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Relative Biological Effectiveness of Energetic Heavy Ions for Intestinal Tumorigenesis Shows Male Preponderance and Radiation Type and Energy Dependence in APC$^{1638N/+}$ Mice

Shubhankar Suman, PhD,* Santosh Kumar, PhD,* Bo-Hyun Moon, MS,* Steve J. Strawn, MS,* Hemang Thakor, MS,* Ziling Fan, MS,* Jerry W. Shay, PhD,† Albert J. Fornace, Jr, MD,*,# and Kamal Datta, MD*

**Department of Biochemistry and Molecular & Cellular Biology and Lombardi Comprehensive Cancer Center, Georgetown University, Washington, District of Columbia; †Department of Cell Biology, UT Southwestern Medical Center, Dallas, Texas; and #Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia*

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Summary

Radiation is a known risk factor for colorectal cancer (CRC). However, much uncertainty exists over estimates of CRC risk after energetic heavy ion radiation exposures. The relative biological effectiveness of intestinal tumor frequency for energetic ^{12}C, ^{56}Fe, and ^{28}Si ions relative to γ radiation was assessed in a mouse model (APC$^{1638N/+}$) of human CRC. Research into

Purpose: There are uncertainties associated with the prediction of colorectal cancer (CRC) risk from highly energetic heavy ion (HZE) radiation. We undertook a comprehensive assessment of intestinal and colonic tumorigenesis induced after exposure to high linear energy transfer (high-LET) HZE radiation spanning a range of doses and LET in a CRC mouse model and compared the results with the effects of low-LET γ radiation.

Methods and Materials: Male and female APC$^{1638N/+}$ mice (n = 20 mice per group) were whole-body exposed to sham-radiation, γ rays, ^{12}C, ^{28}Si, or ^{56}Fe radiation. For the >1 Gy HZE dose, we used γ-ray equitoxic doses calculated using relative biological effectiveness (RBE) determined previously. The mice were euthanized 150 days after irradiation, and intestinal and colon tumor frequency was scored.

Results: The highest number of tumors was observed after ^{28}Si, followed by ^{56}Fe and ^{12}C radiation, and tumorigenesis showed a male preponderance, especially after ^{28}Si. Analysis showed greater tumorigenesis per unit of radiation (per cGy) at lower doses, suggesting either radiation-induced elimination of target cells or tumorigenesis reaching a saturation point at higher doses. Calculation of RBE for intestinal and colon tumorigenesis showed the highest value with ^{28}Si, and lower doses showed greater RBE relative to higher doses.

Reprint requests to: Kamal Datta, MD, Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, Research Building, Room E518, 3970 Reservoir Rd, NW, Washington, DC 20057. Tel: (202) 687-7956; E-mail: kd257@georgetown.edu

S. Suman and S. Kumar contributed equally to this work.

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energetic heavy ion exposure—associated risk of CRC has implications for safe space exploration.

Conclusions: We have demonstrated that the RBE of heavy ion radiation-induced intestinal and colon tumorigenesis is related to ion energy, LET, gender, and peak RBE is observed at an LET of 69 keV/μm. Our study has implications for understanding risk to astronauts undertaking long duration space missions.

Introduction

An increased risk of colorectal cancer (CRC) after exposure to low linear energy transfer (low-LET) radiation such as γ rays has been widely reported in epidemiologic studies and in animal model studies (1-3). Whereas on earth low-LET radiation is predominant on earth, astronauts traveling into outer space are exposed to high-LET energetic heavy ions (HZE) such as ^{12}C, ^{56}Fe, and ^{28}Si, and the risk of CRC from HZE radiation exposure remains to be established. Energetic heavy ions contribute significantly toward the dose equivalent of galactic cosmic radiation, and it has been predicted that during a Mars mission about 30% of the astronauts' cells will be hit by either the primary or the secondary tracts of heavy ions (4-6). Considering that CRC is still a major form of cancer in the United States and that low-LET radiation is a CRC risk factor, high-LET radiation exposure could potentially pose a greater risk for the development of CRC. Therefore, assessing CRC risks associated with energetic heavy ion exposures is important for the health of astronauts undertaking long-duration space missions and safe exploration of outer space.

Currently, we are unable to accurately predict CRC risk from exposure to HZE ions, mostly because of insufficient in vivo tumorigenesis data. However, with limitations in obtaining in vivo human data on energetic heavy ions-associated CRC, there is an urgent need to accrue animal data necessary to predict CRC risk from long-duration space missions. Adenomatous polyposis coli (APC) mutant mouse models have been extensively used to study the molecular pathogenesis of CRC (7-10). It has been previously shown that exposure to 1.6 Gy and 4 Gy of ^{56}Fe caused higher intestinal tumorigenesis in APC$^{Min/+}$ relative to γ radiation (3). Increased intestinal tumorigenesis was also observed in APC$^{1638N/+}$ mice after ^{56}Fe radiation (11). Considering that spontaneous intestinal tumor frequency is markedly lower in control APC$^{1638N/+}$ (0 to 5 tumors) relative to APC$^{Min/+}$ (30 to 50 tumors) mice, radiation-induced tumorigenesis has a better signal-to-noise ratio in the former relative to the latter mouse model (3, 11). Therefore, we used APC$^{1638N/+}$ mice to undertake a comprehensive study for several HZE ions (^{12}C, ^{56}Fe, ^{28}Si) spanning a range of doses and LETs with the aim of determining calculated RBE values relative to γ rays for intestinal and colonic tumorigenesis. The current study demonstrated a male preponderance for the RBE of intestinal and colonic tumorigenesis, and the RBE of tumorigenesis peaked at an LET of 69 keV/μm, which is the similar LET for the highest RBE for survival reported earlier (12-17).

Methods and Materials

Mice

The APC$^{1638N/+}$ mice on a C57BL/6J background were bred, genotyped, and maintained as described previously (3, 18). Male and female APC$^{1638N/+}$ mice 6 to 8 weeks old were used. The animal procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee at Georgetown University (GU) and at Brookhaven National Laboratory (BNL), and we followed the Guide for the Care and Use of Laboratory Animals for our studies.

Irradiation

Mice transportation, irradiation procedures, and dosimetry have been described previously (3). Briefly, mice are shipped to BNL and exposed to different doses of ^{12}C (0.1, 0.5, 2.0 Gy; energy: 290 MeV/n; LET: 13 keV/μm), ^{56}Fe (0.1, 0.5, 1.6 Gy; energy: 1000 MeV/n; LET: 148 keV/μm), and ^{28}Si (0.1, 0.5, 1.4 Gy; energy: 300 MeV/n; LET: 69 keV/μm;) at NASA Space Radiation Laboratory. Mice for sham-irradiation and γ -irradiation were also shipped to BNL and shipped back to GU with the aim to expose all mice to similar transportation stressors, and γ irradiation was performed on the same day of heavy ion irradiation at GU using a ^{137}Cs source. For doses below 1 Gy (0.1 and 0.5 Gy) we used γ irradiation doses equal to those of the heavy ions. For 2 Gy γ rays, we used equitoxic doses of ^{12}C (2 Gy), ^{56}Fe (1.6 Gy), and ^{28}Si (1.4 Gy) radiation determined by using RBE factors of survival ($LD_{50/30}$ studies) calculated earlier, which were 0.99, 1.25, and 1.40, respectively (16, 17).

Tumor count

Mice were euthanized by CO_2 asphyxiation 150 days after radiation. The small intestine and colon were surgically dissected out and cleaned, and the tumors were counted under a dissection scope as previously described (3). Considering that heavy ion radiation exposures are available 3 times a year for a specified time, and also considering logistic issues such as mice breeding, mice genotyping, and beam size (area with uniform dose), we irradiated mice in smaller groups. Subsequently, the data from multiple radiation exposures were pooled for analysis of statistical significance. Tumor frequency and RBE data from male and female mice in the intestine and colon were analyzed and

plotted, and are presented separately. The words "intestine" and "intestinal" refer to the small intestine.

Statistical analysis and RBE calculation

Radiation-induced tumor frequency was normalized by subtracting spontaneous tumor frequency. The normality of data distribution in each irradiated group was tested by the Shapiro-Wilk test (19). The *P* values (<.05), histograms, and skewness and kurtosis measures with standard errors revealed that the tumor data were not approximately normally distributed. Therefore, equality of variances were tested by a non-parametric Levene's test (20), which reported a *P* value of <.05 showing inequality of variances in the tumor dataset. Given that the data showed nonnormal distribution and unequal variance but we have equal sample size (n = 20 mice per study group), Welch's 1-way analysis of variance with Games-Howell post hoc test (21) was performed to determine significance ($P<.05$ was considered significant) among the different types of radiation-induced tumorigenesis. Statistical comparison between male and female tumor frequency for a given radiation type and dose was performed with the Wilcoxon matched-pairs test, and $P<.05$ was considered significant. All statistical analysis was performed with IBM SPSS Statistics for Macintosh, version 22.0 (IBM Corp., Armonk, NY). Error bars represent mean ± standard error of the mean. In each dose, intestinal and colonic tumor frequency scale (y axis) is kept the same in male and female mice for comparison. For a calculated RBE of heavy ion tumorigenesis relative to γ rays, radiation-induced tumor frequency was first normalized by subtracting spontaneous tumor frequency: (radiation tumor frequency − spontaneous tumor frequency). Subsequently, owing to the difference in doses for different radiation types at the highest doses (2 Gy and equitoxic), the normalized tumor frequency for each dose (Gy) was converted to number of tumors per cGy: (radiation tumor frequency − spontaneous tumor frequency)/radiation dose in cGy. The calculated RBE of heavy ion radiation-induced tumorigenesis is expressed as a ratio of heavy ion and γ radiation−induced tumor frequencies (heavy ion radiation-induced tumor frequency per cGy/γ radiation-induced tumor frequency per cGy).

Results

Increased frequency of intestinal tumors in $APC^{1638N/+}$ mice after HZE radiation

All doses of heavy ion radiation showed increased intestinal tumorigenesis (Fig. 1) (Fig. E1 and Table E1; both

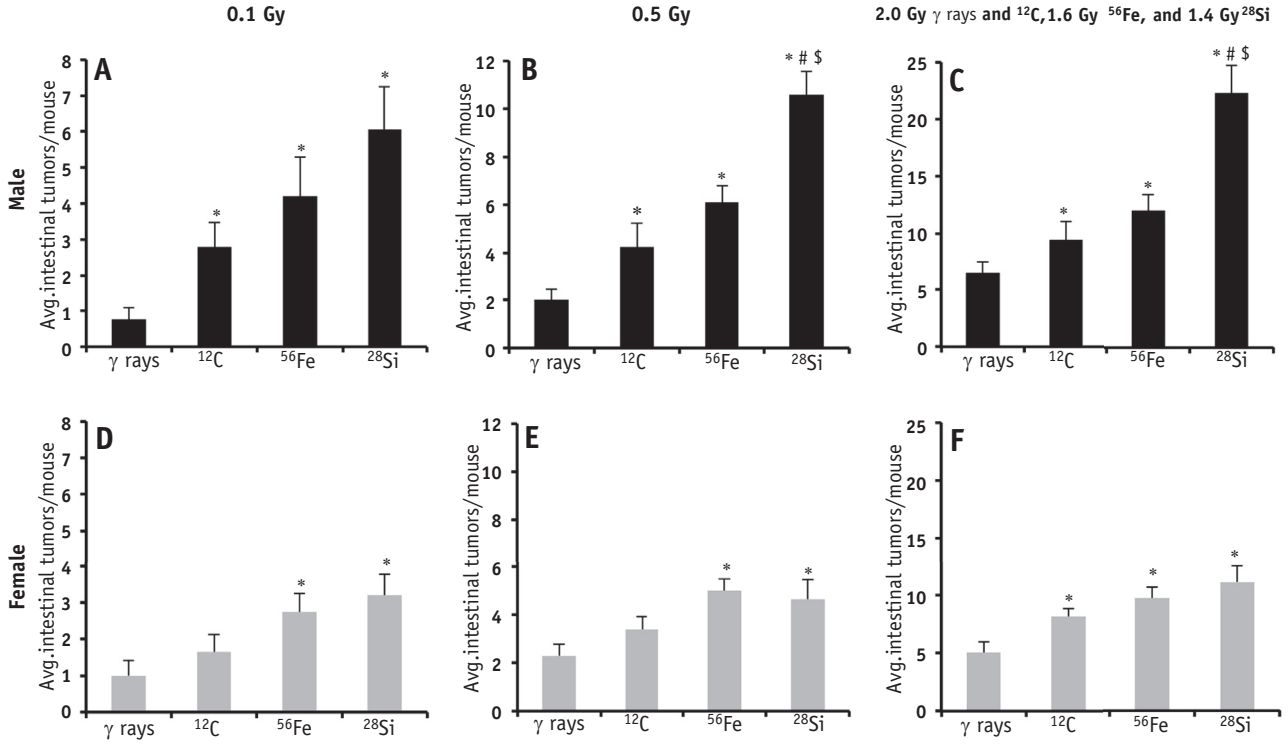

Fig. 1. Increased intestinal tumor frequency after exposure to different types of radiation. Results are presented as average radiogenic tumor incidence per mouse, with spontaneous background caused by the model subtracted. (A, B, C) Higher intestinal tumorigenesis after different doses of ^{12}C, ^{56}Fe, and ^{28}Si relative to γ radiation in male mice. (D, E, F) Intestinal tumor frequency was higher after different doses of ^{12}C, ^{56}Fe and ^{28}Si relative to γ radiation in female mice. Significance ($P<.05$); symbols: *compared with γ radiation, #compared with ^{12}C, $compared with ^{56}Fe.

available online at www.redjournal.org). In male mice, all doses of ^{12}C, ^{56}Fe, and ^{28}Si showed higher intestinal tumor incidence relative to the corresponding doses of γ radiation (Fig. 1 A-C). Tumorigenesis in female mice was also significantly higher after exposure to all doses of ^{12}C, ^{56}Fe, and ^{28}Si except after 0.1 and 0.5 Gy of ^{12}C relative to respective γ radiation doses (Fig. 1 D-F). The highest intestinal tumor frequency was observed after ^{28}Si relative to other radiation types used. Additionally, intestinal tumorigenesis was significantly higher after 0.5 and 1.4 Gy of ^{28}Si relative to ^{12}C and ^{56}Fe radiation in males (Figs. 1B and 1C).

Increased frequency of colonic tumors after heavy ion radiation exposures

Overall, colonic tumorigenesis was also increased after exposure to 3 types of heavy ions (Fig. 2) (Fig. E2 and Table E2; available online at www.redjournal.org). In comparison with γ radiation, tumorigenesis after all doses of ^{12}C, ^{56}Fe, and ^{28}Si radiation was significantly higher except after 2.0 Gy ^{12}C in male mice (Fig. 2 A-C). Tumors did not develop in female mice after 0.1 Gy ^{12}C radiation (Fig. 2D), and the small increase in tumorigenesis after 0.5 Gy ^{12}C was not statistically significant (Fig. 2E).

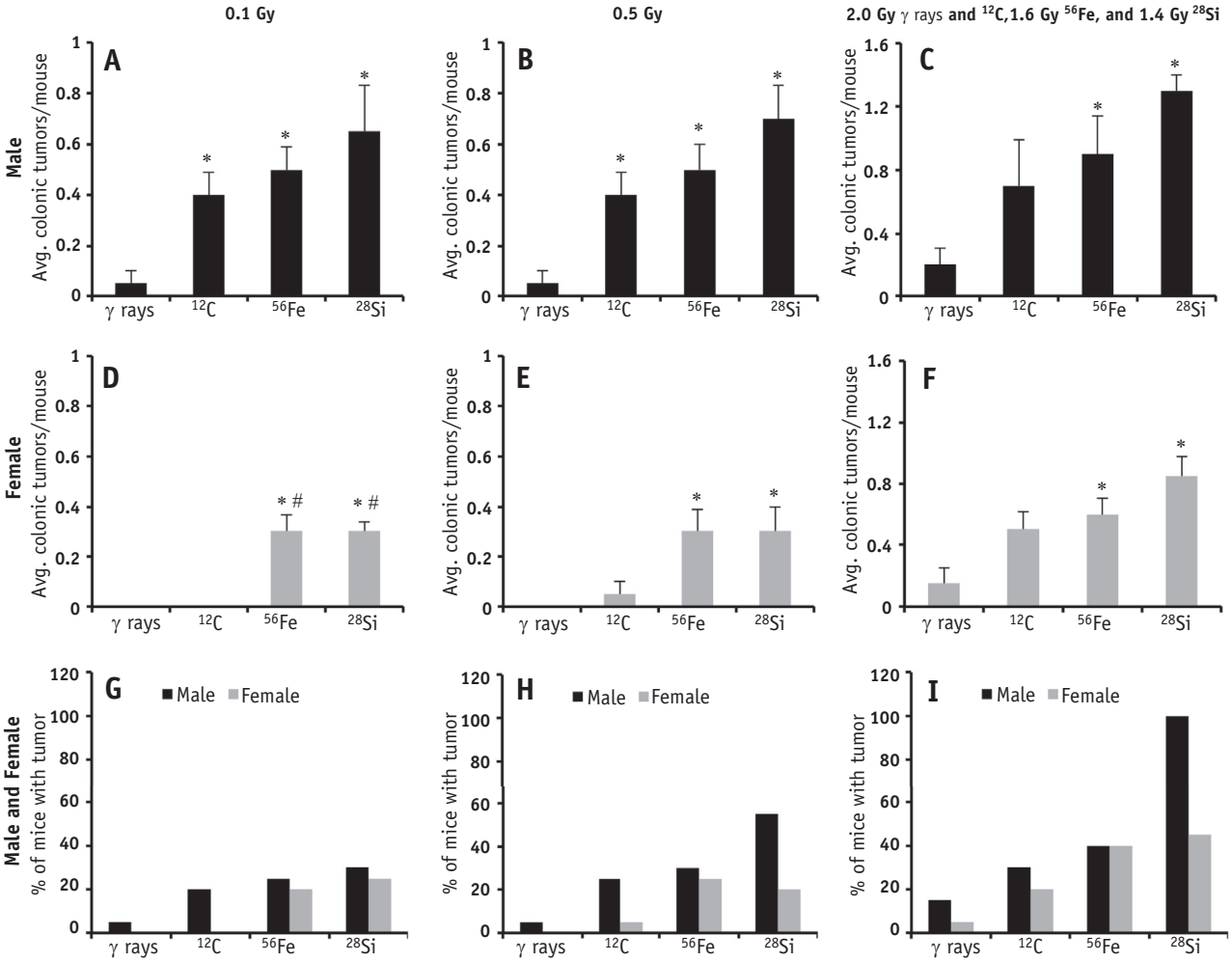

Fig. 2. Increased colon tumor frequency after exposure to different radiation types, presented as average radiogenic tumor incidence per mouse, with spontaneous background subtracted. (A, B, C) Higher tumor frequency in colon after different doses of ^{12}C, ^{56}Fe, and ^{28}Si relative to γ radiation in male mice. (D, E, F) Colon tumorigenesis in female mice after different doses of ^{12}C, ^{56}Fe and ^{28}Si relative to γ radiation. (G, H, I). Since there was no spontaneous tumor in control mice, colon tumorigenesis is expressed as percentage of male and female mice bearing colon tumors after different doses of γ, ^{12}C, ^{56}Fe, and ^{28}Si radiation. Considering that data are presented as percentage of mice bearing tumors relative to total number of mice in each group (total 20 mice/study group), no statistical analysis is shown. Percentage of mice bearing colon tumors = (number of mice with tumor/total number of mice, which is 20 in this study) × 100. Significance ($P<.05$); symbols: *compared with γ radiation, #compared with ^{12}C.

Although we observed higher colon tumorigenesis after 2.0 Gy ^{12}C, it was not statistically significant relative to γ radiation (Fig. 2F). In female mice, colonic tumorigenesis was significantly higher after all doses of ^{56}Fe and ^{28}Si relative to the corresponding doses of γ radiation (Fig. 2 D-F). There was no statistically significant difference in tumorigenesis among comparable doses of ^{12}C, ^{56}Fe, and ^{28}Si radiation except for 0.1 Gy, where tumor frequency was higher after ^{56}Fe and ^{28}Si relative to ^{12}C. Since there was no spontaneous tumor in the colon, we calculated the percentage of mice bearing radiation-induced colonic tumors. In male mice, 5% to 15% had colonic tumors after 0.1 Gy to 2 Gy γ radiation, 20% to 30% had tumors after 0.1 Gy to 2 Gy ^{12}C, 25% to 40% had tumors after 0.1 Gy to 1.6 Gy ^{56}Fe, and 30% to 100% had tumors after 0.1 Gy to 1.4 Gy ^{28}Si (Fig. 2 G-I).

Higher tumorigenesis in male mice relative to female mice after ^{28}Si irradiation

For each radiation dose, tumorigenesis was compared between male and female mice. Intestinal tumorigenesis was significantly higher in male mice relative to female mice at all doses of ^{28}Si radiation (Fig. 3 A-C). However, we did not observe any significant difference in intestinal tumorigenesis between male and female mice after ^{12}C and ^{56}Fe radiation (Fig. 3 A-C). Whereas colonic tumorigenesis was higher in male compared to female mice at all doses of ^{28}Si, we also observed higher colonic tumor frequency in male mice after 0.1 and 0.5 Gy of ^{12}C radiation (Fig. 3 D-F). The differences in colonic tumorigenesis between male and female mice after ^{56}Fe and 2 Gy ^{12}C radiation was not statistically significant (Fig. 3 D-F).

Calculated RBE values for intestinal and colonic tumorigenesis relative to γ radiation

The RBE for intestine and colon tumorigenesis in male and female mice after different doses of ^{12}C, ^{56}Fe, and ^{28}Si were calculated relative to γ radiation (Table E3; available online at www.redjournal.org). The calculated RBE for intestinal tumorigenesis after 0.1 Gy ^{12}C, ^{56}Fe, and ^{28}Si were 3.7, 5.6, and 8.0 in male mice and 1.6, 2.7, and 3.2 in female mice, respectively (Fig. 4A). For 0.5 Gy, our results showed RBE of 2.1, 3.0, and 5.3 in male mice and 1.4, 2.1, and 2.0 in female mice after ^{12}C, ^{56}Fe, and ^{28}Si radiation, respectively (Fig. 4B). For 2-Gy and equitoxic doses, we calculated intestinal tumorigenesis RBE of 1.5, 2.3, and 4.8 in male mice and 1.6, 2.4, and 3.1 in female mice after ^{12}C, ^{56}Fe, and ^{28}Si radiation, respectively (Fig. 4C). We calculated the RBE of colon tumorigenesis for 0.1 Gy (8, 10, and 13 for ^{12}C, ^{56}Fe, and ^{28}Si, respectively) and 0.5 Gy (8, 10, and 14 for ^{12}C, ^{56}Fe, and ^{28}Si, respectively) doses in male mice (Figs. 4D and 4E). Considering that there was no γ radiation-induced colon tumor after 0.1-Gy and 0.5-Gy doses, the RBE for these doses was not calculated in

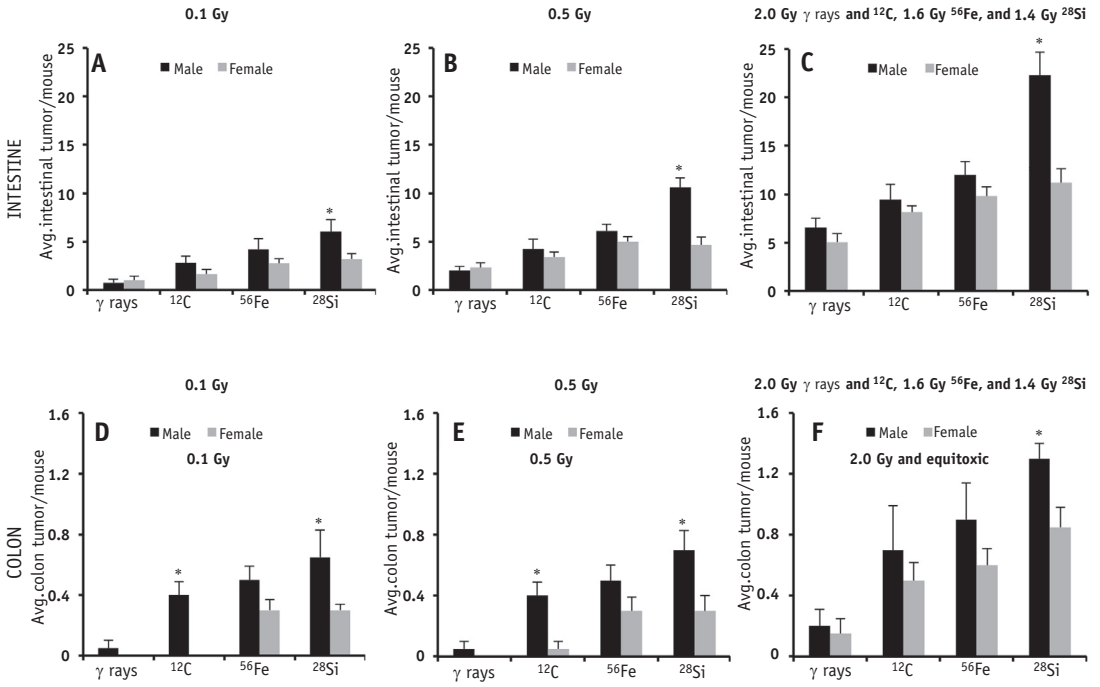

Fig. 3. Sex differences of intestinal and colonic tumorigenesis, presented as average radiogenic tumor incidence per mouse, with spontaneous background subtracted. (A, B, C) Intestinal tumorigenesis in male and female mice after different radiation exposures. Scale in all the doses is kept the same for comparison. (D, E, F) Colonic tumorigenesis in male and female mice after different irradiation. Scale in all the doses is kept same for comparison. Significance ($P<.05$); symbol: *compared with female.

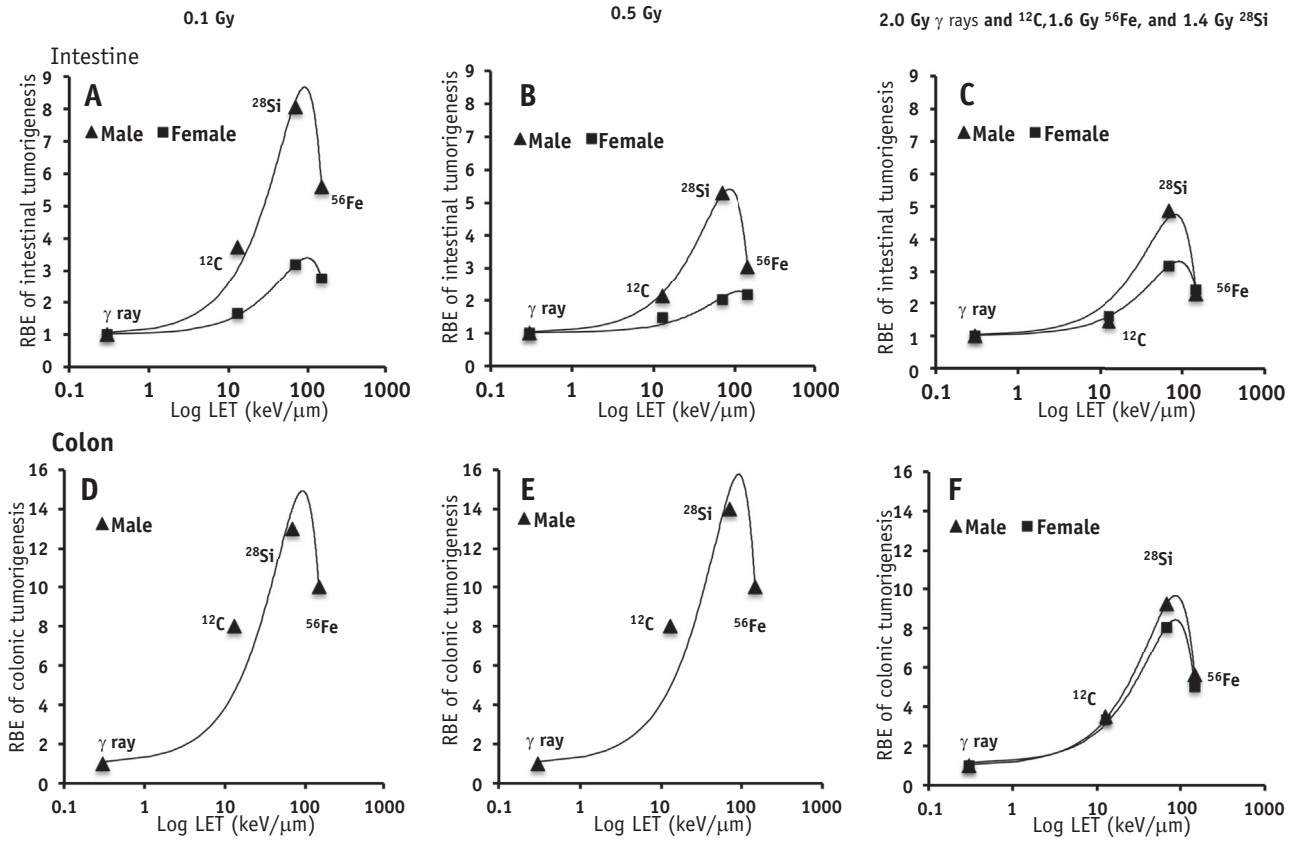

Fig. 4. Relative biological effectiveness (RBE) as a ratio of heavy ion versus γ radiation-induced intestine and colon tumor frequency, presented using smooth fitting curve assuming a single (ion-independent) relationship between RBE and linear energy transfer (LET). (A, B, C) RBE of intestinal tumorigenesis in male and female mice. (D, E, F) RBE of colon tumorigenesis in male and female mice. Considering that there was no colonic tumor in female mice after 0.1 and 0.5 Gy γ radiation, RBE for heavy ions are not shown for these doses.

female mice. For 2-Gy and equitoxic doses, the RBE for colonic tumorigenesis were 3.5, 5.6, and 9.3 for ^{12}C, ^{56}Fe, and ^{28}Si, respectively in male mice and 3.3, 5.0, and 8.1 for ^{12}C, ^{56}Fe, and ^{28}Si, respectively in female mice (Fig. 4F).

Discussion

Because we do not have sufficient human data, an important approach to space travel–associated CRC risk estimation is to determine the RBE of intestinal and colonic tumorigenesis in appropriate animal models for HZE radiation compared with γ radiation. The RBE scaling factor from animal studies can then be used along with the low LET human data on CRC to develop risk prediction models for HZE ions. Whereas our previous studies focused on ^{56}Fe at relatively higher doses (3, 11, 18), the current study was expanded to include 3 HZE ions (^{12}C, ^{28}Si, and ^{56}Fe) and 3 doses spanning a range of energies and LETs to determine the RBE ratio of intestinal and colonic tumor frequency in APC$^{1638N/+}$ mice relative to γ rays.

Studies have shown that radiation-induced tumorigenesis is contingent on several factors, including radiation quality and dose, and high-LET radiation has been reported to induce a higher number of solid tumors relative to low-LET radiation (22-25). In this study, all 3 HZE ions induced higher intestinal and colonic tumorigenesis in mice relative to γ radiation, with ^{28}Si showing the highest response. It is possible that differential tumorigenesis after different irradiation results from varied physical properties such as dose distribution, and energy deposition among these radiation types leading to increased transformation caused by genetic and epigenetic changes in key tumor suppressive/oncogenic pathways (26-32). However, differences in physical properties alone may not fully explain the differential tumorigenesis observed among 3 HZE ions; it is possible that there is involvement of a component of nontargeted effects, which could vary depending on the characteristics of the incident radiation (25, 33, 34).

In both male and female mice, although tumors in excess of control mice after 0.5-Gy and 2.0-Gy dose were more than 0.1 Gy, we observed that tumorigenesis did not increase proportionately relative to radiation dose. For example, the fold increase of normalized intestinal tumor was lower (4.25/2.8 = 1.5-fold and 9.45/2.8 = 3.3-fold) relative to fold increase of radiation dose from 0.1 Gy to 0.5 and 2.0 Gy (0.5/0.1 = 5-fold and 2.0/0.1 = 20-fold) in ^{12}C-irradiated male mice, suggesting a disproportionately lower

increase at higher doses. Indeed, the tumor incidence per unit of ^{12}C radiation (cGy) showed a greater increase after lower doses than after the higher doses (2.8/10 = 0.28 tumors per cGy after 0.1 Gy and 4.25/50 = 0.08 after 0.5 Gy compared with 9.45/200 = 0.05 per cGy after 2 Gy). These results could be interpreted to suggest that saturation effects are at play, which could be due to futile multiple hits of the same cell as well as due to the demise of potential tumorigenic cells after high-dose exposures (35, 36).

The age-adjusted incidence, grade, and mortality of CRC are higher for men than for women, and this is attributed to sex-specific differential exposure to environmental risk factors and endogenous or exogenous protective factors such as estrogen (36-41). Importantly, epidemiologic data from the atom bomb survivor Life Span Study cohort (42) and from an occupational radiation exposure cohort (43) demonstrated that the excess relative risk for CRC is higher in men than in women, and thus male preponderance is also maintained in radiation exposure–associated CRC. Our earlier study in APC$^{1638N/+}$ mice, which was a life-span study, showed higher intestinal tumorigenesis in male mice relative to female mice, specifically after a high dose of γ radiation (44). In the current study, although we observed an increasing trend of tumorigenesis in male mice after γ radiation, it was not statistically significant. This could be due to the lower doses as well as due to the "time-dependent" design (45) of this study, which had a fixed ending time point at 150 days after exposure. Conversely, the frequency of tumorigenesis was significantly higher in male mice relative to female mice after ^{28}Si radiation and to some extent after ^{12}C radiation. Notably, tumorigenesis was not statistically significant between male and female mice exposed to ^{56}Fe, which was consistent with our previous results (11).

Analysis of RBE values supports the notion that particle energy, LET, and RBE are interlinked. When we compared ^{28}Si with ^{56}Fe, we observed higher RBE for ^{28}Si than ^{56}Fe at all doses, which could be due to differences in LET (46, 47). Additionally, a comparison of the results in female APC$^{1638N/+}$ and APC$^{Min/+}$ (3) from our current and previous studies, respectively, after 2-Gy γ rays and equitoxic 1.6-Gy ^{56}Fe showed that the calculated RBE of intestinal tumorigenesis were similar (2.42 in APC$^{1638N/+}$ and 2.80 in APC$^{Min/+}$), suggests consistency of results across 2 models. Our study showing differential RBE after ^{12}C and ^{28}Si at similar energies can be partly explained since LET is linked to the Z-values of the particles, and 2 particles at similar energies have differing LET and thus RBE (15). In contrast, ^{28}Si with a lower Z-value compared to ^{56}Fe showed higher RBE, which may be due to differences in energy, and hence LET, and is consistent with earlier reports (48). Evidence in the literature suggests that RBE shows an upsurge up to a LET of ∼100 keV/μm, and above this the RBE declines (47, 48), which has been attributed to beam and particle characteristics described earlier (35). The RBE of heavy ions is related to the LET, Z-value, and, importantly, to their energy deposition pattern. Overall, our data showed that lower radiation doses have greater RBE relative to higher doses for intestinal and colonic tumorigenesis, and is similar to the reports on heavy ion radiation-induced hepatocellular carcinoma development (25). Developing risk estimates for CRC after energetic heavy ion is a priority for future space missions, and it is, therefore, essential that we determine gastrointestinal tissue–specific biological effects for heavy ions using surrogate endpoints relevant to known human disease processes. Also, our relative comparison of tumorigenesis between γ rays and heavy ion radiation could be further used to model in-depth RBE values at much lower doses for extended and mixed heavy ion radiation field exposures expected during space missions. Finally, our in vivo tumorigenesis data and RBE in a mouse model of human CRC is an important step toward the development of heavy ion exposure–associated CRC risk prediction models and of preventive strategies for humans.

References

1. Preston DL, Shimizu Y, Pierce DA, et al. Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950-1997. 2003. *Radiat Res* 2012;178:AV146-AV172.
2. Henderson TO, Oeffinger KC, Whitton J, et al. Secondary gastrointestinal cancer in childhood cancer survivors: A cohort study. *Ann Intern Med* 2012;156:757-766.
3. Datta K, Suman S, Kallakury BV, Fornace AJJ. Heavy ion radiation exposure triggered higher intestinal tumor frequency and greater beta-catenin activation than gamma radiation in APC(Min/+) mice. *PLoS One* 2013;8:e59295.
4. Curtis SB, Letaw JR. Galactic cosmic rays and cell-hit frequencies outside the magnetosphere. *Adv Space Res* 1989;9:293-298.
5. Setlow RB. The hazards of space travel. *EMBO Rep* 2003;4:1013-1016.
6. Hayatsu K, Hareyama M, Kobayashi S, et al. HZE particle and neutron dosages from cosmic rays on the lunar surface. *J Phys Soc Jpn* 2009;78:149-152.
7. Fodde R, Edelmann W, Yang K, et al. A targeted chain-termination mutation in the mouse Apc gene results in multiple intestinal tumors. *Proc Natl Acad Sci U S A* 1994;91:8969-8973.
8. Suman S, Fornace AJJ, Datta K. Animal models of colorectal cancer in chemoprevention and therapeutics development. In: Ettarh R, editor. Colorectal Cancer–From Prevention to Patient Care. InTech; 2012:277-300.
9. Fodde R, Smits R. Disease model: Familial adenomatous polyposis. *Trends Mol Med* 2001;7:369-373.
10. Rosenberg DW, Giardina C, Tanaka T. Mouse models for the study of colon carcinogenesis. *Carcinogenesis* 2009;30:183-196.
11. Trani D, Nelson SA, Moon BH, et al. High-energy particle-induced tumorigenesis throughout the gastrointestinal tract. *Radiat Res* 2014;181:162-171.
12. Alpen EL, Powers-Risius P, McDonald M. Survival of intestinal crypt cells after exposure to high Z, high-energy charged particles. *Radiat Res* 1980;83:677-687.
13. Aoki M, Furusawa Y, Yamada T. LET dependency of heavy-ion induced apoptosis in V79 cells. *J Radiat Res* 2000;41:163-175.
14. Lett JT. Damage to cellular DNA from particulate radiations, the efficacy of its processing and the radiosensitivity of mammalian cells. Emphasis on DNA double strand breaks and chromatin breaks. *Radiat Environ Biophys* 1992;31:257-277.
15. Rodriguez A, Alpen EL, Powers-Risius P. The RBE-LET relationship for rodent intestinal crypt cell survival, testes weight loss, and

multicellular spheroid cell survival after heavy-ion irradiation. *Radiat Res* 1992;132:184-192.
16. Datta K, Suman S, Trani D, et al. Accelerated hematopoietic toxicity by high energy (56)Fe radiation. *Int J Radiat Biol* 2012;88:213-222.
17. Suman S, Datta K, Trani D, Laiakis EC, Strawn SJ, Fornace AJJ. Relative biological effectiveness of (12)C and (28)Si radiation in C57BL/6J mice. *Radiat Environ Biophys* 2012;51:303-309.
18. Trani D, Datta K, Doiron K, Kallakury B, Fornace AJJ. Enhanced intestinal tumor multiplicity and grade in vivo after HZE exposure: Mouse models for space radiation risk estimates. *Radiat Environ Biophys* 2010;49:389-396.
19. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965;52:591-611.
20. Nordstokke DW, Zumbo BD. A new nonparametric Levene test for equal variances. *Psicologica* 2010;31:401-430.
21. McDonald JH. Handbook of Biological Statistics. 3rd ed. Baltimore: Sparky House Publishing; 2014.
22. Alpen EL, Powers-Risius P, Curtis SB, et al. Tumorigenic potential of high-Z, high-LET charged-particle radiations. *Radiat Res* 1993;136:382-391.
23. Ullrich RL, Jernigan MC, Cosgrove GE, et al. The influence of dose and dose rate on the incidence of neoplastic disease in RFM mice after neutron irradiation. *Radiat Res* 1976;68:115-131.
24. 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.
25. Weil MM, Ray FA, Genik PC, et al. Effects of 28Si ions, 56Fe ions, and protons on the induction of murine acute myeloid leukemia and hepatocellular carcinoma. *PLoS One* 2014;9:e104819.
26. Kronenberg A. Mutation induction in human lymphoid cells by energetic heavy ions. *Adv Space Res* 1994;14:339-346.
27. Kronenberg A. Radiation-induced genomic instability. *Int J Radiat Biol* 1994;66:603-609.
28. Kronenberg A, Gauny S, Criddle K, et al. Heavy ion mutagenesis: Linear energy transfer effects and genetic linkage. *Radiat Environ Biophys* 1995;34:73-78.
29. Ding LH, Shingyoji M, Chen F, et al. Gene expression changes in normal human skin fibroblasts induced by HZE-particle radiation. *Radiat Res* 2005;164:523-526.
30. Ding LH, Shingyoji M, Chen F, et al. Gene expression profiles of normal human fibroblasts after exposure to ionizing radiation: A comparative study of low and high doses. *Radiat Res* 2005;164:17-26.
31. Templin T, Amundson SA, Brenner DJ, et al. Whole mouse blood microRNA as biomarkers for exposure to gamma-rays and (56)Fe ion. *Int J Radiat Biol* 2011;87:653-662.
32. Asaithamby A, Uematsu N, Chatterjee A, et al. Repair of HZE-particle-induced DNA double-strand breaks in normal human fibroblasts. *Radiat Res* 2008;169:437-446.
33. Buonanno M, de Toledo SM, Pain D, et al. Long-term consequences of radiation-induced bystander effects depend on radiation quality and dose and correlate with oxidative stress. *Radiat Res* 2011;175:405-415.
34. Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation? *Oncogene* 2003;22:7094-7099.
35. Alpen EL, Powers-Risius P, Curtis SB, et al. Fluence-based relative biological effectiveness for charged particle carcinogenesis in mouse Harderian gland. *Adv Space Res* 1994;14:573-581.
36. Koo JH, Leong RW. Sex differences in epidemiological, clinical and pathological characteristics of colorectal cancer. *J Gastroenterol Hepatol* 2010;25:33-42.
37. Murphy G, Devesa SS, Cross AJ, et al. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. *Int J Cancer* 2011;128:1668-1675.
38. Majek O, Gondos A, Jansen L, et al. Sex differences in colorectal cancer survival: Population-based analysis of 164,996 colorectal cancer patients in Germany. *PLoS One* 2013;8:e68077.
39. McCashland TM, Brand R, Lyden E, et al. Gender differences in colorectal polyps and tumors. *Am J Gastroenterol* 2001;96:882-886.
40. Rim SH, Seeff L, Ahmed F, et al. Colorectal cancer incidence in the United States, 1999-2004: An updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009;115:1967-1976.
41. Ali RH, Marafie MJ, Bitar MS, et al. Gender-associated genomic differences in colorectal cancer: Clinical insight from feminization of male cancer cells. *Int J Mol Sci* 2014;15:17344-17365.
42. Brenner DJ, Suit HD. Radiation-induced oncogenesis at low and high doses. In: Shrieve DC, Loeffler JS, editors. Human Radiation Injury. Philadelphia: Lippincott Williams & Wilkins; 2011:79-88.
43. Sont WN, Zielinski JM, Ashmore JP, et al. First analysis of cancer incidence and occupational radiation exposure based on the National Dose Registry of Canada. *Am J Epidemiol* 2001;153:309-318.
44. Trani D, Moon BH, Kallakury B, Hartmann DP, Datta K, Fornace AJJ. Sex-dependent differences in intestinal tumorigenesis induced in Apc1638N/+ mice by exposure to gamma rays. *Int J Radiat Oncol Biol Phys* 2013;85:223-229.
45. Bushong SC. Radiologic Science for Technologists Physics, Biology, and Protection. 10th ed. St. Louise: Mosby/Elsevier; 2013.
46. Alpen EL, Powers-Risius P. The relative biological effect of high-Z, high-LET charged particles for spermatogonial killing. *Radiat Res* 1981;88:132-143.
47. Ando K, Koike S, Ohmachi Y, et al. Tumor induction in mice after local irradiation with single doses of either carbon-ion beams or gamma rays. *Int J Radiat Biol* 2014;90:1119-1124.
48. Tsuruoka C, Suzuki M, Kanai T, et al. LET and ion species dependence for cell killing in normal human skin fibroblasts. *Radiat Res* 2005;163:494-500.