6 TARGETING THE 19S PROTEASOMAL SUBUNIT, RPT4, IN COLON CANCER CELLS INDUCES CELL DEATH AND REDUCES CELLULAR PROLIFERATION IN VITRO AND IN VIVO

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Introduction Colorectal cancer (CRC) is the third leading cause of cancer related deaths in the US with half a million deaths worldwide. In recent years much research has centered on the ubiquitin-proteasome pathway (UPP), responsible for the degradation of the majority of intracellular proteins, as a therapeutic target for cancer treatment, with deregulation of the UPP frequently observed in a number of malignancies. Indeed, malignant cells have higher vulnerability to the cytotoxic effects of proteasomal inhibition through their greater dependence on proliferative and anti-apoptotic pathways.

Aims/Background Here we investigated the expression of the 19S proteasome subunit, Rpt4, one of six ATPases of the 19S regulatory subunit involved in the recognition of proteasome substrates and their unfolding and entry into the catalytic core, in tumour samples derived from patients with CRC.

Results Western blotting and immunohistochemical staining of tissue microarrays demonstrated increased expression of Rpt4 in tumour tissue compared to patient matched normal mucosa. Inhibition of Rpt4 expression using siRNA in HCT-116 colorectal cancer cells showed that inhibition of Rpt4 expression leads to reduced cellular proliferation, increased apoptosis and reduced clonogenic survivalin a p53 independent manner. CRL 1807 non-transformed colonocytes remained largely unaffected suggesting that tumour cells may be selectively sensitive to Rpt4 inhibition. Moreover, chemotherapy resistant cell lines were demonstrated to be sensitive to the effects of Rpt4 inhibition. In addition Rpt4 inhibition acted in concert with 5-FU based chemotherapeutic agents by enhancing levels of induced cell death. Interestingly, Rpt4 inhibition led to decreased proteolytic activity of the proteasome. Finally, we could demonstrate that decreased Rpt4 expression could inhibit tumour growth in vivo. HCT-116 luc2 ULTRA (Caliper LS) colorectal cancer cells transfected ex vivo with Rpt4 siRNA and subcutaneously implanted in Balb/c nu-nu immunodeficient mice had significantly increased survival compared to controls. In the same tumour model a cell penetrating peptide based microparticle delivery system administering Rpt4 siRNA via intratumoural injection, demonstrated that in vivo gene silencing of Rpt4 using 0.1 mg/kg siRNA led to reduced tumour volume growth and statistically significant improvement (Log-rank Mantel Cox Test) in survival in these mice.

Conclusion Taken together our data suggest the specific inhibition of Rpt4 function may represent a novel therapeutic target for the treatment of CRC.