[CANCER RESEARCH 63, 2361–2363, May 15, 2003]

Advances in Brief

Adenoma Multiplicity in Irradiated *Apc*^{Min} Mice Is Modified by Chromosome 16 Segments from BALB/c[1]

Natalie L. Degg, Michael M. Weil, Alan Edwards, Jackie Haines, Margaret Coster, John Moody, Michele Ellender, Roger Cox, and Andrew Silver[2]

Radiation Effects Department, National Radiological Protection Board, Chilton, Didcot, Oxon, OX11 0RQ, United Kingdom [N. L. D., A. E., J. H., M. C., J. M., M. E., R. C., A. S.]; Department of Pathology and Microbiology, School of Medical Sciences, University Of Bristol, University Walk, Bristol BS8 1TD, United Kingdom [N. L. D.]; and Department of Experimental Radiation Oncology, M. D. Anderson Cancer Center, Houston, Texas 77030 [M. M. W.]

Abstract

Ionizing radiation (IR) is a well-characterized carcinogen in humans and mice. The BALB/c mouse strain is unusually sensitive to IR-induced tissue damage and cancer development in a range of organs, suggestive of a partial defect in DNA damage response. This has been confirmed by finding BALB/c-specific functional polymorphism in *Prkdc*, a gene on mouse chromosome 16 that encodes the catalytic subunit of DNA-dependent protein kinase. *Prkdc*^{BALB} has been associated with increased susceptibility to IR-induced mammary and lymphatic neoplasia. Here, we provide evidence that chromosome 16 segments from BALB/c interact with *Apc*^{Min} (multiple intestinal neoplasia) and specifically enhance IR-induced adenoma development in the upper part of the small intestine.

Introduction

A combination of lifestyle, exposure to environmental carcinogens, and the overall balance between inherited resistance and sensitivity genes is likely to determine the susceptibility of an individual to cancer. The genetic component may serve to limit, or enhance, the effect of exposure to cancer-causing agents through, *e.g.*, variation in carcinogen metabolism, DNA damage response, or the processes that control specific rate-limiting steps in neoplastic cell growth (1). The success or otherwise of particular allelic combinations in inhibiting tumor formation after carcinogen exposure is likely to be driven by specific gene polymorphisms, which determine protein abundance/activity in exposed tissues. The polygenic nature of cancer inheritance in human populations, and the relatively low penetrance of most contributing polymorphic genes, has confounded the identification of genetic modifiers of tumorigenesis. In contrast, rodent models offer experimental opportunities to overcome problems of variability in lifestyle and carcinogen exposure, as well as provide a means of controlling or specifying the genetic component, through the use of induced/engineered mutations and selective breeding. In this way, rodent models can provide important proof of principle evidence on gene–gene and gene–environment interactions in cancer development (2, 3).

IR[3] is a well-characterized carcinogen capable of inducing tumors in both humans and animals (4). Compared with other inbred strains of mice, BALB/c is unusually sensitive to IR-induced tissue damage and more susceptible to IR-induced tumors, particularly those of the breast (5, 6). A recent study identified two polymorphisms specific to the BALB/c strain in the coding region of *Prkdc*, the gene encoding the DNA-PKcs (7). The protein is involved in cellular response to IR, including nonhomologous end joining of DNA double-stranded breaks, apoptosis, and post-IR signal transduction (8). Other roles include control of chromatin structure and telomeric integrity and involvement in V(D)J recombination (9). The unique *Prkdc*^{BALB} variant gene has been associated with decreased DNA-PKcs activity, partially defective repair of DNA double-stranded breaks, and elevated sensitivity to IR-induced mammary tumors (7). In independent studies, *Prkdc*^{BALB} has been linked with partially defective apoptosis in thymocytes and elevated sensitivity to radiation-induced lymphoma (10).

Thus far, a partial defect in DNA-PKcs appears to determine abnormal IR response and tumor development in at least two tissues. Here, we investigate a third tissue in a study of intestinal adenoma induction in *Apc*$^{Min/+}$ mice of different *Prkdc* genotypes. The data identify a clear gene-IR interaction in the small intestine.

Materials and Methods

Animals, Adenoma Induction, and Scoring. A colony of *Apc*$^{Min/+}$ on a C57BL/6 background was established as given in Haines *et al.* (11) and maintained as an inbred line. Female BALB/cByJ (BALB) mice were obtained from Harlan United Kingdom Ltd. (Bicester, United Kingdom) and crossed with male B6$^{Min/+}$ to provide F1 (N1) progeny. N1 *Apc*$^{Min/+}$ males were then backcrossed to female BALB mice to yield N2 progeny, all of which carry at least one copy of the BALB-derived modifier of min (*Mom1*)-dominant resistance allele. Thus, genetic modification of adenoma multiplicity in N2 will be independent of *Mom1*. N2 *Apc*$^{Min/+}$ mice were irradiated with a single whole-body dose of 2 Gy X-rays at 2 days of age; sham-irradiations were performed concurrently. Mice were housed in conventional cages with water, and standard maintenance diet was provided *ad libitum*. Mice were sacrificed, and the intestines were prepared for tumor counting according to Haines *et al.* (11). The small intestine was divided into two equal portions with the upper segment containing all of the duodenum and jejunum. All procedures involving animals were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 and with guidance from the local ethics review committee on animal experiments.

Genotyping by PCR and Statistical Analysis. Genotyping of *Apc*$^{Min/+}$ was performed according to Luongo *et al.* (12); *Prkdc* genotyping used the methods of Yu *et al.* (7). Microsatellite analysis was performed using standard methods. The MapManager QTX program (13) was used to perform interval mapping and permutation testing.

Results

***rkdc*^{BALB} Is Associated with Increased Adenoma Incidence in the Small Intestine after X-Irradiation.** Intestinal adenomas were scored in the upper and lower segments of the small intestine, and large intestine of 2 Gy x-irradiated (84 males, 58 females) and sham-irradiated (60 males, 51 females) N2, BALB X (BALB X B6) *Apc*$^{Min/+}$ mice. Radiation exposure increased adenoma incidence by

Received 11/14/02; accepted 3/26/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by the Commission of European Communities, Contract Grant F14P-CT95-0008, and NIH Grant CA43322. N. L. D. is the recipient of an extramural studentship from the National Radiological Protection Board.

[2] To whom requests for reprints should be addressed, at National Radiological Protection Board, Chilton, Didcot, Oxfordshire, OX11 ORQ, UK. Phone: 01235 822698; Fax: 01235 833891; E-mail: andy.silver@nrpb.org.

[3] The abbreviations used are: IR, ionizing radiation; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; LOD, likelihood of odds; LRS, likelihood ratio statistic; *Min,* multiple intestinal neoplasia.

Table 1 *Mean adenoma number in the intestine of recombinant Min mice*

Intestinal region[a]	0 Gy	2 Gy	Ratio (2:0 Gy)
U.S.I.	10.2 ± 1.2	19.0 ± 1.4	1.86 ± 0.26
L.S.I.	14.0 ± 1.5	23.9 ± 1.4	1.71 ± 0.21
T.S.I.	24.2 ± 2.5	42.9 ± 2.5	1.77 ± 0.21
Total	25.2 ± 2.5	44.5 ± 2.5	1.77 ± 0.20

[a] U.S.I., upper small intestine; L.S.I., lower small intestine; T.S.I., total small intestine; Total, total small + large intestine; S.I. and total, mean adenoma numbers ± SE.

~1.8-fold with a marginally greater increase observed for the upper small intestine relative to the lower small intestine (Table 1). There was no discernible increase in adenoma multiplicity in the large intestine after irradiation. We then investigated whether N2 mice with a *Prkdc*$^{BALB/BALB}$ genotype showed greater adenoma multiplicity than heterozygote mice (*Prkdc*$^{BALB/B6}$). All mice were genotyped for the *Prkdc* locus (7), and the expected 50:50 ratio for *Prkdc*$^{BALB/BALB}$:*Prkdc*$^{BALB/B6}$ was observed in both the 0 and 2 Gy groups (Table 2). Heterozygote *Prkdc* mice showed only a modest increase in adenoma numbers after x-irradiation compared with unirradiated controls (1.11–1.35-fold; Table 2). In contrast, adenoma multiplicity in irradiated *Prkdc*$^{BALB/BALB}$ mice increased substantially (2.21–2.99-fold; Table 2) with the highest postirradiation incidence recorded in the upper part of the small intestine (2.99-fold; Table 2). However, grouping mice by *Prkdc* genotype yielded no statistical significant difference between the ratios (2:0 Gy) in adenoma counts for the upper and lower parts of the intestine.

Interval Mapping and Permutation Testing Reveals One or More Locus on Chromosome 16 Controlling Radiation-induced Adenoma Formation in the Small Intestine. In addition to the variant *Prkdc* markers (7), each mouse was genotyped using five chromosome 16 microsatellites (*D16Mit182, 4, 5, 189,* and *106*). Markers were spaced evenly at ~10 cM intervals along the chromosome (Fig. 1). The MapManager QTX program was used to perform interval mapping and permutation testing. The data were ln-transformed to satisfy normality assumptions, but untransformed data yielded similar results. The following traits were considered total adenoma numbers in the small intestine and numbers in the upper and lower small intestine with and without radiation exposure. No association was seen in the absence of radiation. The strongest association between chromosome 16 loci was found for adenoma numbers in the upper small intestine of radiation exposed mice. This trait was, therefore, used for interval mapping to sublocalize candidate regions. The most significant association occurs at *D16Mit189* with a LRS of 21.6 (LOD = 4.7); at the *Prkdc* locus, the LRS is 17.6 (LOD = 3.8; Fig. 1*A*). By permutation testing, an LRS of 16.1 is highly significant, and *Prkdc* falls within the 2-LOD support interval of the peak LRS. No association was found when adenoma numbers in the lower intestine of radiation exposed mice were used as the phenotype (Fig. 1*B*). When the entire small intestine is considered, the interval mapping plot (Fig. 1*C*) is similar to that obtained for adenoma induction in the upper small intestine (Fig. 1*A*) but at a lower level of significance with peaks at *D16Mit189* (LRS = 12.5) and just distal to *Prkdc* (LRS = 10.2). This suggests that using total adenoma number for the small intestine partially masks the strong association seen for the upper small intestine (Fig. 1, *A–C*). Overall, the results do not distinguish between a single locus and two or more loci on chromosome 16 controlling adenoma induction after radiation exposure. However, these data suggest strongly that different loci control the response to genotoxic insult for the upper and lower regions of the small intestine.

Discussion

Complex interactions between numerous genes, lifestyle, and exposure to environmental carcinogens are believed to determine individual susceptibility to cancer. This susceptibility is likely to be expressed in a tissue-dependent manner, and understanding genetic predisposition requires the identification of the critical genes involved and their roles in particular tissue types. Mouse model systems using well-characterized mutations, such as the codon 850 mutation in the *Apc* gene of *Min* mice, in combination with selective breeding using inbred strains of mice, offer the opportunity to identify cancer modifying genes.

The present study has identified an association between a chromosome 16 encoded locus, or loci, contributed by the BALB/c inbred strain of mouse and increased susceptibility to adenoma induction by

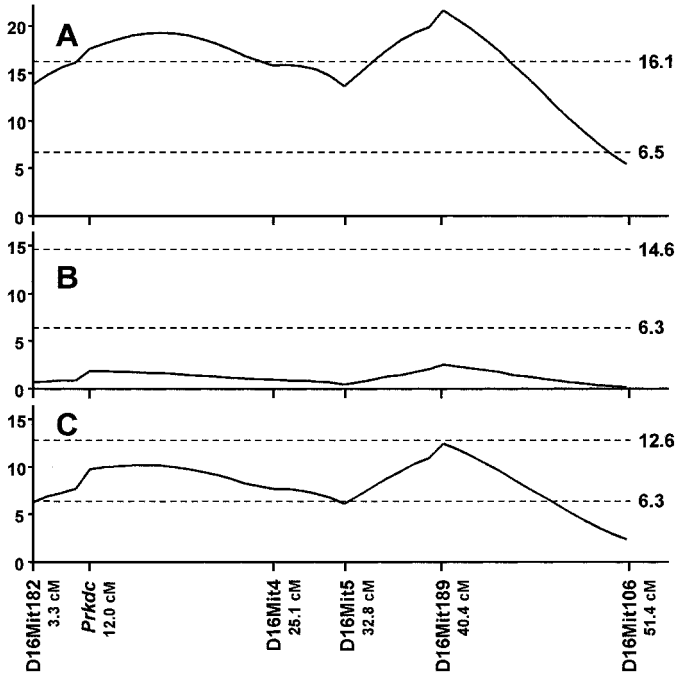

Fig. 1. Plot of the LRS from interval mapping of adenoma numbers in the upper part of the small intestine (*A*), lower part of the small intestine (*B*), and total small intestine (*C*) with markers on chromosome 16. *Horizontal lines*, significant and highly significant values. Genetic marker given as cM from centromere of chromosome 16. Values are taken from Genetic and Physical Maps of the Mouse Genome.[4]

Table 2 *Mean adenoma number and Prkdc genotype of recombinant Min mice*

	Prkdc^{BALB}/*Prkdc*^{BALB}			*Prkdc*^{BALB}/*Prkdc*^{C57BL}		
Intestinal region[a]	0 Gy (n = 55)	2 Gy (n = 69)	Ratio (2:0 Gy)	0 Gy (n = 56)	2 Gy (n = 73)	Ratio (2:0 Gy)
U.S.I.	8.1 ± 1.2	24.2 ± 2.2	2.99 ± 0.52	12.2 ± 1.9	13.6 ± 1.3	1.11 ± 0.20
L.S.I.	11.7 ± 1.9	25.8 ± 2.1	2.21 ± 0.44	16.2 ± 2.2	21.9 ± 1.8	1.35 ± 0.21
T.S.I.	19.8 ± 2.9	50.0 ± 4.0	2.53 ± 0.42	28.4 ± 3.9	35.5 ± 2.7	1.25 ± 0.20
Total	20.9 ± 3.0	51.7 ± 4.1	2.47 ± 0.41	29.4 ± 3.9	37.1 ± 2.8	1.26 ± 0.19

[a] S.I. and total, mean adenoma numbers ± SE.

IR in the small intestine in N2 mice carrying a heterozygous mutation of *Apc*. The upper part of the small intestine, largely the duodenum and jejunum, appeared more prone to IR-induced adenomas than the ileum in mice carrying sensitivity loci of BALB/c origin. The biological basis for differences in sensitivity to radiation-induced adenomas between regions of the gut is unclear and the subject of additional investigations.

Although the *Prkdc* gene falls within the 2-LOD support interval of the peak LRS, the present data do not distinguish between a single locus and two or more loci on chromosome 16 controlling adenoma induction. However, the associations reported previously between murine *Prkdc* genotype and IR response and tumorigenesis (7, 10) provide some indirect support for involvement of this gene. In the present study, the genotypic effects were observed only in x-irradiated mice. This implies that the gene of interest is of specific importance to cellular damage response after radiation exposure, and, on this basis, *Prkdc* must be regarded as a significant candidate. However, other genes should be considered as candidates; in particular, the strong association between the *D16Mit189* genomic region and postirradiation adenoma multiplicity warrants further consideration.

Flanking the *D16Mit189* marker are three physically linked microsatellites, *D16Mit223*, *D16Mit190*, and *D16Mit179*, where BALB/c differs in allele size from the other recorded inbred strains (GenBank accession no. NW_000108).[4] Allele size for the three markers was concordant for inbred strains A/J, C57BL/6J, C3H/HeJ, DBA/2J AKR/J, NON, NOD, and LP/J, supporting the proposition that this region of chromosome 16 also harbors a unique BALB/c haplotype block. The inflammatory associated gene *Adamts1* locates within this region of interest. The gene encodes a metalloproteinase-disintegrin family protein with thrombospondin type 1 motifs (14). Metalloproteinases may contribute to tumor initiation and development by altering the cellular microenvironment in a way that facilitates tumor formation. It has been reported that increased expression of the metalloproteinase *MMP-1* is associated with increased risk of lung cancer in humans (15). In principle, polymorphic variation of this gene in mice may influence the postirradiation cellular microenvironment in intestinal epithelium in a manner that enhances early adenoma formation.

Given the developing knowledge on the multiplicity and complex interaction of murine cancer predisposing loci (3), it is feasible that two modifying genes for radiation-induced adenoma formation encode within the genomic segment spanning ~40 cM on chromosome 16 (Fig. 1). To resolve these outstanding questions, we plan to repeat the backcross experiments using BALB/c- and C57BL/6-derived congenic and recombinant inbred strains with mapped recombination breakpoints on chromosome 16. This approach will allow us to repetitively sample defined chromosomal regions for their effects.

[4] Internet address: http://www-genome.wi.mit.edu/cgi-bin/mouse/index.

In summary, the present study on IR-induced adenoma formation in recombinant $Apc^{Min/+}$ mice strengthens the view that the partially defective DNA-PKcs protein of BALB/c can enhance radiation tumorigenesis in a number of tissues. Although the involvement of other chromosome 16 loci is not excluded, this study further highlights the importance of gene–gene and gene–carcinogen interactions in cancer risk. Evidence that functional polymorphisms of a single murine gene involved in DNA damage response can specifically increase IR cancer risk in a range of tissues enhances the prospects for uncovering similar polymorphisms in human populations.

Acknowledgments

We thank Kevin Whitehill, Pat Hillier, Emma Davies, and Ryzyard Kozlowski for technical support and Chris Paraskeva for useful discussions. We also thank Medical Research Council Radiation and Genome Stability Unit, Harwell, for assistance with X-ray exposures.

References

1. Ponder, B. A. J. Cancer genetics. Nature (Lond.), *411:* 336–341, 2001.
2. Balmain, A., and Nagase, H. Cancer resistance genes in mice: models for the study of tumor modifiers. Trends Genet., *14:* 139–144, 1998.
3. Tripodis, N., Hart, A. A. M., Fijneman, R. J. A., and Demant, P. Complexity of lung cancer modifiers: mapping of thirty genes and twenty-five interactions in half of the mouse genome. J. Natl. Cancer Inst. (Bethesda), *1942:* 69–78, 2001.
4. United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). Sources and Effects of Ionizing Radiation. Report to the General Assembly with Scientific Annexes. Official Record: Fifty-fifth session, Supplement No. 46 (A/55/46). New York: United Nations, 2000.
5. Roderick, T. H. The response of twenty seven inbred strains of mice to daily doses of whole body x-irradiation. Radiat. Res., *20:* 614–631, 1963.
6. Storer, J. B., Mitchell, T. J., and Fry, R. M. Extrapolation of the relative risks of radiogenic neoplasms across mouse strains and to man. Radiat. Res., *114:* 331–353, 1988.
7. Yu, Y., Okayasu, R., Weil, M. W., Silver, A., McCarthy, M., Zabriskie, R., Long, S., Cox, R., and Ullrich, R. L. Elevated breast cancer risk in irradiated BALB/cassociates with unique functional polymorphism of the *Prkdc* (DNA-dependent protein kinase catalytic subunit gene. Cancer Res., *61:* 1820–1824, 2001.
8. Smith, G. C., and Jackson, S. P. The DNA-dependent protein kinase. Genes Dev., *13:* 916–934, 1999.
9. Bailey, S. M., Meyne, J., Chen, D. J., Kurimasa, A., Li, G. C., Lehnert, B. E., and Goodwin, E. H. DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. Proc. Natl. Acad. Sci. USA, *96:* 14899–14904, 1999.
10. Mori, N., Matsumoto, Y., Okumoto, M., Suzuki, N., and Yamate, J. Variation in *Prkdc* encoding the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and susceptibility to radiation-induced apoptosis and lymphogenesis. Oncogene, *20:* 3609–3619, 2001.
11. Haines, J., Dunford, R., Moody, J., Ellender, M., Cox, R., and Silver, A. Loss of heterozygosity in spontaneous and X-ray-induced intestinal tumors arising in F1 hybrid *Min* mice: evidence for sequential loss of Apc^{+} and *Dpc4* in tumor development. Genes Chromosomes Cancer, *28:* 387–394, 2000.
12. Luongo, C., Moser, A. R., Gledhill, S., and Dove, W. F. Loss of *Apc* in intestinal adenomas from *Min* mice. Cancer Res., *54:* 5947–5952, 1994.
13. Manly, K. F., Cudmore, Jr., R. H., and Meer, J. M. MapManager QTX, cross-platform software for genetic mapping. Mamm. Genome, *12:* 930–932, 2001.
14. Kuno, K., Kanada, N., Nakashima, E., Fujiki, F., Ichimura, F., and Matsushima, K. Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. J. Biol. Chem., *272:* 556–562, 1997.
15. Zhu, Y., Spitz, M. R., Lei, L., Mills, G. B., and Wu, X. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. Cancer Res., *61:* 7825–7829, 2001.