Carcinogenesis in *APC*$^{Min/+}$ mice is altered in response to low-dose radiation

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Introduction

The current model for radiation protection is based on the linear-no-threshold (LNT) hypothesis that purports that cancer risk linearly increases with radiation dose. Recent radiobiological work has suggested, however, that the LNT model is inaccurate at low doses (i.e. <100 mGy) and that low-dose radiation (LDR) may induce non-specific adaptive responses. Thus, further work is required to fully determine the role of low doses on cellular homeostasis and carcinogenesis.

Study Objective

The focus of the current study is determine the role of chronic LDR from beta-emission, tritium, and gamma-irradiation, Co60, on carcinogenesis. Specifically, this study will elucidate the cellular molecular changes and resulting pathological changes (i.e. tumorigenesis) in response to LDR. Moreover, this work will compare the molecular and systemic changes resulting from tritium exposure with those from gamma irradiation.

Experimental Design

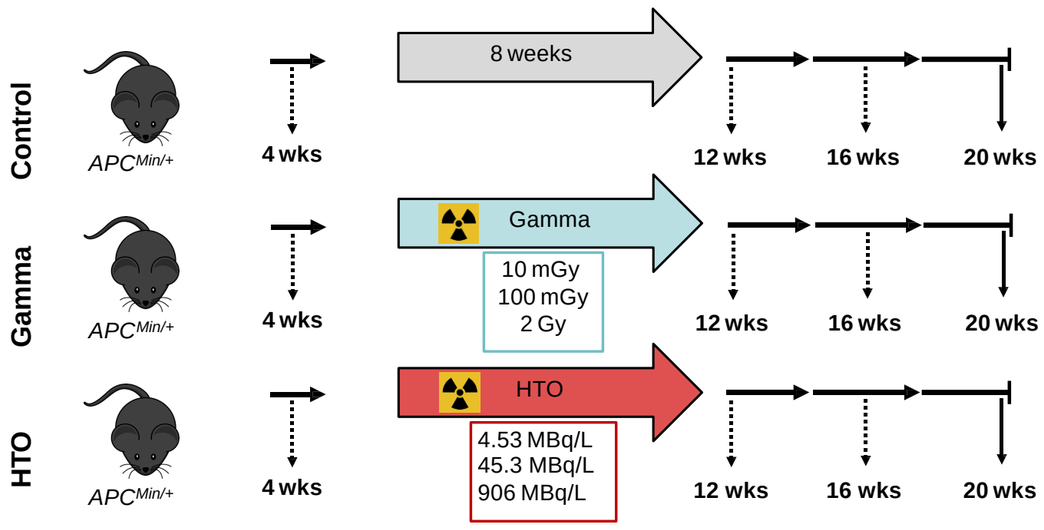

Fig 1. Mice were treated chronically for 8 weeks with either beta- or gamma-radiation, from tritium or Co60, respectively. Four treatment doses were administered: 0 mGy (untreated control),10 mGy (equal to 4.53 MBq/L), 100 mGy (45.3 MBq/L), and 2 Gy (906 MBq/L). The mouse model used, *APC*$^{Min/+}$, spontaneously develop multiple adenomas in the large and small intestine and is an established model for colon cancer research.

Whole Systems Biology Approach

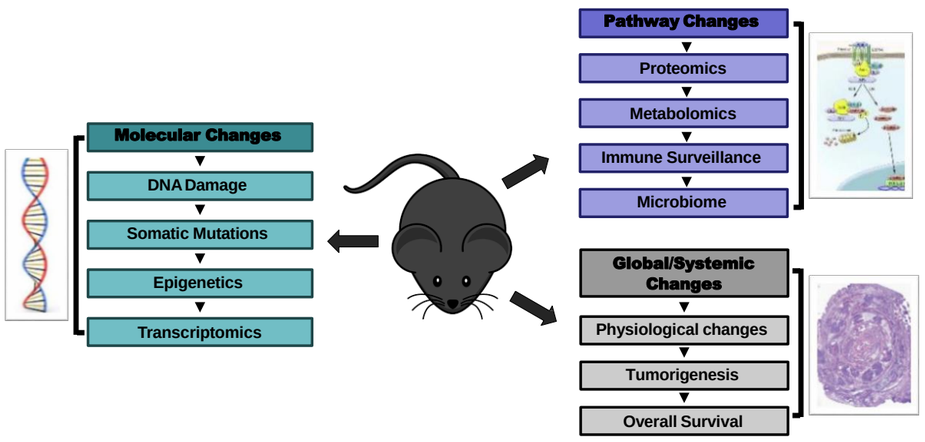

Fig 2. Study outline that takes a holistic approach to examine the molecular, physiological, and systemic changes of the organism in response to LDR.

Results

Low-dose gamma, but not tritium, affects overall mouse survival

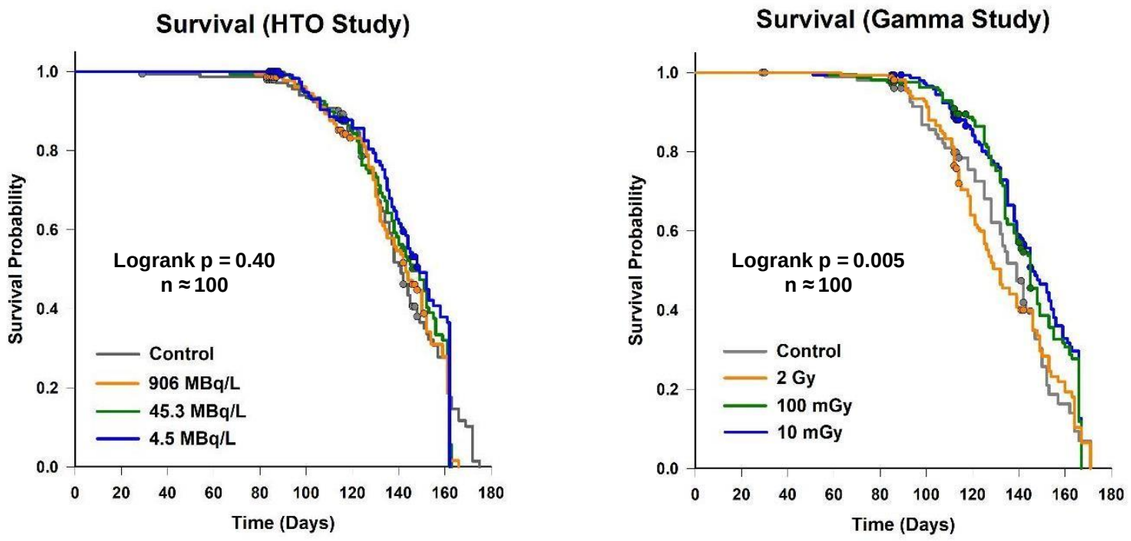

Fig 3. Kaplan-Meier survival curves of mice irradiated with either tritium (HTO) or gamma radiation. No differences were seen between any of the treatment groups for tritium- (HTO) exposed mice. In comparison, gamma-irradiated mice had significantly greater survival for the low-dose cohorts (10 and 100 mGy) compared to 2 Gy and control groups. Statistical significance was determined using Logrank test. n ≈ 100

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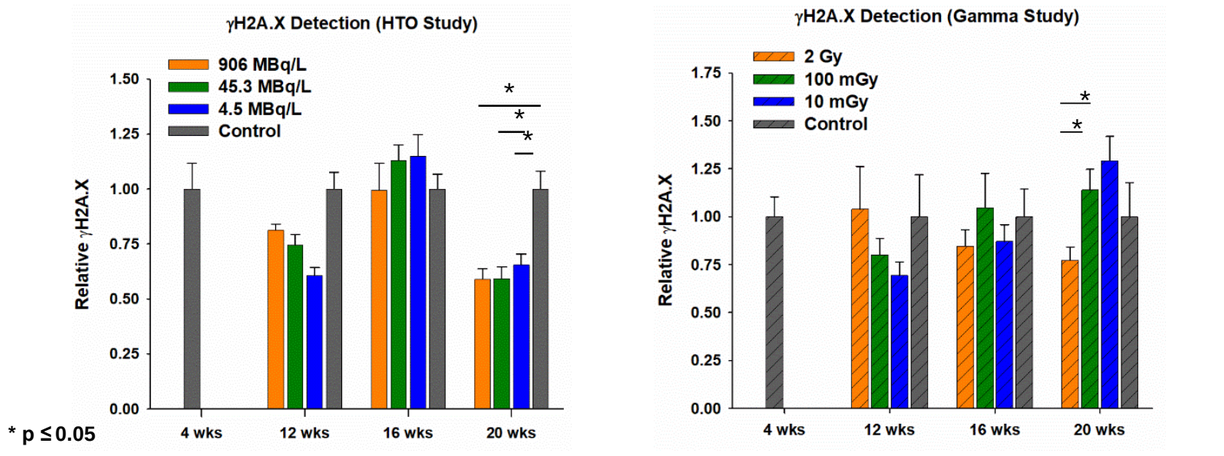
DNA damage is not predictive of overall survival



* p ≤ 0.05

Fig 4. γH2Ax detection by flow cytometry in peripheral blood leukocytes is a marker of global DNA damage. Isolated leukocytes were stained with anti-γH2Ax monoclonal antibody and fluorescence (Alexa488) was detected. For each age and radiation source, control γH2A.X levels were arbitrarily set to 1.0 and γH2A.X levels in 10 mGy, 100 mGy, and 2 Gy cohorts are relative to control. Significance was determined using Student's t test; $p < 0.05$.

Increased tumor size in low-dose tritium cohorts at 12 weeks

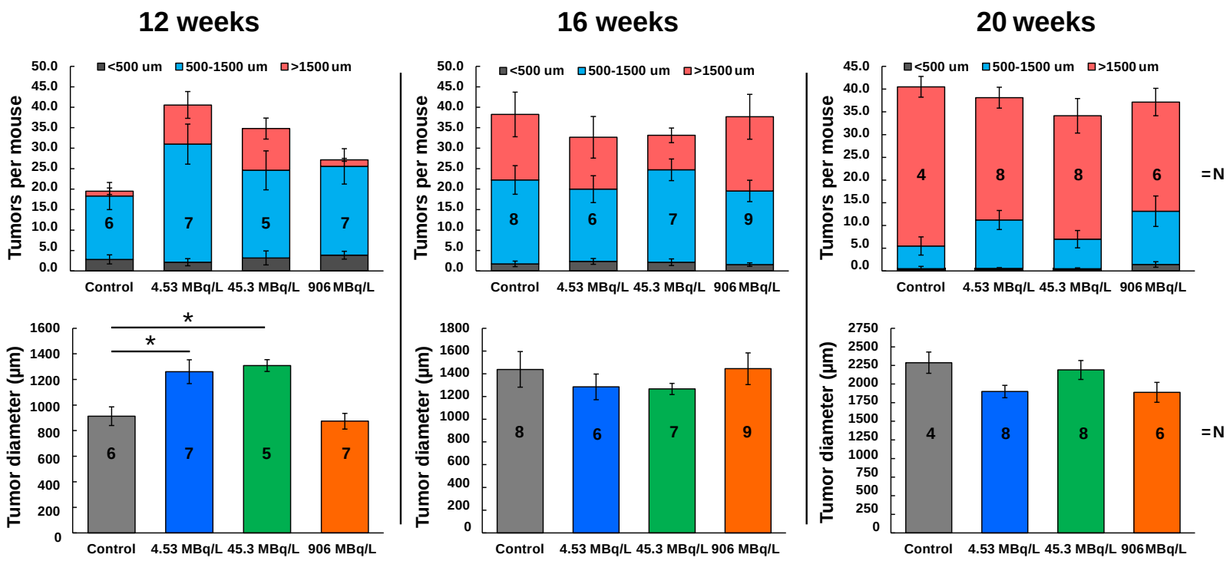


Fig 5. Mean tumor diameters of mice treated with low-dose tritium (4.53 MBq/L and 45.3 MBq/L) were significantly larger at 12 weeks compared to the 2 Gy and control cohorts. No significant difference was seen between treatment groups at 16 or 20 weeks of age. Significance was determined using Student's t test; $p < 0.05$. Tumor scores were determined from histological H&E-stained mouse intestine sections. N values indicated for each group.

Unique DNA-methylation pattern for 4.53 MBq/L cohort

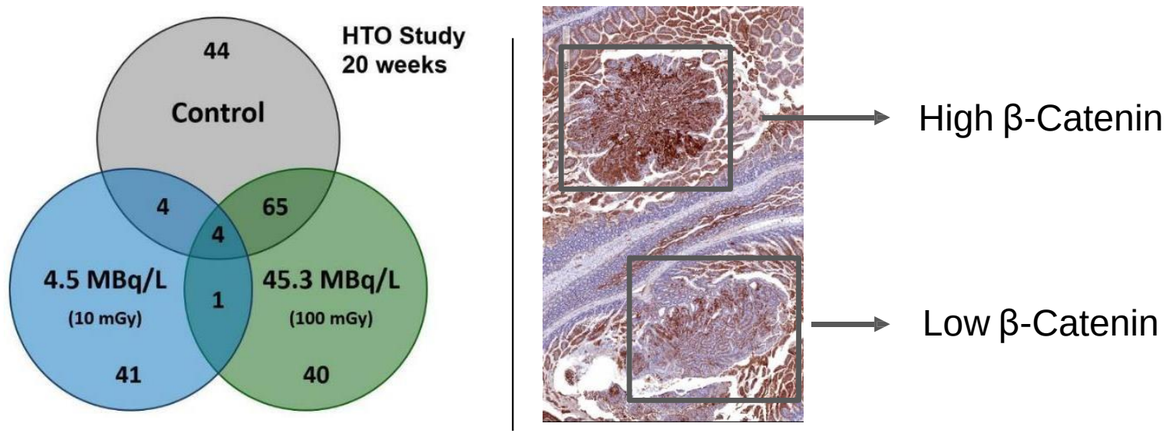


Fig 6. **Left**: RRBS DNA methylation analysis of differentially methylated genes (DMGs) in tumor tissue compared to adjacent, non-tumor tissue. Compared to 45.3 MBq/L and control groups, 10 mGy cohort has a unique methylation pattern demonstrated by limited DMG overlap.
Right: Immunohistochemistry (IHC) staining with anti-β-Catenin shows β-Catenin high and β-Catenin low tumors in the same mouse intestine.

Conclusion

Initial analyses indicated notable changes in epigenetics, gene expression, and tumorigenesis in response to LDR. Survival curves showed that, despite changes at the molecular level, tritium did not affect overall survival, whereas gamma-irradiation produced a hormetic response; suggesting a difference in the relative biological effectiveness (RBE) of tritium versus gamma at low doses. Thus, this work has important implications, not only in understanding the effects of LDR on cellular homeostasis, but in the context of radiation risk assessment.

Future work

Ongoing work will further analyze preserved samples from this study and will attempt to correlate the differences seen at the molecular levels (i.e. DNA methylation, gene expression) to changes at the macroscopic level (i.e. tumorigenesis, survival).

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Funding Sources/Stakeholders

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