DNA repair kinetics in canine osteosarcoma and patient-derived melanoma cells evaluated using γ-H2AX analysis

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Introduction

- Ionizing radiation, which induces cell death through the formation of DNA strand breaks, is a treatment commonly used in the management of cancer.
- Upregulation of DNA repair mechanisms in cancer cell lines can limit the efficacy of radiation therapy.
- γ-H2AX is a histone protein that undergoes phosphorylation in response to DNA double-strand breaks making it a sensitive biomarker of DNA damage in cells exposed to radiation.
- The primary aim of this study is to assess the efficacy of varying radiation doses by measuring the level of dsDNA breaks and repair in canine osteosarcoma cell lines as well as a patient-derived melanoma cell line.

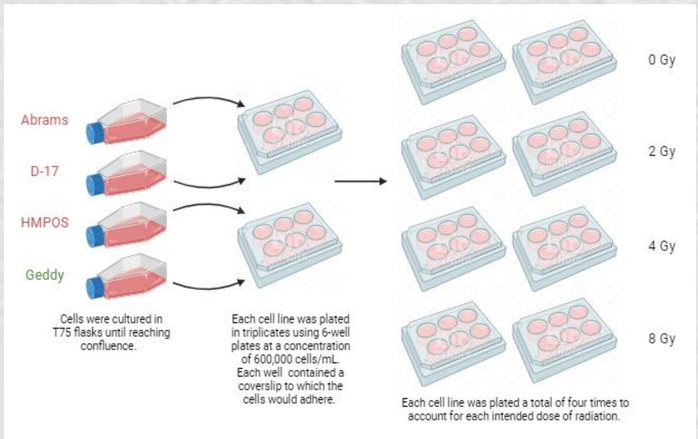
Hypothesis

Higher doses of radiation will induce a greater number of double stranded DNA breaks that will be observable through an increased area of γ-H2AX per area of nucleus.

Methods

Cell Culture

Three canine osteosarcoma cell lines (Abrams, D-17, HMPOS) and one patient-derived melanoma cell line (Geddy) were cultured.



Radiation

A medical linear accelerator (LINAC) machine was used to administer external beam radiation therapy to the plated cell lines at increasing doses of 0, 2, 4, and 8 Gy. The cells were immediately fixed with paraformaldehyde and permeabilized to allow for staining.

Results

Fluorescent Microscopy Imaging

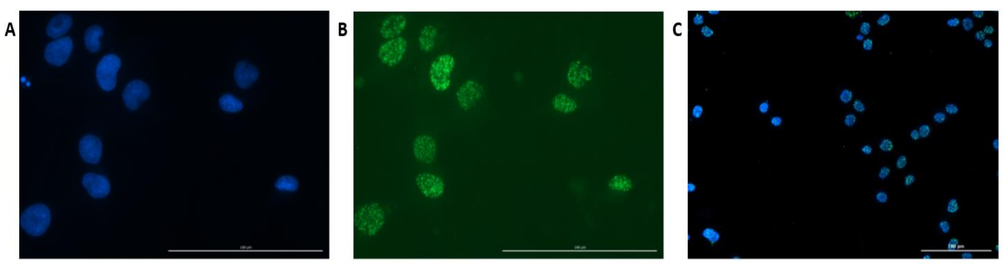


Figure 1. Fluorescent microscopy imaging of cell lines. DNA nuclei were stained with DAPI (A) and individual γ-H2AX foci representing locations of dsDNA breaks were stained using Alexa Fluor 488 antibody (B). Images were processed as z-stack projections and stitched to produce a final image for analysis (C).

Dual-Mask Nuclei Foci Analysis

The area of γ-H2AX foci in each image was quantified as a measure of dsDNA break formation. These were then compared to the area of cell nuclei to quantify the extent of DNA damage that occurred in response to exposure to ionizing radiation. This data was used to generate a dose-response curve for each cell line and compare the efficacy of each dosage of radiation.

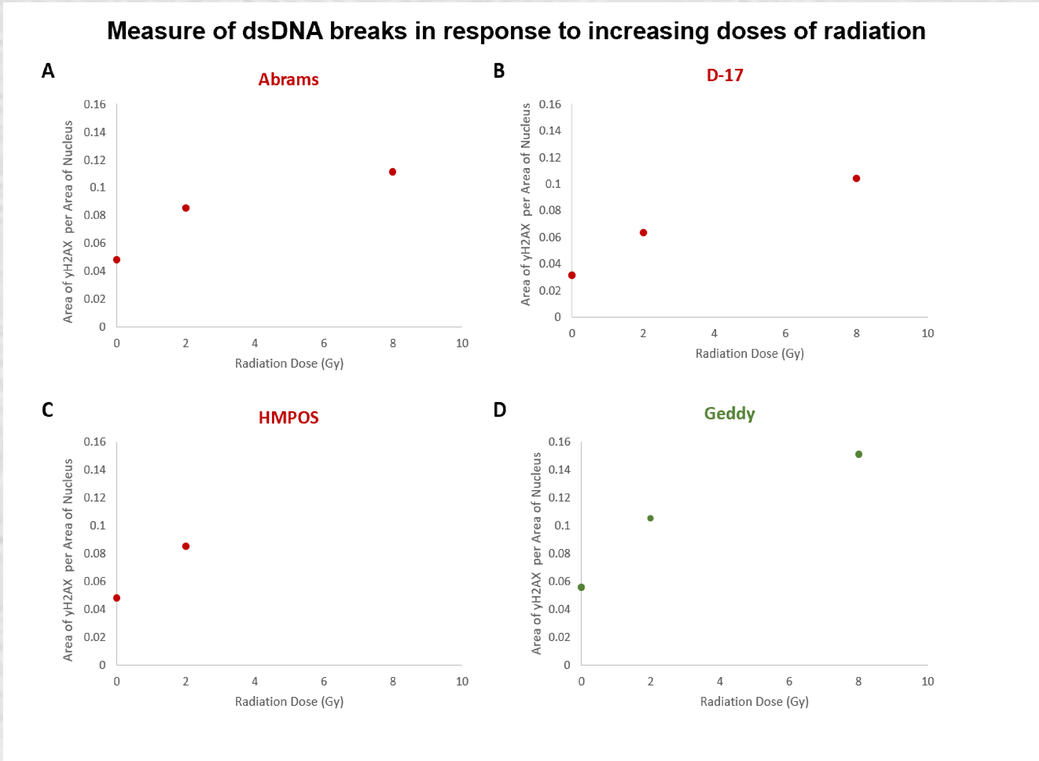


Figure 2. Dose-response of the three osteosarcoma cell lines (A, B, and C) and the one patient-derived melanoma cell line (D) exposed to increasing doses of radiation at intervals of 0, 2, and 8 Gy.

Discussion and Conclusion

- Plates irradiated with a 4 Gy dose of ionizing radiation for all four cell lines were found to have minimal cell adherence following fixation and permeabilization. Due to the low cell density of the slides, the cells were not able to be successfully imaged and analyzed.
- The osteosarcoma cell line HMPOS that was exposed to an 8 Gy dose range did not adequately stain with Alexa Fluor 488. This made it impossible to quantify the level of γ-H2AX phosphorylation that occurred in response to irradiation.
- An increase in the dose of radiation administered resulted in the ratio of the area of γ-H2AX foci to the area of DNA nuclei increasing. This indicates that higher levels of radiation were more effective at inducing dsDNA breaks in the three osteosarcoma cells lines (Abrams, D-17, HMPOS) and the patient-derived melanoma cell line (Geddy).

Future Directions

- Optimize the procedure to ensure successful culture, plating, and fixation of the cells prior to imaging and analysis. This will allow for a complete dose range, including the 4 Gy dosage, to be obtained and factored into further dose-response analysis.
- Repeat the dose-response procedure to account for cell cycle variation among cultured cells.
- Assess the time-response of DNA repair by fixing the cells at intervals of 30 mins and 6 hours post-radiation. This will be done using clinically-relevant radiation dosages of 3 Gy and 8 Gy as controls.
- Different cell lines are expected to display unique repair kinetics that impact their response to radiation. These findings will allow for further elucidation of the mechanisms underlying these dosing regimens and their effectiveness at initiating DNA damage and cell death, generating useful insight into how to strategically administer radiation therapy in a clinical setting.

Acknowledgments

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