Published in final edited form as:

Int J Radiat Oncol Biol Phys. 2015 July 15; 92(4): 829–836. doi:10.1016/j.ijrobp.2015.03.002.

Tumor induction in mice following localized single or fractionated dose irradiation: differences in tumor histotype and genetic susceptibility based on dose scheduling

Elijah F. Edmondson, D.V.M.¹, Nancy R. Hunter, M.S.², Michael M. Weil, Ph.D.¹, and Kathryn A. Mason, M.S.²

¹Colorado State University, Environmental & Radiological Health Sciences Department, Fort Collins, Colorado, 80523, United States of America.

²Department of Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, 77030, United States of America.

Abstract

Purpose—To investigate differences in tumor histotype, incidence, latency, and strain susceptibility in mice exposed to single dose or clinically relevant, fractionated dose γ -ray radiation.

Methods—C3Hf/Kam and C57BL/6J mice were locally irradiated to the right hindlimb with either single large doses between 10 and 70 Gray (Gy) or fractionated doses totaling 40 to 80 Gy delivered at 2 Gy/day fractions, 5 days/week, for 4 to 8 weeks. The mice were closely evaluated for tumor development in the irradiated field for 800 days following irradiation and all tumors were characterized histologically.

Results—A total of 210 tumors were induced within the radiation field in 788 mice. An overall decrease in tumor incidence was observed following fractionated irradiation (16.4%) in comparison to single dose irradiation (36.1%). Sarcomas were the predominant post-irradiation tumor observed ($n = 201$) with carcinomas occurring less frequently ($n = 9$). The proportion of mice developing tumors increased significantly with total dose for both single dose and fractionated schedules and latencies were significantly decreased in mice exposed to larger total doses. C3Hf/Kam mice were more susceptible to tumor induction than C57BL/6J mice following single dose irradiation, however, significant differences in tumor susceptibilities following fractionated radiation were not observed. For both strains of mice, osteosarcomas and hemangiosarcomas were significantly more common following fractionated irradiation whereas fibrosarcomas and malignant fibrous histiocytomas were significantly more common following single dose irradiation.

Corresponding author: Elijah F. Edmondson, 1618 Campus Delivery, Molecular and Radiological Biosciences Building, College of Veterinary and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, United States of America, elijah.edmondson@colostate.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest: none.

Conclusions—This study investigated the tumorigenic effect of acute large doses in comparison to fractionated radiation in which both the dose and delivery schedule were similar to those used in clinical radiotherapy. Differences in tumor histotype following single dose or fractionated radiation exposures provides novel *in vivo* evidence for differences in tumor susceptibility amongst stromal cell populations.

INTRODUCTION

Inbred mouse strains differ in their susceptibilities to various radiogenic tumors including thymic lymphoma, myeloid leukemia, mammary tumors, pulmonary adenocarcinoma, hepatocellular carcinoma, and osteosarcoma (1–9). The strain differences in susceptibilities are thought to be due to the differing genetic backgrounds of the strains and in some cases specific genetic polymorphisms have been identified that may be responsible (7,10–13). Most of these studies on strain differences involve single, acute, whole body exposures, although there are exceptions such as the use of internal emitters in the study of osteosarcoma and the use of dose fractionation to induce thymic lymphomas. The total doses in most, but not all, studies are 3 Gy or less. To the best of our knowledge, research into mouse strain and tumor histotype differences involving fractionated exposures to high total doses, similar to those experienced by radiotherapy patients, have not been reported.

Here we report on tumorigenesis in two inbred murine strains, C3Hf/Kam and C57BL/6J, exposed to single dose or fractionated irradiation of γ -rays up to 70 or 80 Gy delivered to a hindlimb.

METHODS AND MATERIALS

Mice

C57BL/6J and C3Hf/Kam male mice, bred and maintained in the Experimental Radiation Oncology specific-pathogen free mouse colony, were 3–4 months old at the beginning of experiments. The mice, housed 5 per cage, were exposed to 12-hour light dark cycles, and given free access to sterilized pelleted food (Prolab Animal Diet, Purina, Indianapolis, IN) and sterilized water. The facilities were approved by the Association for Assessment and Accreditation of Laboratory Animal Care and in accordance with current regulations of the United States Department of Agriculture and Department of Health and Human Services, and the experimental protocol was approved by and in accordance with guidelines established by the [X].

Irradiation

A preclinical model consisting of development of solid tumors in the limbs of C3H mice exposed locally to ionizing radiation was used to study radiation-induced tumorigenesis (14–17). Right hindlimbs of mice were exposed to local irradiation in air with single doses of γ -rays ranging from 10 to 70 Gy, or with 2-Gy fractions given daily for 5 days per week for a total of 40, 50, 60, 70, and 80 Gy. For single dose radiation, mice were grouped for analysis according to exposures as follows: 10 to 29 Gy, 30 to 39 Gy, 40 to 49 Gy, 50 to 59 Gy, and 60 to 70 Gy as detailed in **Supplementary Table 1**. Only C3Hf/Kam mice were exposed to single dose radiation from 60 to 70 Gy, therefore the results from this dose range were not

included in the statistical analysis comparing tumor incidence between strains. Radiation was delivered from a small-animal irradiator with 2 parallel-opposed ^{137}Cs sources at a dose rate of 6.4 - 8 Gy per minute. During irradiation, unanesthetized mice were immobilized in a jig and the right rear thigh was centered in a circular radiation field 3 cm in diameter.

Assessment of Hindlimb Tumors

Mice were observed for development of tumors in the irradiated limbs at 2 week intervals until 800 days after irradiation. Tumor incidence was defined as the proportion of mice developing hindlimb tumors out of the total number of mice receiving a given dose of radiation. All tumors were analyzed histologically by a blinded veterinary pathologists (blinded to treatment and mouse strain) using 5 μm sections from formalin fixed, paraffin-embedded tissues routinely processed and stained with hematoxylin and eosin. Osteosarcomas were characterized as tumors composed of malignant mesenchymal cells associated with brightly eosinophilic, fibrillar to homogeneous, tumor osteoid matrix (**Figure 1A**). Hemangiosarcomas were composed of atypical, plump endothelial cells forming irregularly anastomosing vascular spaces containing erythrocytes (**Figure 1B**). Fibrosarcomas were composed of spindle-shaped cells separated by variable amounts of lightly eosinophilic collagenous stroma; spindle-shaped cells were arranged in inter-weaving fascicles forming a characteristic herringbone pattern (**Figure 1C**). Malignant fibrous histiocytomas were pleomorphic with fusiform to rounded cells and typically contained numerous multinucleated giant cells (**Figure 1D**). Representative histopathology for additional tumor histotypes can be found in the supplementary materials (**Supplementary Figures 1, 2**). Sarcomas lacking diagnostic features of the previously mentioned subtypes were assigned the diagnosis of undifferentiated sarcoma.

Statistical Analysis

Comparisons were made between tumor histotype, radiation dosing schedule, tumor-free survival times, tumor latencies, and mouse strain. Chi-squared tests were used to compare categorical variables. Logistic regression was used to compare proportions of tumors induced by increasing doses for single dose or fractionated radiation. Kaplan-Meier survival analyses were used to determine differences in tumor-specific survival between strains and differences in tumor specific survival between tumor histotypes and radiation delivery. Tumor latencies between mouse strains, radiation dose groups, and tumor histotypes were compared using analysis of variance. Statistical analyses were performed using STATA software (version 11.2, Statacorp, College Station, TX) and Graphpad Prism (Version 6.0d, GraphPad Software, Inc. La Jolla, CA). All values were considered significant when $p < 0.05$.

RESULTS

Tumor incidence

Following single dose exposures between 10 and 70 Gy, 163 of 451 mice (36.1%) developed hindlimb tumors. Tumor incidences showed a similar increase for each increasing dose group when combining C3Hf/Kam and C57BL/6J mice (**Figure 2A**, $p < 0.001$). Single dose exposures were significantly more effective at inducing hindlimb tumors in comparison

to similar total doses received in 2 Gy fractions ($p < 0.001$). For hindlimbs irradiated with fractionated exposures, 55 tumors were induced in 335 mice (16.4%). The five fractionated dosing groups each showed a consecutive increase in tumor incidence with increasing dose (**Figure 2A**) to culminate in a 38% tumor incidence for the 80 Gy group. Over the dose ranges investigated for single dose irradiation, increases in tumor incidences were observed up to 50 Gy for C57BL/6J mice and up to 60 Gy for C3Hf/Kam mice, with evidence of a plateau in the dose response for larger doses (**Figure 2B**). Over the dose ranges investigated for fractionated irradiation, no evidence for a plateau in the dose response was observed (**Figure 2A, 2B**).

Tumor Latency

Latency was defined as the number of days between the date of irradiation and tumor development. For fractionated irradiation, the day of the final fraction was defined as day 0. For single dose irradiation, the first tumors appeared at 216 days following irradiation for C3Hf/KAM and at 348 days for C57BL/6J. For fractionated irradiation, the first tumors appeared at 256 days following the last fraction for C3Hf/KAM and at 384 days for C57BL/6J. A decrease in latency was observed with increasing dose for both single dose (**Figure 3A**, $p = 0.024$) and fractionated dosing schedules (**Figure 3B**, $p = 0.026$). Following single dose irradiation, the mean tumor latency time for C3Hf/Kam mice was significantly decreased compared to C57BL/6J mice (**Figure 4A**, $p = 0.002$); no significant difference in latency was observed between the two strains following fractionated radiation (**Figure 4B**, $p = 0.858$). The latencies for different sarcoma histotypes were not significantly different (**Figure 5A, 5B**), however, the latency for carcinomas was significantly prolonged in comparison to the latencies for sarcomas as a group (**Supplementary Figure 3**, $p = 0.002$).

Tumor histotype

All 210 tumors observed were categorized histologically, as summarized in **Table 1**. Following fractionated exposures, both C57BL/6J and C3Hf/Kam mice were more likely to develop osteosarcoma ($p < 0.001$) and hemangiosarcomas ($p < 0.001$) than other tumor types (**Figure 5C**). Of the tumors produced by fractionated radiation, 63% were osteosarcoma or hemangiosarcoma; in comparison, these tumor types comprised only 8% of all tumors following single dose exposures. The most common tumors induced by single dose radiation were fibrosarcoma and malignant fibrous histiocytoma, each of which was significantly more common following single dose irradiation ($p < 0.001$). Fibrosarcomas and malignant fibrous histiocytomas comprised 59% of the tumors following single dose exposures in comparison to 7% following fractionated exposures. Additionally, squamous cell carcinomas were more commonly observed in mice given 2 Gy fractions than in mice given single large doses ($p = 0.036$).

Strain Susceptibility

Following single dose exposure, significant differences in tumor incidences (**Figure 2B**, $p < 0.001$) and tumor latencies (**Figure 4A**, $p = 0.002$) were observed between C3Hf/Kam and C57BL/6J mice. For fractionated exposures, tumor incidences and latencies were similarly increased and decreased, respectively, however these changes were not statistically

significant (**Figure 2B, 4B**). For both strains of mice, osteosarcomas and hemangiosarcomas were significantly more common following fractionated irradiation whereas fibrosarcomas and malignant fibrous histiocytomas were significantly more common following single dose irradiation.

DISCUSSION

In this study, we demonstrate that repeated 2 Gy fractions more commonly produce neoplasms arising from endothelial or osteocyte precursors in contrast to single large dose exposures which more commonly produce fibrosarcomas or malignant fibrous histiocytomas. Differences in tumor histotypes following single dose and fractionated radiation exposures provide novel *in vivo* evidence for variability in susceptibility amongst stromal cell populations. Investigations into the cell of origin for sarcomas have identified progenitor cells for the development of specific sarcoma subtypes (18, 19); our work suggests that certain histotype specific progenitor cells may have differential susceptibilities to the late effects of radiation based on dose fractionation.

Sarcomas were one of the original solid tumors to be associated with radiotherapy in the 1920's (20, 21) and continue to be a rare, but highly fatal hazard of modern radiation therapy (22). Conventionally, radiotherapy is delivered as 2 to 2.5 Gy fractions (Monday - Friday) for 1 to 7 weeks (23), which is similar to the fractionation schedule used in our study (2 Gy/day, 5 days/week, for 4 to 8 weeks). Radiation fractionation is thought to increase the efficacy of tumor cell killing by increasing the number of tumor cells irradiated during radiosensitive phases of the cell cycle and by allowing tumor reoxygenation between each fraction which increases the accumulated nonrepairable damage in tumor cells per unit dose (24, 25). Fractionated radiotherapy also has the advantage of allowing non-neoplastic cells time to repair (24, 25) however, accumulated damage to non-targeted cells may lead to a second primary neoplasm. In addition, the protracted time period between fractionated doses allows for the replenishment of radiation depleted cells, including potential target cells for neoplastic transformation, thereby expanding the number of cells at risk. Due to the increased success of cancer therapies, increasing numbers of cancer survivors are at risk for developing second primary malignancies within radiation treatment fields, which include post-radiation sarcomas (22). However, uncertainties surrounding post-irradiation sarcomas exist due to their relative rarity. These uncertainties include the variation in risk by sarcoma histotype, the shape of the dose-response curve, and potential genetic susceptibility.

In the present study, osteosarcomas and hemangiosarcomas were significantly more common following clinically relevant fractionated radiation whereas fibrosarcomas and malignant fibrous histiocytomas were significantly more common following single large doses of radiation. These results are consistent with previous studies in which mice exposed to single or hypofractionated large doses of γ -rays were most commonly diagnosed with fibrosarcomas and malignant fibrous histiocytomas (26, 27). Potential explanations for the observed differences in tumor cell-of-origin following fractionated or single dose irradiation include (a) differential cell type susceptibility to apoptosis, necrosis, or senescence following irradiation, (b) differential cell type repair capabilities, (c) differential post-irradiation immune-modulatory effects on different cell types, or (d) differential cell type

responses to growth factors, cytokines, or hormones following fractionated or single dose irradiation. *In vitro*, changes in transcription in normal human coronary artery endothelial cells exposed to single dose or fractionated radiation have been examined (28, 29). Palayoor et al, demonstrated that exposure of endothelial cell cultures to 5×2 Gy fractions resulted in robust transcriptional changes in comparison to a single 10 Gy dose (29). Genes regulating cell cycle, DNA replication, DNA damage stimulus, DNA repair, and genes related to immune response were significantly altered following exposure to fractionated in comparison to single dose radiation (29).

Similar to the mice in this study, tumors histotypes arising in humans exposed to fractionated radiation are commonly osteosarcoma and angiosarcoma (angiosarcoma is a category which includes hemangiosarcoma and lymphangiosarcoma). Studies of childhood post-irradiation sarcomas reveal that the most frequent second solid cancer occurring in children treated with radiation therapy is osteosarcoma (30). In adult breast cancer patients treated with fractionated radio-therapy (2 Gy/day, 5 weekly fractions) with median doses of 50-55 Gy, the most common tumor was angiosarcoma, followed by undifferentiated sarcoma and osteosarcoma (31).

In our study, the risk of post-irradiation sarcoma following clinically relevant fractionated exposures increased with total doses up to 80 Gy with no evidence of a plateau. Similarly in humans, studies of childhood post-irradiation sarcomas provide clear evidence of increased risks with no evidence of a decrease in slope with doses ≥ 60 Gy (30, 32, 33). For breast cancer patients in adulthood, increased risk of post-irradiation sarcomas are also associated with increasing dose (35). Following single dose irradiation, evidence of a plateau in tumor incidence was observed at doses higher than 50 Gy for C57BL/6J mice and at doses higher than 60 Gy for C3Hf/Kam mice.

The risk of developing sarcomas is influenced by genetic susceptibility in humans and mice, however, only a few specific examples of a genetic susceptibility to radiation related sarcomas are present in the literature (13, 35, 36). Susceptibility to radiation induced osteosarcoma has been associated with a common promoter variant in *Rb1* in mice (13). In humans treated for retinoblastoma with radiotherapy, there is an increased risk of sarcomas in the radiation field (35, 36). Additional evidence for genetic susceptibility to sarcoma can be observed in the differential solid object tumorigenesis in different strains of mice, in which there is a marked difference in tumorigenesis following implanted foreign bodies (37). Genetic susceptibility to radiation induced sarcoma in mice was revealed in the present study as an increased incidence and decreased latencies of post-irradiation sarcomas in the C3Hf/Kam strain compared to C57BL/6 following single dose irradiation. No significant differences were observed between these two strains following fractionated irradiation, however, fewer tumors and different histotypes were induced following fractionated exposures which decreased the power of the statistical analysis and, while not significant, the incidences of tumors were similarly increased in C3Hf/Kam mice compared to C57BL/6 mice (**Figure 1B**).

CONCLUSIONS

Osteosarcoma, hemangiosarcoma, and squamous cell carcinoma are significantly more common in mice following exposure to radiation in fractions of 2 Gy/day. In contrast, fibrosarcomas and malignant fibrous histiocytomas are significantly more common following single large doses of radiation (10 - 70 Gy). Genetic susceptibility to radiation-induced sarcomas was observed as a difference in tumor incidences and latencies between C3Hf/Kam and C57BL/6J mice.

Reference List

1. Kaplan HS, Hirsch BB, Brown MB. Indirect induction of lymphomas in irradiated mice. IV. Genetic evidence of the origin of the tumor cells from the thymic grafts. *Cancer Res.* 1956; 16:434–436. [PubMed: 13330034]
2. Storer JB, Mitchell TJ, Fry RJ. Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. *Radiat Res.* 1988; 114:331–353. [PubMed: 3375429]
3. Szymanska H, Sitarz M, Krysiak E, et al. Genetics of susceptibility to radiation-induced lymphomas, leukemias and lung tumors studied in recombinant congenic strains. *Int J Cancer.* 1999; 83:674–678. [PubMed: 10521806]
4. Rosemann M, Kuosaite V, Nathrath M, et al. The genetics of radiation-induced osteosarcoma. *Radiat Prot Dosimetry.* 2002; 99:257–259. [PubMed: 12194300]
5. Okumoto M, Nishikawa R, Imai S, et al. Genetic analysis of resistance to radiation lymphomagenesis with recombinant inbred strains of mice. *Cancer Res.* 1990; 50:3848–3850. [PubMed: 2354437]
6. Boulton E, Cole C, Knight A, et al. Low-penetrance genetic susceptibility and resistance loci implicated in the relative risk for radiation-induced acute myeloid leukemia in mice. *Blood.* 2003; 101:2349–2354. [PubMed: 12411293]
7. Darakhshan F, Badie C, Moody J, et al. Evidence for complex multigenic inheritance of radiation AML susceptibility in mice revealed using a surrogate phenotypic assay. *Carcinogenesis.* 2006; 27:311–318. [PubMed: 16093251]
8. Ponnaiya B, Cornforth MN, Ullrich RL. Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: the difference is as clear as black and white. *Radiat Res.* 1997; 147:121–125. [PubMed: 9008202]
9. Weil MM, Bedford JS, Bielefeldt-Ohmann H, et al. Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon (56)Fe ions. *Radiat Res.* 2009; 172:213–219. [PubMed: 19630525]
10. Mori N, Matsumoto Y, Okumoto M, et al. Variations in *Prkdc* encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and susceptibility to radiation-induced apoptosis and lymphomagenesis. *Oncogene.* 2001; 20:3609–3619. [PubMed: 11439324]
11. Yu Y, Okayasu R, Weil MM, et al. Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the *Prkdc* (DNA-dependent protein kinase catalytic subunit) gene. *Cancer Res.* 2001; 61:1820–1824. [PubMed: 11280730]
12. Perez-Losada J, Wu D, DelRosario R, et al. Allele-specific deletions in mouse tumors identify *Fbxw7* as germline modifier of tumor susceptibility. *PLoS One.* 2012; 7:e31301. [PubMed: 22348067]
13. Rosemann M, Gonzalez-Vasconcellos I, Domke T, et al. A *Rb1* promoter variant with reduced activity contributes to osteosarcoma susceptibility in irradiated mice. *Mol Cancer.* 2014; 13:182. [PubMed: 25092376]
14. Hunter NR, Guttenberger R, Milas L. Modification of radiation-induced carcinogenesis in mice by misonidazole and WR-2721. *Int J Radiat Oncol Biol Phys.* 1992; 22:795–798. [PubMed: 1312077]
15. Milas L, Hunter N, Stephens LC, et al. Inhibition of radiation carcinogenesis in mice by S-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res.* 1984; 44:5567–5569. [PubMed: 6093999]

16. Suit HD, Sedlacek R, Fagundes L, et al. Time distributions of recurrences of immunogenic and nonimmunogenic tumors following local irradiation. *Radiat Res.* 1978; 73:251–266. [PubMed: 635109]
17. Urano M, Kenton LA, Kahn J. The effect of hyperthermia on the early- and late-appearing mouse foot reactions and on radiation carcinogenesis: Part II. Effect on radiation carcinogenesis (thermal enhancement and oxygen enhancement). *Int J Radiat Oncol Biol Phys.* 1989; 16:437–442. [PubMed: 2921146]
18. Hatley ME, Tang W, Garcia MR. A mouse model of rhabdomyosarcoma originating from the adipocyte lineage. *Cancer Cell.* Oct 16; 2012 22(4):536–546. [PubMed: 23079662]
19. Blum JM, Ano L, Li Z. Distinct and overlapping sarcoma subtypes initiated from muscle stem and progenitor cells. *Cell Reports.* 2013; 5:933–940. [PubMed: 24239359]
20. Beck A. Zur Frage des Roritgensarkoms, zugleich ein Beitrag zur Pathogenese des Sarkoms. *München Med Wchnschr.* 1922; 69:623–624.
21. Martland HS, Humphries RE. Osteogenic sarcoma in dial painters using luminous paint. *Arch Pathol Lab Med.* 1929; 7:406–417.
22. Berrington de Gonzalez A, Kutsenko A, Rajaraman P. Sarcoma risk after radiation exposure. *Clin Sarcoma Res.* 2012; 2:18. [PubMed: 23036235]
23. Demaria S, Formenti SC. Radiation as an immunological adjuvant: current evidence on dose and fractionation. *Front Oncol.* 2012; 2:153. [PubMed: 23112958]
24. Pajonk F, Vlashi E, McBride WH. Radiation Resistance of Cancer Stem Cells: The 4 R's of Radiobiology Revisited. *Stem Cells.* Apr; 2010 28(4):639–648. [PubMed: 20135685]
25. Withers, HR., editor. The four R's of radiotherapy. Academic Press; New York: 1975. Lett, JTAH., editor. *Advances in radiation biology.*
26. Ando K, Koike S, Oohira C. Tumor induction in mice locally irradiated with carbon ions: a retrospective analysis. *J Radiat Res (Tokyo).* 2005; 46:185–190. [PubMed: 15988136]
27. Ando K, Koike S, Ohmachi Y. Tumor induction in mice after local irradiation with single doses of either carbon-ion beams or gamma rays. *Int J Radiat Biol.* Aug.2014 21:1–6.
28. Makinde AY, John-Aryankalayil M, Palayoor ST. Radiation survivors: understanding and exploiting the phenotype following fractionated radiation therapy. *Mol Cancer Res.* Jan; 2013 11(1):5–12. [PubMed: 23175523]
29. Palayoor ST, John-Aryankalayil M, Makinde AY. Differential expression of stress and immune response pathway transcripts and miRNAs in normal human endothelial cells subjected to fractionated or single-dose radiation. *Mol Cancer Res.* Jul; 2014 12(7):1002–15. [PubMed: 24784841]
30. Le, Vu; de, VF.; Shamsaldin, A. Radiation dose, chemotherapy and risk of osteosarcoma after solid tumours during childhood. *Int J Cancer.* 1998; 77:370–377. [PubMed: 9663598]
31. Kirova YM, Vilcoq JR, Asselain B. Radiation-induced sarcomas after radiotherapy for breast carcinoma: a large-scale single-institution review. *Cancer.* 2005; 104:856–863. [PubMed: 15981282]
32. Hawkins MM, Wilson LM, Burton HS. Radiotherapy, alkylating agents, and risk of bone cancer after childhood cancer. *J Natl Cancer Inst.* 1996; 88:270–278. [PubMed: 8614005]
33. Tucker MA, D'Angio GJ, Boice JD Jr. Bone sarcomas linked to radiotherapy and chemotherapy in children. *N Engl J Med.* 1987; 317:588–593. [PubMed: 3475572]
34. Rubino C, Shamsaldin A, Le MG. Radiation dose and risk of soft tissue and bone sarcoma after breast cancer treatment. *Breast Cancer Res Treat.* 2005; 89:277–288. [PubMed: 15754127]
35. Kleinerman RA, Schonfeld SJ, Tucker MA. Sarcomas in hereditary retinoblastoma. *Clin Sarcoma Res.* Oct 4.2012 2(1):15. [PubMed: 23036192]
36. Wong FL, Boice JD Jr, Abramson DH. Cancer incidence after retinoblastoma. Radiation dose and sarcoma risk. *JAMA.* Oct 15; 1997 278(15):1262–7. [PubMed: 9333268]
37. Brand I, Buoen LC, Brand KG. Foreign-body tumors of mice: strain and sex differences in latency and incidence. *J Natl Cancer Inst.* 1977; 58:1443–7. [PubMed: 323507]

SUMMARY

In this study, we demonstrate that repeated 2 Gy fractions more commonly produce neoplasms arising from endothelial or osteocyte precursors in contrast to single large dose exposures which more commonly produce fibrosarcomas or malignant fibrous histiocytomas. There is a general lack of *in vivo* data describing differences in second cancer histotype, incidence, and latency following fractionated irradiation in comparison to single large dose exposures. These results indicate that different cell types respond differently to radiation based on delivery schedule.

Figure 1.

Representative tumor histopathology, hematoxylin and eosin. **(A)** Osteosarcoma. Neoplastic osteocytes surround and are encased in brightly eosinophilic tumor osteoid (arrowhead) which progressively transitions to immature woven bone. Multinucleated osteoclasts are scattered throughout the tumor. **(B)** Hemangiosarcoma. Atypical endothelial cells form irregularly anastomosing vascular channels containing erythrocytes and nuclei of neoplastic cells often bulge into vascular channels (arrowhead). **(C)** Fibrosarcoma. Neoplastic spindle cells are arranged in fascicles that interweave to form a herringbone pattern. Cells are separated by variable amounts of lightly eosinophilic, collagenous stroma and mitotic figures are common (arrowhead). **(D)** Malignant Fibrous Histiocytoma. Pleomorphic cells with fibroblastic and histiocytic differentiation are separated by sparse eosinophilic, collagenous stroma. Moderate to marked anisocytosis and anisokaryosis is observed and multinucleated giant cells are frequent (arrowhead).

Figure 2.

Incidence of hindlimb tumors by radiation dose. **(A)** Incidences of hindlimb tumors are significantly increased in mice exposed to a single large dose of radiation in comparison to mice exposed to fractionated radiation ($p < 0.001$). **(B)** Incidences of hindlimb tumors by radiation dose and mouse strain. C3Hf/Kam mice have a significantly higher incidence of hindlimb tumors following single dose exposures than C57BL/6J mice ($p < 0.001$). No significant difference in tumor incidence is observed between C3Hf/Kam and C57BL/6J mice following fractionated exposures. Single doses are grouped as 10-29, 30-39, 40-49, and 50-59 Gy. Fractionated doses were given as 2 Gy/day, 5 days/week for 4 to 8 weeks and are listed as total doses of 40, 50, 60, 70, and 80 Gy.

Figure 3.

Decreasing tumor latencies with increasing dose following (A) single exposures of 10 to 70 Gy or (B) fractionated exposure of 2 Gy/day fractions given 5 days/week for 4 to 8 weeks. Mice receiving higher total single doses have significantly decreased tumor latencies ($p = 0.024$), measured as the number of days between irradiation and tumor development. Mice receiving higher total fractionated doses also have significantly decreased tumor latencies ($p = 0.026$).

Figure 4.

Tumor latencies following (A) single exposures of 10 to 70 Gy or (B) fractionated exposure of 2 Gy/day fractions given 5 days/week for 4 to 8 weeks separated by mouse strain (C3Hf/Kam or C57BL/6J). C3Hf/Kam mice have significantly decreased tumor latencies in comparison to C57BL/6J mice following single dose exposures ($p = 0.002$), but not following fractionated exposures ($p = 0.858$).

Figure 5.

Tumor histotypes arising in locally γ -irradiated hindlimbs compared by dose schedule. Latency of tumors separated by histotype following (A) single exposures of 10 to 70 Gy or (B) fractionated exposures of 2 Gy/day fractions given 5 days/week for 4 to 8 weeks and (C) tumor incidences separated by dose scheduling. Osteosarcomas ($p < 0.001$), hemangiosarcomas ($p < 0.001$), and squamous cell carcinomas ($p = 0.036$) were significantly more common following fractionated irradiation whereas fibrosarcomas ($p < 0.001$) and malignant fibrous histiocytomas ($p < 0.001$) were significantly more common following single large dose irradiation. Osteosarcoma (OSA), Hemangiosarcoma (HSA), Squamous cell carcinoma (SCC), Rhabdomyosarcoma (RhSA), Undifferentiated sarcoma (US), Malignant fibrous histiocytoma (MFH), Fibrosarcoma (FSA).

Table 1

Histology of γ -irradiation induced tumors of the hindlimbs of locally irradiated mice separated by radiation dose scheduling.

Histologic Diagnosis	Multifractionated Dose (Fx)	Single Dose (SD)	Chi-square p-value
Osteosarcoma	22	9	< 0.001
Hemangiosarcoma	12	4	< 0.001
Squamous Cell Carcinoma	5	4	0.036
Myxosarcoma	1	1	0.430
Rhabdomyosarcoma	2	13	0.255
Undifferentiated Sarcoma	8	32	0.358
Carcinosarcoma	0	1	-
Malignant fibrous histiocytoma	2	40	< 0.001
Fibrosarcoma	2	52	< 0.001
Mice Developing Tumors	54	156	< 0.001
Total Number of Mice	337	452	
C3Hf/Kam	236	304	
C57BL/6J	101	148	

Multifractionated dose given as 2 Gy/day fractions, 5 days/week for 4 to 8 weeks. Single dose irradiation includes acute doses from 10 to 70 Gray. All doses used in each radiation dose schedule are included.