

Assessment of acute bowel toxicity from chemoradiation for bladder cancer using an *in vivo* gastrointestinal crypt assay and *in vitro* organoid system.

Authors: Jessica Gorrill¹, Jia-Ling Ruan¹, Blaz Groselj¹, Jong-Wei Hsu¹, Cheryl L Scudamore², Anne E Kiltie¹

¹CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK/²Mary Lyons Centre, MRC Harwell, Harwell, UK

Background

Radiotherapy combined with a radiosensitiser is effective treatment in muscle-invasive bladder cancer, but can result in severe bowel toxicity. The classical crypt assay can be used to assess gut toxicity *in vivo* following whole abdominal radiation. The recent development of delivering focussed radiation to bladder and surrounding bowel by small animal radiation research platform (SARRP) requires a modification of the classical crypt assay to identify the irradiated lengths of intestine. We aimed to refine the classical assay to produce an accurate and reliable assessment of crypt viability following SARRP irradiation +/- concurrent chemotherapy.

Methods

Ten, 12 or 14 Gy was delivered to the lower abdomen of 5 to 7-week old CD1-nude mice by SARRP, with or without concurrent radiosensitiser (panobinostat, PAN, gemcitabine, GEM). Mice were culled at 3.75 days. Small intestines were processed into three Swiss rolls per mouse, to permit a longitudinal view of the entire small intestine. Following formalin-fixation, paraffin-embedding and H&E staining, irradiated areas were outlined by two independent observers. Crypts were counted using strict criteria and crypt density calculated. Immunohistochemistry was used to assess cell proliferation (BrdU) and apoptosis (cleaved caspase 3) and phloxine-tartrazine staining used to assess Paneth cell viability.

Results

A log-linear crypt survival curve was observed with mock treatment and panobinostat over 10 to 14 Gy. There was no significant reduction in crypt density with panobinostat + IR ($p=0.631$) compared to mock + IR. However, with gemcitabine + IR, there was a significant reduction in crypt density at 12 Gy ($p=0.001$), similar to that observed with 14 Gy alone.

Conclusions

The modified crypt assay is a promising method to determine the effects of chemoradiation combinations on small intestine. We are now developing a murine intestinal organoid system as an *in vitro* drug/radiation testing platform, which might replace the crypt assay.