**Exploring the protein-protein interactions of the p53 apoptosis effector protein PERP**

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**Purpose:** PERP protein is a specific p53-regulated plasma membrane apoptosis effector, which is downregulated in aggressive tumours, including monosomy 3 uveal melanoma. This study aims to define the protein-protein interactions of PERP, to give insight into the signalling pathways involved in its trafficking and apoptosis role. The knowledge gained may help identify novel molecular targets for the treatment of aggressive tumours.

**Methods:** PERP cDNA was cloned into the N-terminal mammalian Halotag vector (Promega) between EcoRI and NotI sites, and the correct, in-frame fusion was confirmed by sequencing. For construct functional validation, Mel202 cells were transfected with Halotag or Halotag-PERP using Turbofect and protein localisation was assessed by live cell imaging using the TMRDirect ligand. Protein lysates were also collected for Western blot analysis to determine whether previously characterised signalling pathways were induced by Halotag-PERP. Protein pull-downs were performed from lysates of transfected Mel202 cells; protein interacting partners removed from the resin using ProTEV Plus (Promega) were digested within an SDS-PAGE gel using Trypsin Gold, identified by LC-MS/MS, and analysed using Maxquant proteomics software. Mass spectrometry results were validated by Western blotting.

**Results:** Halotag-PERP localised to the endoplasmic reticulum (ER) and plasma membrane of Mel202 cells, correlating with an increase in protein levels of endogenous PERP and specific phosphorylation of p53, as previously characterised. A total of 21 proteins were identified as having a fold change of 1.5 or higher in at least 2 independent Halotag-PERP pull-down experiments relative to the Halotag only control, and 6 proteins were identified exclusively in Halotag-PERP pull downs in all experiments. The interaction of PERP with 4 proteins was confirmed by Western blot. Interestingly, two proteins with a fundamental role in maintaining endoplasmic reticulum homeostasis, and one protein involved in plasma membrane protein trafficking were identified as potential interacting partners.

**Conclusions:** The Halotag protein pull-down system was validated as an appropriate system to study the interactions of PERP, with no effect on protein function and localisation by the Halotag. Three novel protein-protein interactions have been identified, indicating a possible role for PERP in ER stress induced apoptosis.