Intestinal Tumorigenesis in Min Mice is Enhanced by X-irradiation in an Age-dependent Manner

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Age dependency/X-irradiation/Intestinal tumorigenesis/Min mouse.

We examined the effect of X-irradiation on intestinal tumorigenesis in Min (multiple intestinal neoplasia) mice. Single whole-body irradiation was given to mice of various ages from newborn to young adults. On the C57BL/6J (B6) background, X-irradiation increased tumor multiplicity of the small intestine exposed at ages from 2–3 days to 24–25 days, with a peak of 2.7-fold increase at 10–12 days of age; exposure at later ages resulted in only a slight increase. X-irradiation also increased colonic tumors; however, the susceptible age period appeared earlier than that of the small intestine; the peak value of 4.6-fold increase was observed in the exposure at around 2–3 days of age. Irradiation at 24 days or later ages showed almost no effect on the colonic tumor induction. On the (B6 x MSM)F1 background, X-irradiation resulted in 2.7-fold increase in the small intestinal tumors, but no increase in the colonic tumors, and besides, the age dependency observed in the small intestinal tumors was much attenuated. Collectively, we conclude that tumorigenic efficacy of X-irradiation in Min mice was determined by the combination of the target organ, the age at exposure, and the genetic background.

INTRODUCTION

The Min mouse is a murine model of human cancer syndrome familial adenomatous polyposis (FAP).1–3) As in human FAP, the mutant mouse is heterozygous for a germ-line nonsense mutation of the Apc gene at codon 850, which produces truncated nonfunctional APC protein.4) Upon inactivation of the remaining normal allele, the mouse develops multiple small intestinal adenomas and sporadic colon tumors. Tumorigenesis in Min mice has been reported to be affected by a diversity of genetic and environmental factors, which include the mouse strain or genetic background,5) mutation/polymorphism of a specific gene,6–9) foods,10,11) drugs,12–14) and many kinds of environmental mutagens.15–20) Min mice are highly susceptible to cytotoxic/genotoxic agents. Shoemaker et al. have shown that N-ethyl-N-nitrosourea (ENU) increased the tumor incidence in the intestine and in the mammary gland even at a small dose that would not induce tumors in normal mice.16) Similarly, Alexander and his coworkers have demonstrated that exposure to the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) increased the tumor burden in the small intestine and the colon directly17,21) or via milk.18) A colon mutagen azoxymethane (AOM) has been reported to enhance tumorigenicity in the colon, but not in the small intestine.19) Besides these chemical mutagens, ionizing radiation has been reported to increase intestinal tumorigenesis in Min mice20) and in addition, intestinal and extraintestinal tumorigenesis in Apc1638N mice, a mouse model of attenuated FAP.22)

One noticeable point commonly seen in the studies with chemical mutagens is that neonatal animals are much more susceptible to tumor induction, indicative of a possible involvement of age-dependent mechanism in the tumorigenic response to exogenous stimuli. To define the phenomenon and reveal the underlying mechanism, however, chemical mutagens have several disadvantages closely related to their tumorigenic process. Some chemical mutagens including PhIP require enzymatic activation to ultimate mutagens.17) It is probable that activity of the metabolizing enzymes might vary among tissues in an animal and also with developmental stages or animal ages in the same tissue. This makes it difficult to determine the actual mutagenic dose in individual tissues involved. In addition, all chemical mutagens should be decomposed or excreted from the body to cease their action. This process will require some duration time, and also involve a decline of the concentration within the animals, either rapid or gradual, until a zero level is attained. Thus, the way of action of the chemical mutagens compromises not only the time points at the treatment but also the actual doses that worked in the treatment. In contrast to the

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chemical mutagens, ionizing radiation, a well known genotoxic/cytotoxic agent that increases the cancer risk both in humans and in mice, is independent of these problems. The insult is given relatively in uniform to every tissues/organs in a body placed in the irradiation field, and stops instantly when animals/organs are removed from the field. Doses and time points of the exposure can be controlled easily and precisely. Thus, ionizing radiation serves as an ideal cytotoxic agent for the analysis of age-related phenomenon, and also for the comparison of the response between different organs/tissues.

Intestinal tumorigenesis in Min mice is strongly affected by the genetic background. Min mutation is fully penetrant on the B6 background, and usually causes 50–100 small intestinal polyps and 0–5 colonic polyps. By contrast, several inbred strains such as AKR/J, MA/MyJ, and CAST/EiJ have been shown to confer resistance to intestinal tumorigenesis induced by Min mutation.\(^5\) Quantitative trait loci (QTL) analysis of these crosses revealed a modifier locus designated Mom1 (modifier of min 1) that maps to the distal mouse chromosome 4.\(^23,24\) Later, an intragenic polymorphism of secretory phospholipase A2 (pla2s) has been proved to partly account for the Mom1 phenotype.\(^25\) Mom1 locus is semidominant for Min-induced intestinal tumorigenesis, one copy of the resistant Mom1 allele reducing the tumor multiplicity approximately to the half.\(^26\) Some strains including AKR/J and CAST/EiJ are highly resistant to Min-induced tumorigenesis that could not be explained only by the Mom1 locus, implicating a contribution of other modifier loci.\(^24,27,28\) It should be noted that the highly resistant genetic background of AKR/J strain not only reduces intestinal tumor multiplicity, but also appears to affect the molecular process leading to the inactivation of Apc, an obligatory step in tumorigenesis in Min mice.\(^27\)

We have found that MSM/Ms strain, an inbred strain derived from Japanese wild mouse Mus musculus molossinus, is highly resistant to Min-induced intestinal tumorigenesis. Indeed, this is one of the most resistant strain that have been reported so far. QTL analysis has revealed several new modifier loci that confer the resistance to the strain (Okamoto et al., manuscript in preparation). In the present study, we examined systematically the effect of X-irradiation on intestinal tumorigenesis in Min mice on the two different genetic backgrounds, sensitive B6 and highly resistant F\(_1\) backgrounds.

**MATERIALS AND METHODS**

**Mice**

B6-Min mice were originally purchased from The Jackson laboratory (Bar Harbor, ME) and propagated at our laboratory. B6 mice were purchased from Clea Japan (Tokyo, Japan). MSM/Ms mice were kindly provided by Dr. T. Shirouhi at National Institute of Genetics (Mishima, Japan). F\(_1\)-Min mice were generated by crossing female B6-Min mice with male MSM/Ms mice. Mice were fed laboratory rodent diet and water ad libitum. All animal experiments were conducted in accordance with the rules and regulations of the Institutional Animal Care and Use Committee.

**Genotyping of Min mutation**

A small piece of an ear was incubated in 100–200 µl of 1 x PCR buffer containing 1.5 mM MgCl\(_2\), 0.45% NP-40, 0.45% Tween 20, and 1 mg/ml of proteinase K (Wako, Japan) at 55°C overnight, and then heated at 95°C for 30–60 min to inactivate proteinase K prior to the use as template DNA. To discriminate the Min allele from the wildtype allele, we carried out PCR-SSCP analysis, using a pair of primers designed to span the Min mutation site. Forward primer (5'-GTATTGCCCAGCTCTTTCTC-3') and reverse primer (5'-ATCTGACAGCTCGTTTGTA-3') were end-labeled with [\(\gamma\)-\(^32\)P]ATP using T4 polynucleotide kinase (New England Biolabs). PCR condition was as follows: 94°C for 5 min, 30 cycles of (94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min), and 72°C for 5 min. PCR products were diluted at 1:10 in 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. After denaturation at 80°C for 5 min, aliquots were applied to a 6% non-denaturing polyacrylamide gel containing 5% glycderin, and electrophoresed in 0.5 x TBE at constant power for 4–5 h.

**X-irradiation**

Single whole-body irradiation of 0.25, 0.5, 1, or 2 Gy was given to mice at 2–3 days (day 2), 10–12 days (day 10), 24–25 days (day 24), 42–44 days (day 42), or 48–49 days (day 48) after birth, using Hitachi X ray apparatus (150kV, 4.5 mA) with a 0.5 mm Cu + 0.5 mm Al filter at a dose rate of 0.27 Gy/min. Min mice and their normal littermates were simultaneously treated and maintained until sacrifice.

**Tumor scoring**

B6-Min and F\(_1\)-Min mice were terminated by cervical dislocation under anesthesia at 16–20 weeks of age and at 35–40 weeks of age, respectively. The colon and the small intestine were removed from the mouse, slit open longitudinally, and rinsed with PBS. The small intestine was divided into three equal segments. The colon and the segments were placed flat on a transparent plastic sheet printed with fine scales in grid, and inspected for tumors under a dissecting microscope.

**Statistical analysis**

The data were firstly tested for significance by Kruskal-Wallis test, and then subjected to the pairwise comparison by two-sided t test and Mann-Whitney’s U test, using a computer software StatView (v5.0, Hulinks). The two test gave essentially the same results. A P-value of <0.05 was considered as significant.
RESULTS

Tumorigenesis on B6 background

We examined the effect of X-irradiation on the tumorigenesis in the small intestine and the colon, two major target organs in spontaneous tumorigenesis in Min mice, on sensitive B6 genetic background. Mice were irradiated at 10–12 days of age with doses ranging from 0.25 to 2 Gy, and scored for intestinal tumors at 16–20 weeks of age. In the small intestine, tumor numbers increased relatively proportionally with increasing doses from 0.25 to 2 Gy (Fig. 1A). As compared with the tumor number of $77.6 \pm 4.8$ (means ± SEM) in unirradiated B6-Min mice, exposure of mice to 1 Gy and 2 Gy of X-rays increased the tumor numbers by a factor of 1.9 and 2.7, respectively. For the colonic tumors, X-irradiation also increased the tumor number in a dose-dependent manner; however, the pattern was not the same with that for the small intestinal tumors (Fig. 1B). Unlike the small intestine, no significant change was found in the tumorigenesis of the colon at around the dose range of 0.25-1 Gy, while a rather rapid increase occurred above the dose level. Exposures to 1 Gy and 2 Gy X-rays increased the colonic tumor number by a factor of 1.8 and 4.8 over the unirradiated mice ($3.5 \pm 0.6$; means ± SEM), respectively. Besides the increase in tumor numbers, there seems to be a tendency that tumors developed in the irradiated animals are slightly larger than those in the unirradiated animals. Tumor distribution patterns along the intestinal tracts of the irradiated mice were very similar to those in the unirradiated mice. There was no tumor induction either in the small intestine or the colon of normal littermates regardless of the X-ray doses.

![Fig. 1. Effect of X-irradiation on tumor multiplicities in the small intestine (A) and the colon (B) of B6-Min mice. Single whole-body irradiation was given to the mice at 10–12 days of age. The average number of tumors for each experimental group is shown as a bar with SEM as a thin error bar. The figure within the bar is the number of mice analyzed for the experimental point. * $P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$; NS, not significant.](image)

![Fig. 2. Relationship between radiation tumorigenesis and age at exposure in B6-Min mice. A., small intestine; B., colon. Mice were given a single X-irradiation of either 1 Gy (light bar) or 2 Gy (dark bar) at various ages. The average number of tumors in the small intestine and the colon is shown as a bar with SEM as a thin error bar. The figure within the bar is the number of mice analyzed for the experimental point. The dashed line represents the average tumor multiplicity of the unirradiated animals. Statistical significance is tested by the comparison of tumor multiplicity of the irradiated group over that of the unirradiated group. * $P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$.](image)
We attempted to determine whether the efficacy of X-irradiation in tumor induction might vary depending on the animal age at exposure. B6-Min mice were exposed to a whole-body irradiation of 1 Gy or 2 Gy at various ages after birth, and examined for tumors at 16–20 weeks of age. As clearly shown in Fig. 2, tumor induction following X-irradiation was dependent on the animal age at exposure, and moreover, the age-effect relationship was different between the small intestine and the colon. The results of the statistical analysis relative to the unirradiated mice are depicted as *(P < 0.05), **(P < 0.01), and ****(P < 0.001) in Fig. 2.

For the small intestine, X-irradiation was effective in tumor induction when given to mice at ages of 2–3 days to 24–25 days, with a peak at 10–12 days of age (Fig 2A). Exposure of mice at 6 weeks or later resulted in much less tumor induction. Although the extent of tumor induction varied depending on the X-ray dose, basic pattern of the age-effect relationship was very similar between the two doses (Fig. 2A). The age-effect relationship for the tumor induction in the colon was somewhat different from that in the small intestine (Fig. 2B); exposure at 24–25 days of age did not induce excess tumors in the colon. Statistical analysis showed that 2 Gy irradiation at 24–25 days or later ages resulted in a significant reduction in colonic tumor number relative to the unirradiated animals; however, this may be incidental, since the probability values were only marginal (P = 0.044 – 0.047).

To clarify the age-effect relationship for the two target organs, we made pairwise comparison of the mean tumor number for every possible combination of the mouse groups irradiated at different ages. The results are summarized in Fig. 3, confirming the remarks in Fig. 2. Collectively, the small intestine and the colon of Min mice are highly susceptible to X-ray-induced tumorigenesis at specific periods of their early postnatal days, and in addition, it seems likely that the susceptible period for the colon is earlier than that for the small intestine.

**Tumorigenesis on F1 background**

When crossed with B6-Min mice, the genetic background of MSM strain reduced the intestinal tumor multiplicity by two orders of magnitude relative to the parental B6-Min mice. Within the ordinary observation period of 20 weeks, the majority of F1-Min mice did not develop visible intestinal tumors. To examine whether the highly resistant genetic background might exert any effect to radiation tumorigene-

![Small intestine (1 Gy)](image1)

![Small intestine (2 Gy)](image2)

![Colon (1 Gy)](image3)

![Colon (2 Gy)](image4)

**Fig. 3.** Statistical analysis of age-effect relationship in radiation tumorigenesis in the small intestine and the colon of B6-Min mice. Pairwise comparison of the tumor multiplicity was made using two-sided t test and Mann-Whitney’s U test in every possible combination of the groups irradiated at different ages for each dose and organ. The two tests gave statistically almost the same results except for the case of the small intestine (2 Gy), where day 2 vs. day 10 was not significant in t test, while significant in U test. In several cases, t test was more sensitive than U test, although either test gave statistically significant difference. Probability of the difference in tumor multiplicity estimated with t test is shown for each pair as a figure by the side of a line or an arrow. An arrow represents the pair of significant difference, the thickness corresponding to the significant level of P < 0.05, 0.01, and 0.001, respectively. # represents P < 0.0001.
sis and age-effect relationship, we generated F1-Min mice and carried out irradiation experiments. Tumor scoring was made at 35–40 weeks of age, instead of 16–20 weeks of age as was in B6-Min mice, since tumor growth in F1-Min mice was much slower than that in B6-Min mice.

X-irradiation given to the F1-Min mice at 10–12 days of age showed a dose-dependent increase in tumor multiplicity in the small intestine (Fig. 4). The dose dependency was seen independently of the size threshold for tumor scoring. Tumors ≥0.2 mm are regarded to include almost all dysplas-

Fig. 4. Effect of X-irradiation on small intestinal tumorigenesis in (B6 x MSM)F1-Min mice. Mice were exposed to a single whole body irradiation at 10–12 days of age. The average tumor multiplicity is shown as an open bar (≥0.2 mm in diameter) and an dark bar (≥1.0 mm in diameter), each with SEM as a thin error bar. The figure within the bar is the number of mice analyzed for the experimental group. ** and *** indicate significant increase in tumor multiplicities in irradiated mice compared with the unirradiated mice at $P < 0.01$ and $P < 0.001$, respectively.

Fig. 5. Relationship between small intestinal tumor multiplicity and age at exposure in (B6 x MSM)F1-Min mice. The average tumor multiplicity is shown as an open bar (≥0.2 mm in diameter) and an dark bar (≥1.0 mm in diameter) with SEM as a thin error bar. The figure within the bar is the number of mice analyzed for the experimental group. The solid line and the dashed line represent the average tumor number in the unirradiated mice, as calculated for tumors ≥1.0 mm in diameter and ≥0.2 mm in diameter, respectively.

Table 1. Probability test of the efficacy of X-ray dose and age at exposure in intestinal tumorigenesis of Min mice under the two different genetic backgrounds

| Genetic background | Factor    | Small intestine | Colon       |
|--------------------|-----------|-----------------|-------------|
| B6-Min             | X-ray dose| $P < 0.0001$    | $P < 0.0001$|
|                    | Age effect|                 |             |
|                    | 1 Gy      | $P < 0.0001$    | $P = 0.026$ |
|                    | 2 Gy      | $P < 0.0001$    | $P < 0.0001$|
| F1-Min             | X-ray dose|                 |             |
|                    | ≥1.0mm    | $P = 0.0004$    | $P = 0.98$  |
|                    | ≥0.2mm    | $P < 0.0001$    | $P = 0.99$  |
|                    | Age effect|                 |             |
|                    | ≥1.0mm    | $P = 0.0009$    | $P = 0.50$  |
|                    | ≥0.2mm    | $P < 0.0001$    | $P = 0.90$  |
|                    | Dose and age effect|     |             |
|                    | ≥1.0mm    | $P = 0.0008$    | $P = 0.54$  |
|                    | ≥0.2mm    | $P < 0.0001$    | $P = 0.82$  |

Probability of the contribution of each factor to radiation-induced tumorigenesis in the small intestine and the colon was determined by Kruskal-Wallis test for the data sets as specified in the table.
tic lesions visible under a dissecting microscope, while tumors ≥ 1.0 mm are the ordinary measure for tumor scoring and also used for B6-Min mice. Exposure to 0.5 Gy irradiation increased tumor multiplicity by a factor of 2, and that to 1-2 Gy, by a factor of 2.7-3.1. These values are comparable to those observed for the small intestinal tumors in B6-Min mice. In contrast to the small intestine, there was no such excess induction of colonic tumors following X-irradiation; unirradiated F1-Min mice developed 1.8 colon tumors in average, while those exposed to 0.5 to 2 Gy irradiation developed 1.0-1.8 colon tumors.

Tumor numbers in the small intestine varied depending on the age at exposure, as was seen in B6-Min mice, but the age dependency was much attenuated (Fig. 5). The highest tumor induction was observed at 10–12 day irradiation, and afterwards tumor multiplicities showed a gradual decline with increasing ages at exposure, although the decrease was only small. When tumor numbers were compared in the measure of tumors ≥ 1.0 mm, irradiation at 2–3 days of age and at 10–12 days of age, but not at 42–44 days of age, showed significant induction in small intestinal tumors relative to the unirradiated mice (P < 0.001). When compared in the measure of tumors ≥ 0.2 mm, however, all irradiated animals showed significantly higher tumor numbers than the unirradiated animals (P < 0.01 for 42–44 day group and P < 0.001 for the other groups). There was no substantial increase by X-irradiation in the tumor number of the colon of F1-Min mice exposed at the various ages. Kruskal-Wallis test also indicated that tumorigenesis in the colon of F1-Min mice was not influenced by X-irradiation (Table 1).

DISCUSSION

The data presented here demonstrate that X-irradiation enhances tumor induction in the small intestine and the colon of Min mice in dose dependent manners when given at specific early postnatal ages. Exposure at later ages was ineffective to tumor induction in either organ. Very similar age dependency has been reported by several workers, using ENU,16) PhIP,17) and AOM.19) Shoemaker et al. have demonstrated that ENU treatment of Min mice at 5–14 days of age resulted in 3.8-fold increase in intestinal tumorigenesis.16) Treatment at later ages resulted in a gradual loss of the enhancement. We also showed here that the highest tumor induction in the small intestine occurred in mice irradiated at 10–12 days of age, and at later ages the tumor induction gradually decreased, reaching to the level of unirradiated animals by 6–7 weeks of age. Interestingly, both of the X-ray exposure and the ENU treatment showed that the animal age of 2 weeks was more susceptible to intestinal tumorigenesis than the age before 1 week, although the difference was not significant. They assessed the ENU tumorigenicity by the sum of the tumor number in the specified region of the small intestine (approximately one third of the entire small intestine) and that in the entire colon, whereas we scored for tumors along the entire small intestine and the colon separately. Nevertheless, age-effect relationship in the small intestine with X-irradiation accords very well with that from the ENU treatments. This is probably because Min mice develop much more tumors in the small intestine than in the colon, thereby possible contribution of the colon tumors to the total intestinal tumor number being relatively small. Steffensen et al. have examined for the susceptible period of a food mutagen PhIP in tumor induction in the small intestine and the colon of Min mice by exposing mice prenatally and postnataally. They found that Min mice were susceptible to intestinal tumorigenesis by PhIP when treated before day 12 after birth, while the susceptibility had been lost by day 36.17) Similarly, colon-specific carcinogen AOM induced higher number of dysplastic lesions in the colon when treated at 1–2 weeks of age than at 4–5 weeks of age, although AOM treatment at 4–5 weeks of age still resulted in 10-fold higher lesions than untreated animals.19)

All these data suggest that the age-related difference in the susceptibility to exogenous mutagens is a common feature of tumorigenesis in Min mice. In addition, similar age-related difference has been found in the intestine of normal mice treated with ENU, although the tumor incidence was very low compared with that of Min mice.16) This raises a possibility that early postnatal or perinatal period of mouse life may be critical in intestinal tumor development, and because of the germline mutation in one copy of the Apc gene, tumorigenic lesions induced by exogenous mutagens could be readily detected by monitoring the intestinal tract of Min mice. Thus, the mice would serve as a good model not only in the detection and identification of the molecular events leading to the tumor induction, but also in the study of various aspects of intestinal tumorigenesis including age-dependency in the susceptibility to exogenous mutagens.

At present, little is known about the mechanism that leads to the high tumor induction only in the exposure at a specific, early postnatal life. A variety of possible mechanisms have so far been proposed, which include differential activity of metabolizing and/or detoxifying enzymes, difference in DNA repair systems, changes in immunological status and intestinal microflora, hormonal influences, and developmental changes in the proliferation/differentiation status of the crypt components. Due to the lack of experimental data on the early postnatal life, we can not yet support or preclude any of these possibilities. Steffensen et al. found that higher susceptibility of 12-day mice relative to 36-day mice in the PhIP treatment partially parallels the higher PhIP-DNA adduct level, indicative of a possible involvement of metabolizing/detoxifying enzymes in the age-related difference in tumor induction by PhIP.17) However, they also found several inconsistency between tumor numbers and PhIP-DNA adduct levels. In contrast to the chemical mutagens, X-irradiation exerts its effect directly to the target molecule/cells
without any enzymatic intervention, but nevertheless, we have found almost the same age-effect relationship in X-irradiation with that seen in the PhIP and ENU experiments. This suggests the involvement of a mechanism/process other than the enzymatic metabolism, which should be active specifically in the perinatal and/or postnatal ages and also operative under the various mutagenic stimuli.

The development of the intestinal tract organs begins in the mid-gestation period, and by the time of birth the basic structure of intestinal mucosa including crypt/villus architecture is generally established. Histogenesis of the organs, however, continues after birth, until a steady state of the adult intestines is attained. The number of crypts in the small and large intestines rapidly increases with increasing age over the first 6 weeks of life. The number of cells in the individual crypts also increases drastically during the early postnatal life; intestinal crypt population for the newborn mouse is less than one fifth of the adult mouse. Cell kinetic studies have revealed that intestinal mucosa of the mouse undergo significant kinetic changes during the early postnatal life. Mitotic index for the small intestine shows an unique, typical up and down at these days; it remains low for the first 48 hrs after birth, and then rises to a high level which is maintained for 2 weeks, followed by a sudden fall and subsequently an abrupt rise again, reaching an adult value.

During the early postnatal days of the relatively high mitotic indices, intestinal crypt stem cells undergo symmetrical cell division and reproduce themselves to increase the crypt number and crypt population. By contrast, crypt stem cells in the adult intestine undergo asymmetrical cell division to maintain a steady state of tissue homeostasis. Potten et al. have demonstrated that stem cells in the adult small intestine are well protected against genetic defects by means of the selective segregation of template DNA, where the old or template strands are transferred into the lineage-ancestral stem cells, and newly-synthesized strands, into the daughter cells. As for the colon, there is no available data showing that the same molecular mechanism is also working in the crypt stem cells, although the mechanism seems to be tightly linked to the asymmetric cell division. Considering the apparent correspondence in the timing of symmetric cell division and high susceptibility in the preweaning ages, together with the protection mechanism linked to the asymmetric cell division, we are tempted to speculate that the developmental change in stem cell status or proliferation characteristics might have any causal relationship with the age dependency in intestinal tumor induction. Interestingly, DNA methyltransferase inhibitor 5-aza-deoxycytidine has been shown to markedly reduce the intestinal tumor multiplicity of Min mice when given during the early postnatal ages, while the treatment after 50 days of age had lost the tumor suppressive effect.

Increased tumor induction following X-irradiation at early postnatal days was also seen in the small intestine of F1-Min mice, although the age dependency was not so evident as was seen in B6-Min mice. This may be related to the difference in spontaneous tumorigenesis between the two mouse strains. Tumor induction in the small intestine of B6-Min mice reaches a plateau by 12–13 weeks of age, and afterwards new tumors rarely develop, while tumorigenesis in the small intestine of F1-Min mice proceeds much more slowly. The majority of tumors in F1-Min mice are still less than 1 mm in diameter even at 20 weeks of age, and moreover, new tumors often develop over 30 weeks of age (unpublished data). Considering the possible relationship between intestinal histogenesis and age-related susceptibility to X-rays, we speculate that the prolonged pattern in spontaneous tumorigenesis may have some relevance to the slower loss of the enhancement in radiation-induced tumorigenesis in the small intestine of F1-Min mice.

X-irradiation did not induce excess tumors in the colon of F1-Min mice, which is in contrast to the small intestine of F1-Min mice, and also to the colon and the small intestine of B6-Min mice. This indicates that radiation tumorigenesis and its age effect are dependent on the target organ and also on the genetic background. At present, little is known on the molecular mechanism that makes the colon of F1-Min mice resistant to radiation tumorigenesis. However, it is possible that the genetic background MSM strain might be involved in the phenomenon. We have found that this mouse strain possesses several modifier loci that affect spontaneous tumorigenesis in the intestines in an organ-specific manner (Okamoto et al., manuscript in preparation). Thus, it seems likely that radiation tumorigenesis is also affected by organ-specific modifier loci. MSM strain is genetically remote from B6 strain, and thus highly polymorphic in SSLP markers and possibly in a variety of genes involved in the histogenesis of the intestines, cell growth characteristics, and cellular responses to radiation. Some of these polymorphisms may lead to the suppression of tumor induction in the colon of irradiated F1-Min mice.

In the present work we have found several differences in radiation tumorigenesis between the small intestine and the colon. On the sensitive B6 background, small intestinal tumors were induced by X-irradiation even at a small dose of 0.25 Gy, and increased relatively proportionally with increasing doses. By contrast, colonic tumor induction did not occur at such small doses, showing a rapid induction at higher doses. In addition, the susceptible period for the colonic tumor induction appeared earlier than that for the small intestine. Similar observation has been reported in the PhIP treatment, where the susceptible period for the small intestine was from day 3 to day 12 after birth, while that for the colon was from day 3 before birth to day 3 after birth. More profound difference was seen in F1-Min mice, where X-irradiation induced tumors in the small intestine in a dose-dependant manner, but did not induce excess tumors in the
colon. The small intestine and the colon are of the same developmental origin and under the control of WNT signaling pathway,\(^41,42\) and also have similar architecture or crypt as a proliferation unit. However, they differ in many aspects including cell proliferation kinetics\(^29\) and responses to exogenous stimuli.\(^33,43,44\) Crypt stem cells in the small intestine are extremely radiosensitive and undergo apoptosis without any sign of DNA repair, while those in the colon are relatively resistant to radiation-induced apoptosis.\(^34,43,44\) The resistance has now been ascribed to the expression of\(^{bcl-2}\) gene in the crypt stem cells of the colon.\(^45\) In addition, induction of\(^{p21}\) after irradiation has been shown to be much higher and prolonged in the colon than in the small intestine.\(^46\)

In summary, we have found that the animal age at exposure is an important determinant in the efficacy of X-irradiation on the intestinal tumorigenesis of Min mice, and besides, the tumorigenic response also depends on the target organ and the genetic background. There have been little data on the cellular responses to radiation in developing animals, since the almost all works so far have used adult animals. Cellular response to radiation, either cell death or tumorigenesis, is supposed to be influenced by cellular context such as proliferation status, tissue type, and genetic background. Further analyses in\(^{such}\) approaches would provide insights into the mechanism underlying the age dependency in radiation tumorigenesis, and also would help to address the mechanism involved in the radiation tumorigenesis.

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