Comet assay to compare biological effectiveness of various radiation sources in canine bladder cancer

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Introduction

- Radiation therapy and surgery are the primary treatments for tumors in both humans and dogs. The main goal of radiation therapy is to kill tumor cells via DNA damage, while causing minimal damage to normal cells.
- DNA damage occurs as single or double stranded breaks. Single stranded breaks are more likely to be repaired by intracellular mechanisms. Most often cell death occurs via mitotic catastrophe or apoptosis after being irradiated.
- Different types of radiation may interact with tissues in dissimilar ways.

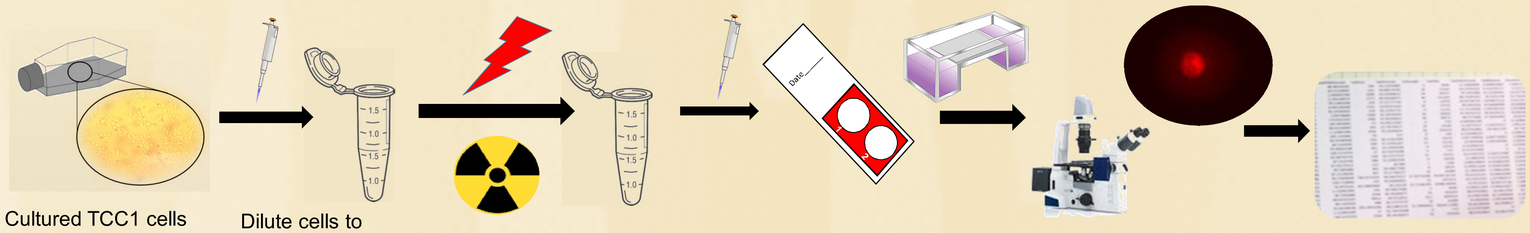
Rationale

- Despite the common use of radiation therapy in dogs, DNA strand breakage in regards to radiation source, has not been directly studied in these patients.
- In the present study, to test the effect of different radiation sources, we are using one of the most commonly seen canine urinary bladder tumors (Transitional cell carcinoma, TCC). To observe the difference in relative biologic effectiveness, the quantity of single and double stranded DNA breaks will be analyzed using a comet assay after being exposed to 8 Gy of radiation from different modalities. In our study, we will be using high and low energy photons, high and low energy electrons, alpha particles, and neutrons.
- By observing the data, we will attempt to more accurately quantify the relative biologic effect of these different radiation sources in canine bladder cancer cells.

Hypothesis

- We hypothesize that low linear energy transfer (LET) radiation, such as photons and high energy electrons, will cause less DNA damage than high LET radiation, such as neutrons or alpha particles.
- High LET radiation is suspected to produce more double stranded DNA breaks, while low LET will most likely produce more single stranded DNA breaks.

Materials and Methods



Each radiated microcentrifuge tube will be exposed to a different source of radiation: high energy photons from a linear accelerator, low energy photons from a Xoft Axxent source, Beta particles from $^{177}LuCl_3$ provided by MU Research Reactor (MURR), mixed neutron field from MURR thermal neutron beam, and alpha particles with recoil lithium nuclei from the boron neutron capture reaction.

Results

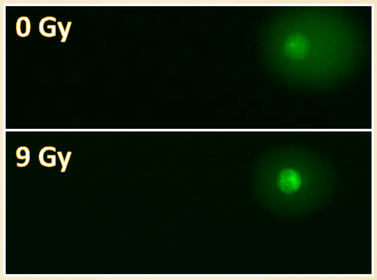


Figure 1. Our results show no significant difference between cells treated with 9 Gy and cells not irradiated.

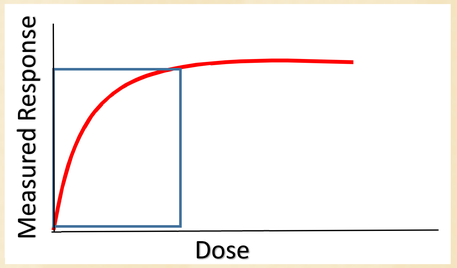


Figure 2. We expect our dose response curve to have a steeper slope than the standard curve. The blue box represents the area of interest to our study.

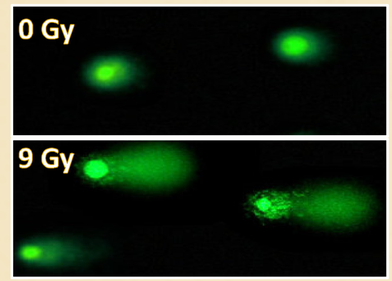


Figure 3. Expected results showing an increase in DNA damage with 9 Gy of radiation compared to cells not irradiated.

- While validating the comet assay and generating a dose response curve, we expect to see an increase in DNA damage as the dose of radiation increases from 0 Gy to 9 Gy. Currently, we are unable to identify a difference between cells not irradiated and cells treated with 9 Gy. Our data collected is shown in figure 1. Figure 3 is an example of our expected results. When drawing our dose response curve, we will use the percent tail DNA.
- When analyzing high LET radiation, we expect to see a higher ratio of comet tail length and intensity compared to the head. Additionally, we expect to see tails present in the neutral solution where none are present in cells treated with low LET Radiation.

Future Directions

By comparing various radiation sources, we will attempt to more accurately quantify the relative biologic effect of these different radiation sources in canine bladder cancer cells. This project should standardize the procedure for follow up studies of other tumors found in the veterinary field. As a far reaching goal, we hope to establish the comet assay in the COREL laboratory. After validation, the comet assay is a time efficient, cost effective, reliable test that will assist in future projects when perfected.

Acknowledgements

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