Does Suppressing Inflammation Response In Lung Radiotherapy Protect the Cancer Cells?

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BACKGROUND

- Radiation-induced lung injury (inflammation/fibrosis) limits effective dosing in lung cancer radiotherapy. (1)
- A novel RHAMM-mimetic peptide designed to sequester pro-inflammatory hyaluronic acid (HA) fragments has been shown to protect healthy lung tissue from radiation. (2)
- Question: Does this peptide also protect lung cancer from radiation?

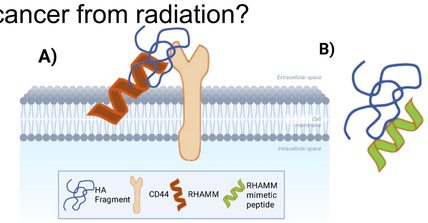

Figure 1. (A) CD44 and RHAMM interaction with HA **(B)** RHAMM mimetic peptide binding to HA fragment

OBJECTIVES

a) Physics: Verify the radiation dose delivered to wells in a tissue culture 12 well plate.

b) Biology: Verify RHAMM expressions in lung adenocarcinoma cells (A549) and evaluate the effects of peptide on A549 radiation sensitivity.

Summary and Conclusions

- Some A549 cells express RHAMM and indifferent to whether it has been exposed to radiation.
- Addition of peptide at 5 μM did not change the radiation survival curve (up to 10 Gy).
- Using the peptide up to 50μM to suppress radiation induced lung inflammation does not affect lung cancer cell killing.
- Future: 3D cultures and additional cell lines.

References

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METHODS a) Physics

- Gafchromic film (EBT3) was cross calibrated with a farmers ionization chamber and analyzed via Python. (3)
- The film was placed under a 12 well plate to verify dose delivered to cells.

Figure 2. Orthovoltage machine (200kV): film irradiation calibration.

METHODS b) Biology

- A549 cells plated in 12 well plates in triplicate conditions and irradiated via an orthovoltage X-ray unit (200 kV).
- Clonogenic assay with cell survival fitted using the Linear-Quadratic (LQ) equation (4):

$$S = e^{-(\alpha D + \beta D^2)}$$

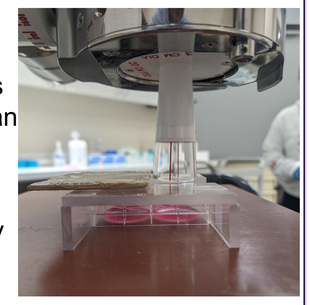


Figure 4. Orthovoltage x-ray unit used to radiate the lung cancer cells in a 12 well plate.

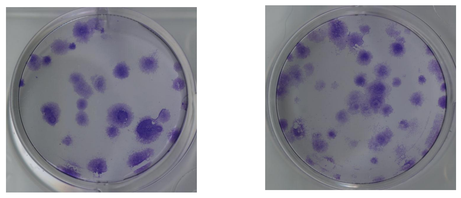
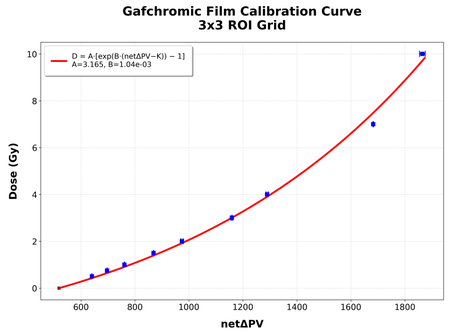


Figure 5. Sample colony formation 10 days after radiation for 0 and 4 Gy. Seeding density of 75, and 300 respectively.

RESULTS a) Physics



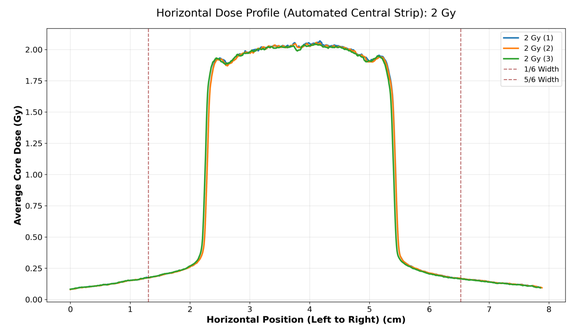


Figure 3. (Left) Sample scan of film under 12 well plate column given 2 Gy **(Middle)** Calibration curve for GAFchromic film used to verify dose profile of radiation delivered to lung cancer cells **(Right)** Dose profile across a 12-well plate column for all 3 wells at a dose of 2 Gy.

- Irradiated wells had a uniform dose profile with 95.6% flatness and mean difference of 5.5% across wells.

RESULTS b) Biology

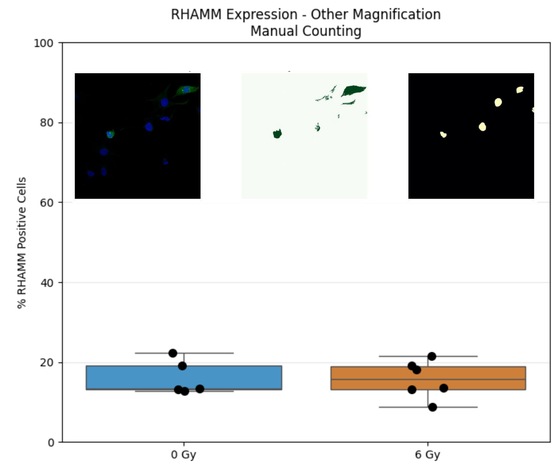


Figure 6. RHAMM Expression of A549 cells with sample image of staining/image processing

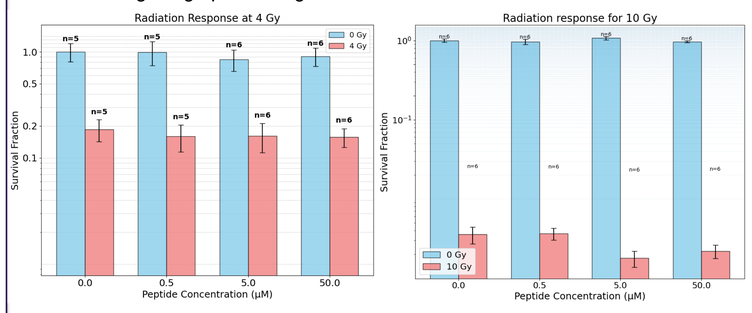


Figure 7. Survival Fraction of cells at 4 and 10 Gy with 0-50 μM of peptide

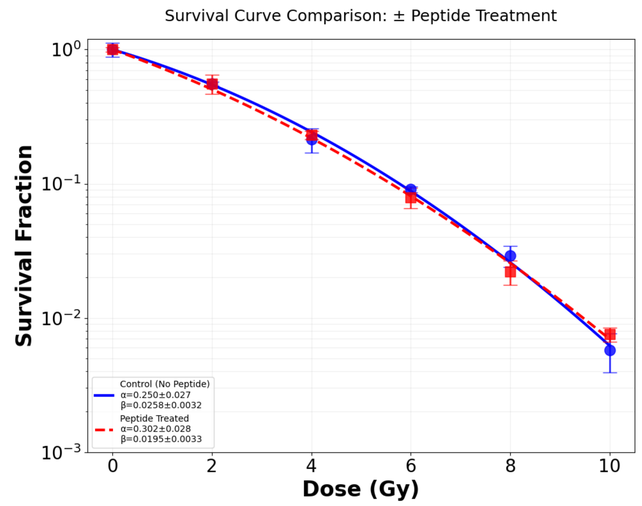


Figure 8. Survival fractions plotted at all doses (0-10 Gy) and peptide concentrations 5 μM

- Sensitivity enhancement ratio of **1.03 +/- 0.21**.
- Survival curve fitted parameters:

	α [Gy⁻¹]	β [Gy⁻²]
Control	0.250 ± 0.027	0.0258 ± 0.0032
Peptide	0.3020 ± 0.0278	0.0195 ± 0.0033

- Survival curve agrees with previously reported values for A549 cells (5, 6)
  $\alpha \approx 0.24 - 0.30\ \mathrm{Gy}^{-1}$, and $\beta \approx 0.02 - 0.03\ \mathrm{Gy}^{-2}$

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