Purpose/Background: Chemoradiotherapy (CRT) is often employed to treat locally advanced rectal cancer with highly variable response, emphasizing the necessity for predictive response biomarkers. Our initial proteomic and immunohistochemical work demonstrated that acid ceramidase (AC) expression correlated with poorer CRT responses in rectal cancer. We described that higher AC expression correlates with radioresistance in colorectal cancer cells and improved radiosensitivity through siRNA inhibition of AC. The mechanisms behind AC expression, radioresistance and apoptosis remain unknown in colorectal cancer. AC is known to affect apoptosis and the enzyme poly (ADP-ribose) polymerase-1 (PARP-1) is a DNA repair enzyme that is also cleaved into specific fragments during apoptosis.

Aims: To elucidate a potential mechanism linking AC expression with radioresistance in colorectal cancer cells.

Methods/Interventions: Differential AC protein expression of four colorectal cell lines was confirmed by Western blotting. Radiosensitivity of these cell lines was examined using standard clonogenic assays by counting individual colony survival post-exposure to increasing doses of ionizing radiation. siRNA knockdown of AC was performed with further clonogenic assays to establish the impact of AC inhibition on radiosensitivity. HT29 and HCT cells were then treated with non-targeting control siRNA and AC siRNA, irradiated at increased doses of radiation then harvested at specific time points (2,6,24h). Western blotting was then performed to detect the presence of specific PARP-1 cleavage fragments in the different treatments as specific apoptotic markers.

Results/Outcome(s): Clonogenic assays confirmed that cell lines with greater cellular AC protein expression (LIM 1215/MDS18) demonstrated higher colony survival compared to those with lower AC expression (HT29/HCT 116) post irradiation. siRNA AC knockdown improved radiosensitivity by reducing colony formation efficiency (CFE) in three cell lines: HT29 (0.52 CFE control vs 0.13 CFE knockdown at 1G p=0.00004); HCT (0.24 CFE control vs 0.09 CFE knockdown at 1Gy p=0.026); LIM 1215 (0.88 CFE control vs 0.43 CFE knockdown at 0.25Gy p=0.001). Western blotting confirmed that HT29 and HCT cells treated with AC siRNA displayed significantly higher levels of the 24kD PARP-1 cleavage compared to control therefore indicating increased apoptosis.

Conclusions/Discussion: Higher AC expression correlates with radioresistance in multiple colorectal cell lines and we successfully improved radiosensitivity through biological (siRNA) inhibition of AC. Initial mechanistic work has confirmed that siRNA inhibition of AC causes increased apoptosis in HT29 and HCT cells following ionizing radiation, suggesting a role of AC expression and radioresistance through reduction in apoptosis. Further work is required which could potentially allow AC to serve as a predictive CRT response biomarker in rectal cancer patients.