Published in final edited form as:

Radiat Environ Biophys. 2010 August ; 49(3): 389–396. doi:10.1007/s00411-010-0292-2.

Enhanced Intestinal Tumor Multiplicity and Grade *in vivo* after HZE Exposure: Mouse Models for Space Radiation Risk Estimates

Daniela Trani^{1,*}, Kamal Datta^{1,3,*}, Kathryn Doiron¹, Bhaskar Kallakury², and Albert J. Fornace Jr.^{1,3}

¹Department of Biochemistry and Molecular & Cell Biology and Lombardi Comprehensive Cancer Center, Georgetown University, Room E504 Research Building, 3970 Reservoir Rd., NW, Washington, DC 20057-1468, USA

²Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, 119 Basic Science Building, Washington, DC 20057-1468, USA

Abstract

Carcinogenesis induced by space radiation is considered a major risk factor in manned interplanetary and other extended missions. The models presently used to estimate the risk for cancer induction following deep space radiation exposure are based on data from A-bomb survivor cohorts and do not account for important biological differences existing between high-linear energy transfer (LET) and low-LET-induced DNA damage. High-energy and charge (HZE) radiation, the main component of galactic cosmic rays (CGR), causes highly complex DNA damage compared to low-LET radiation, which may lead to increased frequency of chromosomal rearrangements, and contribute to carcinogenic risk in astronauts. Gastrointestinal (GI) tumors are frequent in the United States, and colorectal cancer (CRC) is the third most common cancer accounting for 10% of all cancer deaths. On the basis of the aforementioned epidemiological observations and the frequency of spontaneous precancerous GI lesions in the general population, even a modest increase in incidence by space radiation exposure could have a significant effect on health risk estimates for future manned space flights. Ground-based research is necessary to reduce the uncertainties associated with projected cancer risk estimates and to gain insights into molecular mechanisms involved in space radiation-induced carcinogenesis. We investigated *in vivo* differential effects of γ -rays and HZE ions on intestinal tumorigenesis using two different murine models, ApcMin/+ and Apc1638 N/+. We showed that γ - and/or HZE exposure significantly enhances development and progression of intestinal tumors in a mutant-line-specific manner, and identified suitable models for *in vivo* studies of space radiation-induced intestinal tumorigenesis.

Keywords

Apc; intestinal tumorigenesis; space radiation; risk estimates

³corresponding authors: phone: 202 687-7843, fax: 202 687 3140, af294@georgetown.edu, phone: 202 687-7956, fax: 202 687-3140, kd257@georgetown.edu.

*These authors contributed equally to this work

Introduction

As advances in science and technology have made the perspective of long-term interplanetary missions more realistic, it has become important to investigate health risks related to human exposure to the space environment. Carcinogenesis induced by space radiation is considered a major risk factor in manned long-term missions to the Moon or to the exploration of Mars. Ground-based research aimed to gain more insights into the molecular mechanisms involved in space radiation-induced carcinogenesis will contribute in reducing the present uncertainties associated with projected cancer risk estimates (Durante and Cucinotta 2008).

Gastrointestinal (GI) tumors are frequent in the United States, and colorectal cancer (CRC) is the third most common cancer accounting for 10% of all cancer deaths. It has been estimated that CRC has caused approximately 14,900 new cases and 50,000 deaths in the United States in 2008 (American Cancer Society Statistics 2008). Precancerous lesions are present in about 10% of adults at age 40 and their incidence increases to ~25% in older people (Grady and Markowitz 2002). In humans, mutations in the adenomatous polyposis coli (APC) gene occur early in the development of both sporadic and familial colon cancer (familial adenomatous polyposis, FAP), and APC mutation in normal human population varies between 1 in 13,528 and 1 in 43,700 (Bisgaard et al. 1994; Cachon-Gonzalez et al. 1991). APC mutations are commonly followed by mutations in other key oncogenes and tumor suppressor genes, such as K-ras, p53 and transforming growth factor- β (TGF- β) (Kinzler and Vogelstein 1996; Grady and Markowitz 2002). This multistep process is associated with the progression of untreated colorectal adenomatous polyps to carcinomas. The tumor suppressor protein encoded by the APC gene has been shown, among several functions, to be implicated in the Wingless (Wg)/WNT signaling pathway as a negative regulator of β -catenin-mediated transcriptional activation (Nathke 2004). Involvement of APC in WNT pathway is crucial for the maintenance of gut homeostasis, and molecular imbalances due to mutations or loss of APC gene are tightly related to development of intestinal cancers in humans, and in murine models of colorectal cancer as well (Uronis and Threadgill 2009; Taketo 2006; Taketo and Edelmann 2009).

Ionizing radiation (IR) is a known risk factor for CRC based on studies such as the A-bomb survivor cohort (Thompson et al. 1994; Preston et al. 2007; Pawel et al. 2008). On the basis of these epidemiological observations and the frequency of spontaneous precancerous GI lesions in the general population, we can argue that an even modest increase in incidence by space radiation exposure could have a significant effect on health risk estimates for future manned space flights. High-energy and charge (HZE) nuclei, along with high-energy protons, represent the major component of galactic cosmic rays and importantly contribute to radiation risk in space (Cucinotta and Durante 2006). Risk from galactic cosmic radiation is also relevant for trans-continental flight personal, especially in combination with solar particle events (primarily protons). Extrapolation of risks associated with space radiation from epidemiological studies on individuals exposed to IR accidentally or therapeutically on Earth relies on application of scaling factors, which account for differences in radiation dose and dose rate (dose and dose rate effectiveness factor, DDRE), and radiation quality (radiation quality factor, Q). However, physical differences between sparsely IR, such as X- and γ -rays, and HZE ions and high-energy protons, produce major qualitative differences in their biological effects, which have been not extensively investigated in vivo. Ground-based in vivo experiments are, therefore, necessary (1) for improving estimates of quality factors for different radiation types and weighting factors (W_R) associated with exposure of different tissues, (2) to correctly understand the risks associated with space radiation exposure and (3) to gain insights into specific mechanisms involved in space radiation-induced colon carcinogenesis and control the related risks.

In order to compare intestinal tumorigenesis induced by γ rays and HZE ions, two different murine models of human colon cancer were selected, the Apc multiple intestinal neoplasia (Min) model (Moser et al. 1995) and the Apc1638 N model (Fodde et al. 1994). Min mice carry a nonsense mutation at codon 850 of the murine Apc allele. Mice homozygous for the Min mutation are embryonic lethal, while ApcMin/+ mice spontaneously develop up to about 100 intestinal adenomas within the entire life span, which is approximately 5–6 months. Furthermore, ApcMin/+ females are also predisposed to spontaneous mammary tumors. The number of intestinal lesions is highly dependent on different alleles of specific Apc-modifier loci, which lead to phenotypic variation observed in different inbred strains. Murine strains like AKR/J have been shown to be relatively resistant to intestinal tumor development because they are homozygous for the Modifier of Min-1 (Mom1) resistant allele: in the pure AKR/J background, ApcMin/+ mice develop as few as 1–4 lesions in the entire life span and can survive up to 1 year (Shoemaker et al. 1998; Kwong et al. 2007). Conversely, C57BL/6J mice are homozygous for the Mom-1 sensitive allele. The ApcMin model has been employed in many laboratories to investigate the role of Apc and interacting genes in the initiation of intestinal and mammary tumorigenesis. Study performed in F1 progenies from (AKRxC57BL/6 J-ApcMin) crossings, which have a longer life span compared to ApcMin in the pure C57BL/6 J background, may allow to investigate tumor progression in ApcMin mice following radiation exposure. The Apc1638 N mouse model carries a frameshift mutation at a position corresponding to amino acid 1638 of the Apc gene and was generated by Fodde et al. introducing a neomycin phosphotransferase cassette (PGK-NEO) at the targeted site (Fodde et al. 1994). Like mice homozygous for the Min mutation, mice homozygous for the Apc1638 N mutation are also embryonic lethal. However, in the pure C57BL/6 J background, Apc1638 N/+ mice develop only about 2–6 intestinal adenomas during their lifetime and can live longer than 1 year. Compared to ApcMin, the Apc1638N may represent a better model to investigate both space radiation–induced intestinal tumor development and progression due to the higher signal to noise ratio. It has been previously observed that whole-body exposure to X-rays induced in C57BL/6J-Apc1638N a significant increase in small intestinal tumor multiplicity compared to untreated controls (van der Houven van Oordt et al. 1997). The Apc1638 N mutation is also related to development of extra-intestinal malignancies, such as desmoid-like lesions, a clinical manifestation also reported in patients affected by Gardner's syndrome (Smits et al. 1998; Fotiadis et al. 2005).

Gardner's syndrome is a familial form of polyposis characterized by the development of multiple polyps in the colon and in other organs. The extracolonic tumors may include thyroid cancer, osteomas of the skull, epidermoid cysts, fibromas and sebaceous cysts, as well as the occurrence of desmoid tumors in approximately 15% of all Gardner's patients. The presence of very high number of tumors in the colon predispose to development of colon cancer if the colon is not surgically removed. Patients with Gardner's syndrome may develop also polyps in the stomach, duodenum, spleen, kidneys, liver, mesentery and small bowel.

In this work, we investigated possible differential effects of radiation exposure on the development and progression of intestinal tumors in both ApcMin and Apc1638 N mice. Our aim is to characterize these two murine models of human colon cancer in order to assess their suitability for in vivo studies of space radiation–induced intestinal tumorigenesis estimates. For this purpose, we analyzed intestinal tumor multiplicity and grade in C57BL/6 J-ApcMin, F1(AKRxC57BL/6 J-ApcMin) and C57BL/6 J-Apc1638 N mice exposed whole body to a dose of 5 Gy of γ rays. In addition, C57BL/6J-ApcMin and F1(AKRxC57BL/6J ApcMin) mice were also exposed to 4 Gy of 1 GeV/n ^{56}Fe ions, and intestinal tumor multiplicity and grade were assessed.

Materials and methods

Mouse husbandry and genotyping

Ten- to twelve-week-old wild-type (wt) female C57BL/6 J, wt AKR/J female and C57BL/6J-APC^{min/+} male mice were purchased from Jackson Laboratories (Bar Harbor, ME). Male mice C57BL/6J-Apc1638 N/+ were obtained from the Mouse Model for Human Cancer Consortium of the National Cancer Institute (Frederick, MD). Breeding of Apc^{Min/+} and Apc1638 N/+ mice was done at the Georgetown University animal facility, which is an Association for Assessment and Accreditation of Laboratory and Animal Care International (AAALACI) accredited facility. All animals were housed in a separate room with 12-h dark and light cycle maintained at 22°C in 50% humidity, and certified rodent diet was provided with filtered water ad libitum.

Wild-type AKR/J or C57BL/6J females were mated with males C57BL/6J-Apc^{Min/+}. C57BL/6J-Apc^{Min/+} female mice, and F1(AKRxC57BL/6 J-Apc^{Min/+}) female and male hybrids were employed in the present study. Similarly, C57BL/6 J wt females were mated with C57BL/6J-Apc1638N/+, and heterozygous F1 male mice were used in this study. All animal procedures were performed in accordance with a protocol approved by the Georgetown University Animal Care and Use Committee (GUACUC) and Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council, and US National Academy of Sciences was followed.

The animals were genotyped by PCR of tail DNA. Genotyping of Apc^{Min/+} mice was done as per protocol in the Jackson Laboratory web site. For Apc1638 N/+ genotyping, the following primers were used: primer A, TGCCAGCACAGAATAGGCTG; primer B, TGGGAAGGATTGGAGCTGCTG; and primer C, GTTGTCATCCAGGTCTGGTG. The reaction was performed in a 20-ml reaction mixture containing 100 ng of DNA, 0.33 mM primers A, B and C, 1.2 mM MgCl₂, 0.2 mM each dNTP and 0.5 units of Taq polymerase. Cycling conditions were 5 min at 94°C (1 cycle); 1 min at 94°C, 2 min at 55°C, and 1 min at 72°C (35 cycles); and 5 min at 72°C (1 cycle). The presence of the wild-type allele generates a 300-bp PCR fragment (primers A and C) and the mutant allele a 400-bp PCR fragment (primers A and B).

Radiation exposures

Six- to eight-week-old C57BL/6J-Apc^{Min/+} female mice and F1(AKRxC57BL/6J-Apc^{Min/+}) female and male hybrids were exposed whole body to either 5 Gy of γ rays or 4 Gy of 1 GeV/n ⁵⁶Fe ions. Gamma exposures were performed at Georgetown University, while for exposure to Fe ions, animals were irradiated at NASA Space Research Laboratory's (NSRL) linear accelerator at Brookhaven National Laboratory (BNL). Female and male mice of the same genotype and background were left untreated and used as age- and sex-matched controls. Similarly, 6- to 8-week-old C57BL/6 J-Apc1638 N/+ male mice were exposed whole body to 5 Gy of γ rays and age-matched controls were left untreated.

For γ -exposures, 10 mice at a time were placed in a well-ventilated pie-shaped clear box and placed in a ¹³⁷Cs irradiator. At the NSRL, each mouse was placed in a small transparent rectangular Lucite box (7.6 cm 9 3.8 cm 9 3.8 cm) with multiple holes, and 10 of these boxes were slid into a sample holder made of lowdensity foam. The sample holder was then placed in the path of the beam (20 cm 9 20 cm). Mice were shipped by the vendor or from GU directly to BNL; after irradiation at the NSRL, all mice were shipped back to GU by courier for same day delivery in a temperature-controlled truck. Control untreated mice were kept in the same conditions.

Analysis of intestinal tumors

Animals were euthanized by CO₂ asphyxiation at 100 days (C57BL/6 J-ApcMin/+), 120–140 days (F1(AKRxC57BL/6J-ApcMin/+), or 150 days of age (C57BL/6 J-Apc1638 N/+) after exposure. For each of the mutants, the post-irradiation time was close to the corresponding average life span. Age-matched controls were included for all strains and radiation treatments. The intestinal tract from the duodenum to the colon was then removed. The small intestine was cut in 3 sections of equal length and flushed with 1X phosphate-buffered saline (PBS) solution. The colon was left whole and also flushed. The segments were laid on black paper and cut open longitudinally. Tumors in each section were counted under a dissecting microscope at 6.3X magnification. Tumors were always scored by the same operator without previous knowledge of the specific treatment. Tumors were counted in at least 10 animals for each experimental group, and average number of tumors was estimated. When comparing two groups, statistical significance was determined by two-tailed, unpaired student's t test. P values < 0.05 were considered significant, and fold change was calculated with a 95% confidence interval. Tumors were dissected and fixed overnight in 10% neutral-buffered formalin and subsequently transferred to 70% ethanol. Representative tumors from each mouse were embedded in paraffin, and 5-mm thick sections were prepared and hematoxylin & eosin (H&E) stained using standard protocol. Tumors were analyzed and classified by a board-certified pathologist using standard criteria (Boivin et al. 2003).

Results

Tumor incidence and grade in C57BL/6 J-APC+/min mice exposed to γ rays or 1 GeV/n ⁵⁶Fe

Exposure to 5 Gy of γ -rays and equitoxic 4 Gy, determined by LD50/30 study in C57BL/6J mice, of 1 GeV/n ⁵⁶Fe ions resulted in significantly higher tumor incidence than unirradiated control mice (Fig. 1a). Average number of tumors in the small intestine of unirradiated Min mice was 43 ± 5 (range 35–51 tumors; n = 20). However, when mice were irradiated with 5 Gy of γ -rays, average tumor number increased to 62 ± 8 (range 48–78 tumors, n = 20), which was 1.5 fold more (P < 0.001) than control (95% confidence interval (CI): 1.33–1.56). Importantly, exposure to ⁵⁶Fe radiation caused a greater increase in tumor incidence (average 95 ± 15 ; range 68–123 tumors, n = 30). When compared to tumor incidence in control, the fold change was 2.2 (P < 0.001; 95% CI: 2.04–2.39). Gross distribution of tumors along the intestinal tract showed markedly greater number of tumors per unit area in ⁵⁶Fe-irradiated mice suggesting increased tumor initiation and/or accelerated tumor development with this type of radiation (Fig. 1b). Histopathological examination of H&E stained section of the tumors showed no difference in tumor grade. In control as well as in γ rays and ⁵⁶Fe-irradiated samples, adenomatous proliferation with low-grade dysplasia was observed (Fig. 1c). In these mice, most of the tumors are in small intestine and very few tumors (1–3) are observed in colon, and no marked differences between radiation types were observed (unpublished observation).

Intestinal tumor development and grade in F1(AKR/JxC57BL/6 J-ApcMin/+) mice exposed to γ rays or 1 GeV/n ⁵⁶Fe

We observed a 1.8-fold increase in total intestinal tumor multiplicity (P < 0.001) (with a 95% CI: 1.48–2.21) after exposure of F1(AKRxC57BL/6 J-ApcMin) mice to 5 Gy of γ rays (Fig. 2a). Gamma-exposed animals developed an average tumor number of 49 ± 13 (range 20–76 tumors; n = 24). Age-matched control mice developed an average of 27 ± 6 intestinal tumors (range 17–39 tumors; n = 20) along the whole intestinal tract. Similarly, exposure to 4 Gy of 1 GeV/n ⁵⁶Fe ions resulted in an average total intestinal tumor number of 42 ± 4 (range 34–53 tumors; n = 10) and 1.5-fold increase (P < 0.001) (with a 95% CI: 1.39–1.76).

in total tumor burden compared to age-matched controls. Differences between low- versus high-LET effects resulted not significant in F1(AKRxC57BL/6 J-ApcMin) mice. For both irradiated and control animals, most of the lesions were observed in the small intestine with very few lesions detected in the colon (data not shown). Our study also revealed that radiation-induced intestinal tumors were characterized by higher-grade dysplasia compared to spontaneous lesions developed in age-matched control mice. Representative histopathology of intestinal tumors from control, γ and Fe-exposed F1(AKRxC57BL/6 J-ApcMin) mice are shown respectively in Fig. 2b, c and d. Tumors from untreated mice were classified as tubulovillous adenomas mostly with low-grade dysplasia (Fig. 2b), while invasive (Fig. 2c) and in situ (Fig. 2d) carcinomas were observed in both animals exposed to 5 Gy of γ rays and 4 Gy of 1 GeV/n ^{56}Fe ions. No gender-related differences were observed following exposure of F1(AKRxC57BL/6J-ApcMin) mice to γ rays or Fe ions.

Intestinal tumor incidence, distribution and grade in C57BL/6 J-Apc1638 N/+ exposed to γ rays

We observed a significant 16-fold increase ($P < 0.001$) (with a 95% CI:11.97–25.12) in total intestinal tumor burden following exposure to 5 Gy of γ rays. This finding is consistent with data previously published by van der Houven van Oordt et al. that showed a significant increase in total intestinal tumor burden induced by exposure to 5 Gy of X-rays (van der Houven van Oordt et al. 1997). In our study, irradiated Apc1638 N male mice showed an average total tumor multiplicity of 66 ± 13 (range 49–83 tumors; $n = 10$) along the whole intestinal tract, while the average total intestinal tumor number in age-matched untreated controls was of 4 ± 2 (range 2–7, $n = 10$) (Fig. 3a). Furthermore, we observed that IR exposure induced a different tumor distribution along the intestinal tract when compared to distribution of spontaneous lesions in unirradiated controls. As shown in Fig. 3a, duodenum, jejunum and colon displayed the highest relative tumor increase at 150 days after irradiation. In regard to large intestinal tumor multiplicity, we report here that a single adenoma was found in the large intestine of only one age-matched control, while γ -irradiated Apc1638 N mice showed an average of 4.7 ± 2.0 adenomas (range 2–8 tumors) in the colon and, therefore, a substantial 47-fold increase compared to unirradiated animals. Histopathological examination of adenomas dissected from both irradiated animals 150 days after exposure and age-matched controls revealed that radiation induced a small increase in the adenoma/carcinoma ratio, mostly in the duodenum. In Fig. 3b, a representative adenocarcinoma from an irradiated Apc1638 N is shown. Frequently, large colonic adenomas (size: 3–5 mm in diameter) were observed in the colon of irradiated animals at 150 days after exposure (Fig. 3c).

Discussion

In the present study, we observed a significant increase in intestinal tumor burden in all ApcMin mutants exposed to 5 Gy of γ rays—1.5 fold in C57BL/6 J-ApcMin and 1.8-fold in F1(AKRxC57BL/6 J-ApcMin) mice. Our observations are in agreement with data previously published from X-irradiated ApcMin mice (Okamoto and Yonekawa 2005; Haines et al. 2000; Ellender et al. 2006). We also sought to assess in vivo how exposure to high-linear energy transfer (LET) radiation, such as 1 GeV/n ^{56}Fe ions, may affect intestinal tumorigenesis compared to low-LET radiation, like γ rays. We showed for the first time an increase in tumor burden in both C57BL/6J-ApcMin and F1(AKRxC57BL/6J-ApcMin) mice after exposure to 4 Gy of 1 GeV/n ^{56}Fe ions compared to age-matched controls. However, while the 2.2-fold increase observed in C57BL/6 J-ApcMin mice after iron exposure was significantly higher compared to 1.5 fold induced by γ rays, there were equivalent increases induced by low- and high-LET radiation in F1(AKRxC57BL/6 J-ApcMin) mice. In regard to the different effects of low- and high-LET reported here for intestinal tumorigenesis in the

pure C57BL/6J versus the F1(AKRxC57BL/6 J) mice, it is important to emphasize that the number of spontaneous intestinal lesions in ApcMin mutants is highly dependent on the genetic background. Indeed, different alleles of specific Apc-modifier loci are responsible for phenotypic variation observed in different inbred strains. Some murine strains like AKR/J have been shown to be relatively resistant to intestinal tumor development because they are homozygous for the Modifier of Min-1 (Mom1) resistant allele: in the pure AKR/J background, ApcMin/+ mice develop as few as 1–4 lesions in the entire life span and can survive up to 1 year (Shoemaker et al. 1998; Kwong et al. 2007). This intrinsic resistance may play an important role also in predisposition to radiation-induced intestinal tumorigenesis. Overall, our data in C57BL6J-ApcMin mice represent the first in vivo evidence for enhanced biological effectiveness of HZE ions compared to γ rays in regard to intestinal tumor induction.

ApcMin is considered the standard model for the development of intestinal tumorigenesis risk assessment and has been employed in studies with several known carcinogens. However, we investigated the effect of radiation exposure also in a different Apc mutant, Apc1638 N, which develops <10 intestinal adenomas during their lifetime and can live longer than 1 year. Apc1638 N mice spontaneously develop advanced lesions in the small intestinal tract and may serve as a model, complementary to ApcMin, to investigate space radiation-induced intestinal tumor progression. In a previous study, van der Houven van Oordt et al. (1997) reported a greater relative increase in total GI tumor multiplicity in Apc1638 N mice exposed to 5 Gy of X rays. In our study, we exposed C57BL/6 J-Apc1638 N mice to 5 Gy of γ rays and we report here a 16-fold increase—consistent with findings by van der Houven van Oordt et al. —in overall intestinal tumor multiplicity. However, we report for the first time that irradiated mice developed multiple colonic adenomas, while most unirradiated showed no colonic tumors. Based on the high signal to noise ratio described for both small and large intestine adenomas in Apc1638 N mice following exposure to γ rays, Apc1638 mutant appears a promising candidate to serve as a model for space radiation-induced intestinal tumorigenesis estimates along with ApcMin mutant.

We observed also that radiation exposure elicits different responses along the GI tract in different mutants. In contrast to Apc1638 N mice, which develop most of the lesions in duodenum, jejunum and colon, tumors in ApcMin mice, regardless of the background, show highest number in the ileum, followed by jejunum. Conversely, very few polyps were found in the duodenum and in the large intestine of irradiated ApcMin mice. This evidence supports the hypothesis that the site of specific chain terminating mutations in Apc sequence may be responsible for the tumor distribution and severity of the corresponding phenotype (Fodde et al. 1994), similar to that observed among patients with FAP (Bertario et al. 2003). In addition to differences in intestinal tumor multiplicity and distribution, the strains employed in our study displayed different susceptibilities to develop low- versus high-grade dysplasia adenomas in irradiated animals compared to corresponding controls. Both Apc1638 N and F1(AKRxC57BL/6J ApcMin) develop in situ and invasive carcinomas following radiation exposure, while tumors from exposed as well as unirradiated C57BL/6J ApcMin were classified as adenomatous proliferation with low-grade dysplasia. Interestingly, in the case of Apc1638 N, an increase in adenocarcinoma rate was observed in the duodenum, and it is possible that malignant tumors would develop elsewhere at longer times after irradiation considering high-grade dysplasia observed. Thus, the Apc1638 N model may have suitability to investigate tumor progression following exposure to radiation. Furthermore, our tumor multiplicity data and histopathological observations suggest that both specific mutation site and presence of modifier genes, such as Mom-1 present in the form of a sensitive (Mom-1^S) and a resistant allele (Mom-1^R) in by F1(AKRxC57BL/6J-ApcMin) mice, may contribute to the development and progression of intestinal tumors induced by low- or high-LET radiation.

The models presently used to estimate the risk for cancer induction following exposure to space radiation are based on data from A-bomb survivor cohorts and do not account for important biological differences existing between high-LET- and low-LET-induced DNA damage. Indeed, HZE radiation causes highly complex DNA damage compared to low-LET radiation and produces a high frequency of interchromosomal exchanges exposed cells, which may well contribute to genomic instability and tumorigenesis. Our findings contribute to the understanding of quality factors for space radiation effects in vivo in regard to intestinal tumorigenesis. Furthermore, they clearly underscore the importance of selecting appropriate mouse models for studies of molecular pathways involved in initiation and/or progression of space radiation-induced intestinal cancer. In this context, we conclude that the ApcMin provides a valid model to explore in vivo radiation effects in the presence of a mutation, closely related to the APC mutation carried by the majority of patients with FAP. ApcMin mice also have the advantage of quickly developing adenomas to test potential countermeasures for space radiation-induced tumorigenesis. Overall, with our investigation into C57BL6J-ApcMin mutant, we provide the first in vivo evidence for a potential role of space radiation as a risk factor for intestinal tumor development and progression. Loss of heterozygosity (LOH) studies for Apc locus in F1(AKRxC57BL/6JApcMin) are likely to provide important information about specific genetic events involved in the loss of the wild-type allele in tumors induced by different types of radiation. However, based on the very high signal to noise ratio that we reported for the first time for both small intestinal and colonic adenomas in Apc1638 N following exposure to γ -rays, Apc1638 N mutant appears a promising candidate to serve as an alternative model for space radiation-induced intestinal tumorigenesis estimates.

Acknowledgments

The work was supported by NASA Grant NNX-7AH70G. Daniela Trani was partially supported by the National Space Biomedical Research Institute (NSBRI) through NASA NCC9-58. The authors are grateful to Adam Rusek, the NASA Space Radiation Laboratory (NSRL) and the Brookhaven National Laboratory (BNL) staff for valuable assistance. The authors wish to thank Deborah Berry and the team of the Histopathology and Tissue Shared Resource at Georgetown University for technical support.

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Fig. 1. Intestinal tumor incidence and grade in APCmin/+ mice. APCmin/+ mice were exposed to γ rays and HZE radiation, and intestinal tumors were counted. **a** Greater numbers of intestinal tumors were observed after either 5 Gy γ - or equitoxic dose (4 Gy) of HZE-irradiated mice. **b** Photograph showing more tumors per unit area of the intestinal surface with HZE. **c** Histopathology of intestinal tumors showing low-grade dysplasia in control as well as γ and HZE-irradiated mice

Fig. 2. Intestinal tumor incidence and grade in F1(AKR/JxC57BL/6 J-ApcMin/+) mice exposed to ionizing radiation. **a** Total intestinal tumor number showing increased intestinal multiplicity in animals exposed to either 5 Gy of γ rays or 4 Gy of ^{56}Fe ions relative to control. Standard deviations are indicated. **b** In unirradiated (Control) animals, most of the tumors were classified mostly as tubulovillous adenomas with low-grade dysplasia. In situ carcinomas (**c**) and invasive carcinomas (**d**) were observed in both γ - and iron-irradiated animals

Fig. 3. Intestinal tumor incidence, distribution and grade in C57BL/6 J-Apc1638 N/+ exposed to γ rays. **a** Consistent with previous observations from van der Hoven van Oordt et al. (1997, Carcinogenesis, 18, 2197–2203), a significant increase was observed in total GI tumor multiplicity after exposure to 5 Gy of γ rays. Distribution of tumors along the GI tract is also shown. Standard deviations are indicated. **b** Photomicrograph of adenocarcinoma from the small intestine in an Apc1638 N mouse 5 months after exposure to 5 Gy of γ rays (original magnification X 100). **c** Large colonic adenoma from the same animal. In the larger panel, higher detail of adenomatous hyperproliferation of the intestinal mucosa is shown. Original magnifications are X 40 (small frame) and X 100 (large frame).