDM2 in normal and cancerous cell lines.

Results: We present localisation images that show different expression for RBBP6 and p53 in cancerous and normal cell lines. We also present colocalisation images of p53 and RBBP6 in cancerous cell lines that indicate that p53 and RBBP6 localise in similar regions of the cell. The calculated Manders coefficients were above 0.75 in both MCF7 and A549 cells and the Pearson's coefficient was found to be 0.759 for MCF7 and 0.630 for A549 cells. The co-immunoprecipitation assays indicate MDM2, p53 and RBBP6 from a complex as they are eluted together when anti-p53, anti-RBBP6 and anti-MDM2 are each separately used as probes.

Discussion and Conclusion: We present data here that shows p53 and RBBP6 are localised in similar areas of the cells, particularly in cancerous cell lines and therefore could potentially interact *in vivo*. The *in vitro* data presented suggests that MDM2, p53 and RBBP6 interact and form a complex, therefore supporting the theory that RBBP6 could act as a facilitator for p53 and MDM2 interaction and ultimately p53 degradation. This further supports RBBP6 as a prospective therapy target.

Keywords: RBBP6, p53, MDM2, colocalisation, Co-IP, cancer