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Biology Contribution

Tumor Induction in Mice After Localized Single- or Fractionated-Dose Irradiation: Differences in Tumor Histotype and Genetic Susceptibility Based on Dose Scheduling

Elijah F. Edmondson, DVM,* Nancy R. Hunter, MS,† Michael M. Weil, PhD,* and Kathryn A. Mason, MS†

**Environmental and Radiological Health Sciences Department, Colorado State University, Fort Collins, Colorado; and †Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas*

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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 after fractionated irradiation in comparison with single large-dose exposures. These results indicate that different cell types respond differently

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

Methods and Materials: C3Hf/Kam and C57BL/6J mice were locally irradiated to the right hindlimb with either single large doses between 10 and 70 Gy or fractionated doses totaling 40 to 80 Gy delivered at 2-Gy/d fractions, 5 d/wk, for 4 to 8 weeks. The mice were closely evaluated for tumor development in the irradiated field for 800 days after 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 after fractionated irradiation (16.4%) in comparison with single-dose irradiation (36.1%). Sarcomas were the predominant postirradiation 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 after single-dose irradiation; however, significant differences in tumor susceptibilities after fractionated radiation were not observed. For both strains of mice, osteosarcomas and hemangiosarcomas were significantly more common after fractionated irradiation, whereas fibrosarcomas and malignant fibrous histiocytomas were significantly more common after single-dose irradiation.

Reprint requests to: Elijah F. Edmondson, DVM, Colorado State University, College of Veterinary and Biomedical Sciences, Molecular and Radiological Biosciences Building, 1618 Campus Delivery, Fort Collins, CO 80523. Tel: (970) 491-7038; E-mail: elijah.edmondson@colostate.edu

Conflict of interest: none.

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to radiation, depending on delivery schedule.

Conclusions: This study investigated the tumorigenic effect of acute large doses in comparison with fractionated radiation in which both the dose and delivery schedule were similar to those used in clinical radiation therapy. Differences in tumor histotype after single-dose or fractionated radiation exposures provide novel in vivo evidence for differences in tumor susceptibility among 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 radiation therapy patients, have not been reported.

Here we report on tumorigenesis in 2 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 to 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 US 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 University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee.

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 Table E1 (available on at www.redjournal.org). 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 to 8 Gy/min. 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 veterinary pathologist (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 (Fig. 1A). Hemangiosarcomas were composed of atypical, plump endothelial cells forming irregularly anastomosing vascular spaces containing erythrocytes (Fig. 1B). Fibrosarcomas were composed of spindle-shaped cells separated by variable amounts of lightly eosinophilic collagenous stroma; spindle-shaped cells were arranged in interweaving fascicles forming a characteristic herringbone pattern (Fig. 1C). Malignant fibrous histiocytomas were pleomorphic with fusiform to rounded cells and typically contained numerous multinucleated giant cells (Fig. 1D). Representative histopathology for additional tumor histotypes can be found in Figures E1 and E2 (available online at www.redjournal.org). Sarcomas lacking diagnostic features of the previously mentioned subtypes were assigned the diagnosis of undifferentiated sarcoma.