MSc DEFENCE Monday, September 30th, 2019
Student: Anam (Nan Nan) Liu
Title: A SEMI-AUTOMATED ASSAY FOR RADIATION-INDUCED INTESTINAL DAMAGE IN MICE
Time and location: 1:00 PM; Dorothy Lam Board Room, BC Cancer Research Centre, 675 W 10th Ave, Vancouver, BC
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ABSTRACT

Purpose: The effectiveness of radiotherapy to eradicate cancers is limited by normal tissue that is inevitably included in the irradiated volume. Drugs are being developed with the hope of increasing the therapeutic ratio between tumour cell eradication and normal tissue damage. Improvements in assays to measure normal tissue toxicity are needed. This thesis summarizes the development of a semi-automated intestinal assay with three analysis models - *crypt proliferation* assessing S-phase cells, *villi morphology* assessing villi size and *DNA damage* assessing DNA double strand breaks (DSBs). We use computer automation to address issues such as subjective assessment of damage.

Methods: Mice were irradiated with 0-15 Gy x-rays and euthanized at 48 h, 3.5 d and 1–24 h for proliferation, morphology and DNA damage models respectively. Mice received 5-Ethynyl-2'-deoxyuridine (EdU) to stain S-phase cells. A 5 cm length of the jejunum was collected, cut open, wrapped to form a swirl and frozen for cryosectioning. Cryosections (10 µm) of the jejunal swirl were stained and imaged using a robotic microscope; images were processed using ImageJ software. EdU was identified via histochemical staining and the proliferation model reports the proportion of pixels positive for EdU per length of the jejunum. Endothelial cells were identified using immunostaining for CD31, with the villi morphology model reporting the length of villi capillary beds. γH2AX was stained to quantify DSBs, with the DNA damage model reporting the amount of DSBs. A statistical power and assay sensitivity analysis were performed to estimate the detectable effect size and robustness to variability.

Results: Proliferation and length of villi were inversely related to radiation dose and the intensity of γH2AX was inversely related to time post-irradiation. Applying to future drug evaluations, power analysis estimates that both proliferation and morphology models can detect EdU and villi length alterations >20%, while the DNA damage model can detect γH2AX modifications >10%. Sensitivity analysis showed that the proliferation model could withstand <30% random error while both morphology and damage models could withstand <15%.

Conclusion: A semi-automated intestinal assay was developed with three models applicable to assessing the effects of radiation dose-modifying drugs in intestinal tissue.