Intestinal and extra-intestinal tumor multiplicities in the \textit{Apc1638N} mouse model after exposure to X-rays

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Seven-week-old \textit{Apc1638N} mice were exposed to a single dose of 5 Gy total-body X-irradiation resulting in a 8-fold increase in the number of intestinal tumors and a reduction of the lifespan to an average of 6 months. The distribution of tumors along the intestinal tract as well as the adenoma/carcinoma ratio, were similar between non-irradiated and irradiated animals. Semi-quantitative PCR analysis of intestinal-tumor DNA revealed that 10 out of 14 tumors had lost the wild-type \textit{Apc} allele. However, in contrast to spontaneous \textit{Apc1638N} intestinal tumors in which the LOH event at the \textit{Apc} locus involves the entire chromosome 18 (1), in 6 out of 10 tumors derived from X-irradiated animals the \textit{Apc} loss is associated with only a partial intrachromosomal deletion. The remaining tumors have lost all chromosome 18 markers tested. In addition to the intestinal tumors, female \textit{Apc1638N} mice are susceptible to the development of mammary tumors. Upon X-irradiation, \textit{Apc1638N} mice show a striking 15-fold increase in mammary tumors. Moreover, \textit{Apc1638N} mice spontaneously develop other extra-intestinal neoplasia, such as desmoid-like lesions similar to those associated with familial adenomatous polyposis (FAP), the human syndrome caused by germline mutations in the \textit{APC} gene. Spontaneous desmoid growth is sex-dependent, as male \textit{Apc1638N} mice develop 3-fold more desmoids than female mice. Interestingly, X-irradiation seemed to increase the number of desmoids per animal nearly twofold only in female \textit{Apc1638N} mice. Five out of 9 desmoids found in \textit{Apc1638N} mice exposed to X-ray displayed loss of the wild-type \textit{Apc} allele.

Introduction

Mutations in the human adenomatous polyposis coli (\textit{APC*}) gene are involved in both sporadic (2) and familial colon cancer (familial adenomatous polyposis, FAP) (3,4). Individuals carrying a germline mutation in the \textit{APC} gene develop hundreds to thousands of colorectal adenomatous polyps, some of which will progress to carcinomas if left untreated (3,4). Other manifestations of the disease include an increased risk for upper gastrointestinal (GI) tract tumors (5), mandibular osteomas, retinal dysplasia, epidermal cysts (6) and desmoid tumors (7). Desmoids constitute one of the clinically most relevant extra-colonic manifestation of FAP. These potentially life-threatening fibromatous lesions typically arise in the body wall and the bowel mesentery after surgery (8).

The \textit{APC} gene encodes a large (312 kDa) protein, which has been shown to bind to several proteins and macromolecular structures including the microtubulin cytoskeleton (9,10), hDLG, a human homolog of the \textit{Drosophila} discs large tumor suppressor protein (11), \textit{β}-catenin (12,13) and glycogen synthase kinase 3\textit{β} (GSK3\textit{β}) (14). The binding to \textit{β}-catenin and GSK3\textit{β} indicated a possible involvement of \textit{APC} in the WNT-signal-transduction pathway (15). In fact, recent results have further elucidated the role of \textit{APC} in the WNT-pathway as being a negative regulator of \textit{β}-catenin-mediated transcriptional activation (16,17). In addition, it has been postulated that \textit{APC} is correlated with apoptosis (18,19) and cell-cycle progression (20).

In order to study the molecular basis of \textit{FAP} in an \textit{in-vivo} laboratory model, a mouse lineage carrying a targeted 3' mutation in the endogenous \textit{Apc} gene has been generated (21). Mice homozygous for the targeted frameshift mutation at a position corresponding to amino acid 1638 (\textit{Apc1638N}), are embryonic lethal. Heterozygous \textit{Apc1638N} mice develop 5–6 intestinal adenomas per animal during their lifetime. Compared with other mouse models that carry a mutated \textit{Apc} gene as \textit{Min} (multiple intestinal neoplasia) (22,23) and \textit{Apc}Δ\textsubscript{716} (24), the \textit{Apc1638N} lineage displays a relatively mild intestinal phenotype and, as such, it represents an excellent model to study both the initiation and progression of intestinal neoplasia.

One way to further examine the intestinal tumor development in \textit{Apc1638N} mice is to analyze the effect of a carcinogen on this process. In man as well as mice, ionizing radiation contributes significantly to the risk of developing a variety of malignancies including gastrointestinal tumors (25,26). The large intestine and the stomach display a relative increased risk for tumor development, in contrast to the small intestine, where only relative low numbers of X-ray-induced tumors are observed (27).

To study the effect of ionizing radiation on the initiation and progression of intestinal tumors, \textit{Apc1638N} mice were exposed to total-body X-irradiation. In addition, considering the array of extra-colonic neoplasia detected in the \textit{FAP}-patients, it was of interest to investigate the effect of X-irradiation on the extra-intestinal tumor types in the \textit{Apc1638N} mice.

Material and methods

\textbf{Animals, tumor induction and tissue samples}\n
The \textit{Apc1638N} mice used in this study have been back-crossed to C57Bl/6Jco (B6) mice to F9–F13. Mice were housed conventionally, with water and standard lab chow \textit{ad libitum}. At the age of 7 weeks heterozygous \textit{Apc1638N} (female \textit{n} = 16, male \textit{n} = 14) and control \textit{Apc\textsuperscript{+}} (wild type) mice (female \textit{n} = 24, male \textit{n} = 15) were exposed to a single dose of 5 Gy total-body X-irradiation (Smart 225, Andrex; dose rate: 0.1 Gy/min). Mice were

\textsuperscript{*}Abbreviations: APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; H&E, hematoxylin and eosin; LOH, loss of heterozygosity; PCR, polymerase chain reaction; GI, gastrointestinal; GSK3β, glycogen synthase kinase 3β; Min, multiple intestinal neoplasia.

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examined twice a week for signs of illness and killed when moribund or 250 days after exposure to X-ray. Control untreated Apc1638N mice (female \( n = 8 \), male \( n = 19 \)) were killed at age 6 months, at the time at which most irradiated Apc1638N mice became moribund (this is the age-matched control group). In addition, 24 untreated moribund Apc1638N mice (female \( n = 6 \), male \( n = 18 \)), killed at the age 8–16 months, were included for some analyses (this is the moribund control group). At autopsy, mammary tumors and intestinal lesions were scored macroscopically. A total of 27 of 30 X-irradiated Apc1638N mice were available for intestinal tumor counts. In order to score the intestinal lesions the entire intestinal tract was removed, opened longitudinally and spread out flat on a filter paper in sections of ~10 cm. In addition, in order to determine the effects of X-irradiation on desmoid numbers and sizes, a different group of Apc1638N mice (female \( n = 10 \), male \( n = 7 \)) were exposed to a single dose of X-irradiation, while a second group of Apc1638N mice (female \( n = 12 \), male \( n = 13 \)) were kept untreated as a control. The animals were killed when moribund or at the age of 6 months. For the determination of desmoid multiplicity, lesions located on the back were excluded.

At autopsy all (tumor) tissues were collected and fixed for 24 h in 10% phosphate-buffered formalin or stored at ~80°C. After formalin-fixation, the tissues were transferred to 70% ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) prior to microscopic analysis. Macrscopically distinct and separately resected intestinal tumors from 24 X-irradiated Apc1638N mice (\( n = 51 \)) and from eight untreated age-matched Apc1638N mice (\( n = 16 \)) were analyzed microscopically. Tumors from irradiated Apc1638N mice originated approximately from the duodenum (\( n = 32 \)), jejunum (\( n = 10 \)), ileum (\( n = 4 \)) and from the large intestine (\( n = 5 \)). From non-irradiated age-matched Apc1638N mice tumors originated from the periampullary region (\( n = 4 \), duodenum (\( n = 6 \), jejunum (\( n = 5 \)) and ileum (\( n = 1 \)).

Average numbers and sizes of intestinal tumors and desmoid-like lesions were evaluated statistically with Student’s t-test.

Tumor DNA isolation and loss of heterozygosity (LOH) analysis

Tumor DNA was isolated from paraffin sections as described (1). Loss of the wild-type Apc allele was analyzed by co-amplifying the wild-type and targeted Apc alleles with three primers: a shared forward primer (Apc-A 5’-TGC CAG CAC AGA ATA GGC TG-3’) and two allele-specific reverse primers, Apc-C2 and Neo3 (1). Amplification from primers Apc-A and Apc-C2 results in a 103-bp product diagnostic of the wild-type allele, whereas the combination of primers Apc-A and Neo3 yields a product of 115-bp diagnostic of the targeted allele. Amplification was performed using a touchdown protocol with annealing temperatures of 60°C, 58°C and 56°C. The amplified samples were resolved on denaturing 6% polyacrylamide gels, and quantified by Phosphorimager analysis (Molecular Dynamics). Allelic ratios were calculated as described previously (1). The mean allelic ratio of at least three controls was used to generate a comparative ratio >1.0 by dividing the mean control allelic ratio by the tumor allelic ratio. A comparative ratio >1.5 was interpreted as significant, i.e. indicative of loss of the wild-type Apc allele.

As previously reported, some chromosome 18 dinucleotide repeat markers flanking the targeted Apc locus still retain the original 129/Ola alleles from the embryonic stem cell line, E14, that was employed for the targeting experiments even after more than eight generations of back-crossing to B6 (1). Markers D18Mit17, D18Mit58 and D18Mit124 were still informative in the generations used in this study. Amplification and LOH analysis of dinucleotide-repeat markers were performed as described (1). Loss was established based on the same criteria as described for the Apc locus.

Southern blot analysis

For Southern blot analysis, 10 μg of total high-molecular-weight genomic DNA was digested with restriction enzymes as recommended by the supplier, separated on agarose gels and transferred to N’ Hybond (Amersham). Filters were hybridized with \(^{32}P\)-labeled probes and washed according to standard procedures. Probes used for the determination of the cell type of the lymphomas were as follows: the constant region of the T-cell receptor β2 gene (86TS) (28) and the joining region β2 of the T-cell receptor β2 gene (21B) (29).

Results

Survival of X-irradiated Apc1638N mice

Groups of 7-week-old female and male B6Apc1638N mice and B6 wild-type (Apc\(^+\)) mice were exposed to a single dose of 5 Gy total-body X-irradiation. Apc1638N mice were highly sensitive to X-irradiation: the majority of the animals succumbed at the age of 5–7 months, generally because of intestinal tumors, whereas non-exposed Apc1638N mice become moribund at the age of 8–16 months (R.Smits and R.Fodde, personal communication). Of the exposed Apc\(^+\) mice only two (5%) developed a non-Apc-related tumor during the observation period of 250 days (Figure 1). At the final autopsy at 250 days after exposure to X-rays, one intestinal tumor (adenocarcinoma) was found in a control irradiated Apc\(^+\) mouse, which reflects the reported development of rare intestinal tumors in B6 mice after a single high dose of X-rays (30). All X-irradiated Apc1638N mice developed multiple intestinal tumors, especially around the periampullary region. As massive tumor development at this site impeded separate counting of the lesions, this region was excluded from the analysis of tumor multiplicities. X-ray-treated Apc1638N mice presented with a mean overall intestinal tumor multiplicity of 21 (range 5–34 tumors). Age-matched untreated Apc1638N mice revealed an average tumor multiplicity of 2.7 with a range of 0–8 tumors per mouse (three out of 27 were without tumor) (Table 1), which is comparable to the tumor multiplicity of 2.6 (range 0–7 tumors) found for moribund (age 8–16 months) untreated Apc1638N mice (1). Thus, X-irradiated Apc1638N mice display a significant 8-fold increase of intestinal tumors compared with non-exposed Apc1638N mice (\( \alpha < 0.001 \)).

The size of the intestinal tumors in the X-irradiated Apc1638N mice, however, was reduced by 43% (2 ± 1 mm in diameter; range 1–7 mm) when compared with moribund untreated Apc1638N mice (3.5 ± 2 mm in diameter; range 1–12 mm) (\( \alpha < 0.01 \)) (Table 1). The age-matched untreated group had similar small-sized intestinal tumors (2.5 ± 1 mm in diameter; range 1–5 mm) as the X-irradiated Apc1638N mice. In contrast to the increased tumor multiplicity, no difference was observed for the tumor distribution along the length of the intestinal tract between unexposed and X-ray-treated Apc1638N mice (Figure 2). However, after X-irradiation Apc1638N mice displayed a small relative increase of tumors located in the jejunum (42% versus 27–29%) and a concomitant decrease of duodenal tumors (50% versus 65–66%) when compared with untreated Apc1638N mice of both groups (Figure 2). The relative incidence of large intestine tumors was not increased after X-irradiation.

In order to assess the degree of malignancy of the intestinal tumors in X-irradiated Apc1638N mice, histological sections were prepared and classified using standard criteria (1,31). A
randomly chosen group of 51, separately excised, intestinal tumors isolated from 24 mice were studied. Histological analysis revealed that five sections contained two or more lesions. Of these 67 tumors 16 (21%) were classified as tubular/tubulovillar adenomas with mostly severe dysplasia and invasive carcinomas in X-ray-treated, untreated Apc1638N mice. The degree of dysplasia and the adenoma/carcinomas ratio were comparable to the age-matched untreated Apc1638N mice. In the untreated moribund group, however, a nearly twofold increased multiplicity of invasive adenoma/carcinomas. As the desmoids were first discovered during the course of the experiment, a second group of Apc1638N mice along the intestinal tract, excluding the periampulary region. Filled bars = number of intestinal tumors in moribund Apc1638N mice exposed to 5 Gy total-body X-irradiation (n = 27); hatched bars = number of intestinal tumors in moribund untreated Apc1638N mice (n = 24); open bars = number of intestinal tumors in age-matched untreated Apc1638N mice (n = 27). Standard deviations are indicated.

**Extra-intestinal tumor types in Apc1638N mice after exposure to X-rays**

Besides the intestinal tumors, Apc1638N mice developed other malignancies, such as desmoid-like lesions and mammary carcinomas. As the desmoids were first discovered during the course of the experiment, a second group of Apc1638N mice was X-irradiated or kept untreated to determine tumor numbers and sizes (Materials and methods). Apc1638N desmoid tumors are multifocal, located both subcutaneously as well as in different muscle tissues. The lesions usually consist of a dense meshwork of thick bundles of collagen fibers, which mostly have the glassy appearance of hyalinization (Figure 3a). In addition, the number of lesions per animal appears to be sex-dependent, since male mice develop approximately three times as many desmoids as female Apc1638N mice. Interestingly, upon X-irradiation the number of desmoids per animal nearly doubled for female Apc1638N mice (p < 0.01) from 11.2 to an average of 20.2 desmoids/animal, whereas it was slightly, albeit not significantly, reduced in male Apc1638N mice from 39.5 to 31.0 desmoids/animal. The sizes and the histology of the lesions, however, remained unaffected by the X-ray treatment (Table I).

Surprisingly, X-ray treatment also resulted in an at least 15-fold increase in mammary tumors in female Apc1638N mice. Ten out of 16 (63%) X-irradiated Apc1638N females developed mammary tumors, sometimes at multiple sites, whereas no

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**Table I. Tumor types in Apc1638N mice**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>X-irradiated Apc1638N mice (n = 27)</th>
<th>Untreated age-matched Apc1638N mice (n = 27)</th>
<th>Untreated moribund Apc1638N mice (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%a, Tumors per animal</td>
<td>Size in mm</td>
<td>%a, Tumors per animal</td>
</tr>
<tr>
<td></td>
<td>meanb, rangec</td>
<td></td>
<td>meanb, rangec</td>
</tr>
<tr>
<td>Intestinal tumorsd</td>
<td>100%</td>
<td>21.0 ± 8.0</td>
<td>5–34</td>
</tr>
<tr>
<td>Mammary tumors</td>
<td>63%</td>
<td>0.8 ± 0.8</td>
<td>0–3</td>
</tr>
<tr>
<td>Desmoids (females)</td>
<td>100%</td>
<td>20.2 ± 7.6</td>
<td>11–30</td>
</tr>
<tr>
<td>Desmoids (males)</td>
<td>100%</td>
<td>31.0 ± 9.4</td>
<td>19–43</td>
</tr>
</tbody>
</table>

The tumor types detected in unexposed Apc1638N mice and after a single dose of 5 Gy total-body X-irradiation. ND, not determined; –, no tumors detected.

%a % of tumor-bearing animals.

b Average number of tumors per animal or average tumor size in mm diameter.

c Range of number of tumors per animal or range of size in mm diameter.

d Excluding the periamillary region.

e Only female Apc1638N mice are affected (p = 16; n = 25; R.Smits, personal communication).

f Exposed to X-ray n = 10, unexposed n = 12.

g Exposed to X-ray n = 7, unexposed n = 13.

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**Intestinal Tumor Distribution in Apc1638N Mice**

**Fig. 2.** Distribution of tumors in Apc1638N mice along the intestinal tract, excluding the periamillary region. Filled bars = number of intestinal tumors in Apc1638N mice exposed to 5 Gy total-body X-irradiation (n = 27); hatched bars = number of intestinal tumors in moribund untreated Apc1638N mice (n = 24); open bars = number of intestinal tumors in age-matched untreated Apc1638N mice (n = 27). Standard deviations are indicated.

**Table II. Adenoma/carcinoma frequencies in Apc1638N mice**

<table>
<thead>
<tr>
<th>Apc1638N Mice</th>
<th>Intestinal tumor type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenoma Mild/moderate</td>
</tr>
<tr>
<td>X-ray treated (n = 57)</td>
<td>21</td>
</tr>
<tr>
<td>Untreated age-matched (n = 16)</td>
<td>19</td>
</tr>
<tr>
<td>Untreated moribund (n = 57)</td>
<td>22</td>
</tr>
</tbody>
</table>

Percentages of intestinal adenomas with mild/moderate dysplasia, adenomas with severe dysplasia and invasive carcinomas in X-ray-treated, untreated age-matched and untreated moribund Apc1638N mice.
mammary lesions were detected either in age-matched non-exposed Apc1638N mice or in X-irradiated Apc+ mice (Table I). Only one out of 25 (4%) untreated moribund Apc1638N females had developed a mammary tumor (R.Smits, personal communication). The spontaneous and the X-ray-induced mammary tumors were classified as mammary adenosquamous carcinomas, a tumor type also reported as adenoacanthoma (32). The tumors were composed of numerous island of tumor cells. Many of these islands showed squamous differentiation and keratinization was prominent. In some cases this produced keratin-filled cysts within the tumor (Figure 3b).

After X-irradiation two out of 30 Apc1638N mice and two out of 39 Apc+ mice had developed a clonal T-cell lymphoma (as identified by clonal T-cell receptor β gene rearrangements; data not shown), indicating that the Apc1638N allele does not confer a predisposition to lymphoma development in mice.

Loss of the wild-type allele of Apc and chromosome 18 dinucleotide repeat markers in Apc1638N tumors

Somatic loss of the wild-type Apc allele appears to be an obligatory step for intestinal adenoma formation in the Min (33) and Apc1638N mouse models (1). In order to investigate whether this is also the case for Apc1638N X-ray-induced intestinal tumors, semi-quantitative PCR analyses were performed on DNA of 14 tumors isolated from 12 mice (three carcinomas, eight tubular adenomas and three tubulovillar adenomas). Ten of 14 tumors (regardless of the tumor type) had lost the wild-type allele of Apc which is similar to the previously published frequency of loss in spontaneous intestinal tumors of Apc1638N mice (1).

Ionizing radiation causes DNA-damage, which can lead to chromosomal deletions, amplifications and/or rearrangements (34). Since in the majority of the spontaneous Apc1638N intestinal tumors LOH at the wild-type Apc-locus reflects the integral loss of chromosome 18 (1), we investigated the possibility that in some of the X-ray-induced Apc1638N lesions the Apc LOH results from intrachromosomal deletions. To this aim, LOH analysis was performed on intestinal tumor DNA using the Apc-flanking dinucleotide-repeat markers D18Mit17, D18Mit58 and D18Mit124, which were found to be heterozygous for the 129/Ola and B6 alleles in the generations used in this study (see Materials and methods) (Figure 4). All tumors with loss of Apc were tested for loss of marker D18Mit58 and eight out of 10 displayed LOH at D18Mit58. However, two intestinal tumors (nr. 248 and 250) retained D18Mit58 and D18Mit17. The tumors were further analyzed for LOH events at the more distally located marker D18Mit124, which was lost in three intestinal tumors, but retained in six other tumors. Tumor 372 was not informative at this locus.

Similar analyses carried out on DNA of desmoid-like lesions showed that five out of nine lesions (isolated from eight mice) had lost the wild-type allele of Apc. LOH analysis of dinucleotide repeat markers showed that one (nr. 267) of these five desmoids with Apc loss had retained the initially tested marker D18Mit58. Further analysis also revealed retention of markers D18Mit124 and D18Mit17. In the remaining four
desmoids, marker D18Mit124 was lost in addition to D18Mit58. These results clearly show that in X-irradiated Apc1638N mice loss of Apc in neither intestinal tumors nor in desmoids was necessarily associated with the loss of all of chromosome 18. However, some tumors might still have lost the entire chromosome 18, as LOH events have been identified for all markers tested.

Discussion

Apc1638N mice appear to be highly sensitive to X-rays, as a total-body exposure to 5 Gy X-rays significantly increases tumor development causing a reduction of the lifespan to an average of 6 months (Figure 1). In contrast to the low intestinal tumor multiplicity in non-exposed Apc1638N mice (only 2.7 tumors per mouse, excluding those located around the periampullary region), X-ray-treated Apc1638N mice developed a mean of 21 tumors per mouse, which amounts to a significant 8-fold increase in intestinal tumor multiplicity (Figure 2).

It can be postulated that there are two major mechanisms by which ionizing radiation can lead to increased tumor multiplicities. X-rays directly damage DNA, causing chromosomal aberrations, which may affect genes contributing to tumor development (34,35). Alternatively, it is known that after exposure to ionizing radiation multiple metabolic pathways are impaired (36), some of which regulate the accurate duplication and distribution of DNA to the progeny cells. This impairment persists even over many generations resulting in delayed genomic instability. Furthermore, X-ray-induced damage can elicit massive apoptosis followed by a regenerative proliferative response of the surviving cells, as has been described for the stem cells in the small intestine (37).

In contrast to the loss of the entire chromosome 18 as observed in spontaneous intestinal tumors of Min (33) and Apc1638N (1), after X-irradiation six out of 10 Apc1638N intestinal tumors with loss of Apc displayed only partial chromosome 18 loss. Recent results obtained with the Min mouse model also showed that some γ-ray-induced intestinal tumors had lost only parts of chromosome 18 (38). Assuming that Apc loss is the first hit required for tumor development, the data indicate that some of these Apc1638N and Min tumors are initiated directly by the radiation-induced DNA-damage. The results presented here also support the conclusions of Luongo and Dove (38) that loss of other genes located on chromosome 18 such as Mcc and Dec is not necessary for Apc-driven intestinal tumor development.

Yet, although only a limited set of dinucleotide repeat markers were investigated, it might be that some intestinal tumors (four out of 10) of X-irradiated Apc1638N mice still had lost chromosome 18 together with Apc. Accordingly, similar studies on γ-ray-treated Min mice showed loss of all markers tested in a significant number of the intestinal tumors (38). This could be a direct effect of X-ray-induced gross chromosomal mutations leading to the loss of entire chromosomes. Alternatively, the exposure to X-rays may thus induce epigenetic changes increasing the ‘naturally’ occurring spontaneous loss of chromosomes, as is frequently observed in mouse cells (39–41). On the other hand, the X-ray treatment might damage cells that have already lost chromosome 18 and lead to other mutations supporting tumor growth.

Moreover, small intestinal stem cells, the postulated precursors for tumor development, are very sensitive to X-ray-induced apoptosis (42). This massive death is rapidly followed by an extensive and persistent (>8 days) proliferative response originating from the more radiation-resistant clonogenic cells, which are the first progenitors of the stem cells (37,42). This unscheduled proliferation of the clonogenic cells, which will lead to the repopulation of the empty stem cell compartment, may favor the acquisition of mutations because of the interference with normal metabolic processes, some of which govern DNA-integrity.

In this context it is interesting to note that APC has been described to be involved in apoptosis (18,19), indicating the possibility that a reduced amount of the APC protein might not be enough for induction of some apoptotic processes. If so, the haploinsufficiency for the Apc protein in Apc1638N mice (1) might partially protect the intestinal stem cells from an otherwise inevitable apoptotic death after X-ray exposure, thus contributing to the pool of potential tumor-forming stem cells that are X-ray-damaged.

In contrast to the increase in intestinal tumor multiplicity, the overall tumor distribution along the intestinal tract in the Apc1638N mice was not severely affected by the X-ray treatment. The development of large tumor masses at the periampullary region probably reflects the already high spontaneous tumor incidence at this site (1). The radio-sensitive large intestine displayed only a small, if any, increase in tumor number. The lack of any overt shift in tumor development to this site is most likely because of the fact that extremely high local doses of radiation are needed to significantly increase the tumor incidence in this part of the intestine (27). Also the degree of dysplasia and malignancy remained constant in X-irradiated compared with age-matched untreated Apc1638N mice (Table II). This suggests that in our mouse model X-rays act at an early timepoint in tumor development. This is supported by the finding that half of the tumors present with partial chromosome 18 deletions, which implies that X-ray is the direct cause for tumor initiation. However, a significant number of the tumors have possibly lost the entire chromosome18, either due to X-ray-induced (epi)genetic damage or because the X-rays hit an already initiated cell, thereby promoting its outgrowth to a tumor.

In addition to the intestinal tumors, Apc1638N mice spontaneously develop desmoid-like lesions with a 100% penetrance (Table I). Five out of nine desmoids derived from X-irradiated Apc1638N mice had lost the wild-type allele of the Apc gene, supporting the hypothesis that these murine lesions are directly related to the loss of a functional Apc protein. Interestingly, the development of desmoids is sex-dependent since untreated male Apc1638N mice have three times more desmoids than females (Table I). In contrast, after X-irradiation an opposite sex-related susceptibility was observed. The number of desmoids was only increased in female Apc1638N mice, whereas a slight reduction in their multiplicity was observed in male Apc1638N mice. Possibly the X-ray treatment changes hormone levels or functions, thereby stimulating desmoid development only in female Apc1638N mice. Further studies are underway to investigate the characteristics and sex-dependency of desmoid growth in the Apc1638N mouse model (R.Smits et al., manuscript in preparation).

A total of 63% of X-irradiated Apc1638N females developed mammary tumors, compared with none of the age-matched and only 4% of the moribund untreated Apc1638N females (Table I). These data show that exposure to ionizing radiation increases the mammary tumor multiplicity at least 15-fold. All
Apc1638N females who developed mammary tumors were virgin animals, indicating that the hormonal stimulation of pregnancy and lactation was not required for Apc1638N mammary carcinogenesis. The neoplasms were characterized as adenomas and a 15-fold increase in mammary adenosquamous Apc is a very effective carcinogenic agent in mice. A partial loss of a functional Apc protein in general significantly contributes to the susceptibility for these mammary tumors. In conclusion, we have demonstrated that exposure to X-rays is a very effective carcinogenic agent in Apc1638N mice, as it severely reduced their survival and strongly enhanced tumor multiplicities. The mice showed an 8-fold increase in intestinal neoplasms and a 15-fold increase in mammary adenomas and carcinomas. In contrast, however, the number of desmoids appeared to be only increased in female mice. The majority of the intestinal tumors as well as the desmoids displayed LOH at the Apc locus, which in several cases is associated with an intrachromosomal deletion.

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