SHORT COMMUNICATION

Heterozygosity for a mutation in Brca1 or Atm does not increase susceptibility to ENU-induced mammary tumors in Apc\(^{\text{Min/}+}\) mice

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The proteins encoded by BRCA1 and ATM may be important in DNA repair and maintenance of genomic integrity. Women heterozygous for a mutation in BRCA1 have an increased incidence of breast cancer. Some evidence also suggests that female carriers of ATM mutations may be susceptible to breast cancer. However, mice carrying one mutant allele of Brca1 or Atm are not highly susceptible to breast cancer. We proposed that heterozygosity for a mutant allele of Brca1 or Atm may confer a decreased ability to repair DNA damage. Such a defect might lead to a heightened sensitivity to tumor development in susceptible animal models. Therefore, mice predisposed to mammary tumor development might show an increased susceptibility if they also carry an Atm or Brca1 mutation.

C57BL/6J (B6) Min/+ mice are predisposed to mammary and intestinal tumors and exposure to the point mutagen ethylnitrosourea (ENU) markedly increases mammary tumor multiplicity and incidence. To test our hypothesis, B6.Min/+ male mice were crossed with 129S6/SvEvTac females heterozygous for a mutant allele of either Brca1 or Atm. Female progeny from each cross were treated with ENU and followed for tumor development. Only Min/+ F1 females developed mammary tumors and heterozygosity for a mutant Brca1 or Atm allele had no effect on mammary or intestinal tumor incidence and multiplicity. These results suggest that heterozygosity for a mutation in Brca1 or Atm does not affect Min-induced tumorigenesis in mice under these conditions. Additionally, exposure to a somatic point mutagen does not increase tumor development in mice carrying Brca1 or Atm mutations.

The identification of genetic alterations that confer an increased risk of breast cancer is required for effective treatment and prevention of the disease. Defects in genes that control the cellular response to DNA damage might influence cancer development by causing an elevated mutation rate or increased genomic instability. The gene products encoded by the BRCA1 tumor suppressor gene (1–6) and the ATM gene (7–10) have been implicated in mediating the DNA damage response. Mutations in BRCA1, and to a lesser extent ATM, have also been associated with an increased risk of breast cancer in women (11–14).

The BRCA1 gene encodes a 220 kDa nuclear protein (13). BRCA1 has been shown to have a role in maintaining genomic integrity through involvement in homologous recombination, transcription-coupled repair and double-strand break repair. Brca1 function is required for early embryonic development in the mouse. BRCA1 has also been shown to play a role in transcriptional regulation through interactions with RNA polymerase II or transcription factors. In addition, several domains in BRCA1 are involved in the addition of ubiquitin to other proteins (reviewed in ref. 15).

Generation of a mouse model of BRCA1-induced neoplasia has been attempted through gene targeting of the mouse homolog of BRCA1 (1,3,16,17). Such mutations are embryonic lethals when homozygous, and heterozygous animals are not obviously susceptible to mammary tumor development. One explanation for the lack of mammary tumors in Brca1\(^{+/}\) mice is that further mutational events are required and the relatively short lifespan of the laboratory mouse does not allow enough time for these events to occur. This idea is supported by the observation that mice carrying both a conditional allele and null allele of Brca1 develop mammary tumors following mammary gland-specific deletion of the conditional allele (18). However, tumors developed in a small number of mice only after a long latency period. Thus, loss of Brca1 function is not sufficient for tumor development and further events are required. Introduction of a p53 mutation increases mammary tumor multiplicity in the conditional mutants, as well as in mice heterozygous for Brca1 mutation (19). Tumors lacking BRCA1 frequently have multiple genetic alterations. These observations led to the hypothesis that loss of BRCA1 would allow these genetic alterations to accumulate and that loss of p53 function would allow the cells to escape apoptosis and continue to proliferate and accumulate mutations. Tumors could arise as a result of the accumulated loss of growth control through loss or alteration of tumor suppressor genes.

Homozygosity for mutations in ATM results in the disorder ataxia telangiectasia (AT) (7). AT patients are extremely sensitive to the effects of ionizing radiation and are highly susceptible to malignancies, particularly lymphomas. Cells from AT patients also exhibit hypersensitivity to ionizing radiation, genomic instability and defects in cell cycle checkpoint responses (20–22). The clinical and cellular manifestations of the disease are consistent with a defect in DNA double-strand break repair or maintenance of genomic stability. ATM has been shown to play an important role in the activation of proteins, such as BRCA1, that are involved in the repair of DNA damage. Thus, cells that lack ATM function cannot respond appropriately to DNA damage and would continue to proliferate, resulting in mutations or alterations in DNA.

Epidemiological studies involving families of AT patients

Abbreviations: AT, ataxia telangiectasia; ENU, ethylnitrosourea.

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have suggested that females who carry one mutant allele of ATM may be at an increased risk of developing breast cancer (12,23). Interestingly, there is some evidence that early exposure to irradiation may increase the risk of mammary tumors in ATM heterozygotes. However, other studies do not support an elevated risk of breast cancer in ATM mutation carriers (24–26). Further, there is speculation that ATM may act as a low penetrance susceptibility gene (27). In this case, ATM mutations might predispose women to breast cancer only in concert with other mutations. It would be useful to develop an animal model that could be used to explore some of the questions raised by these human studies.

Atm-deficient mice have been generated by gene targeting (8,9,28). Homozygous mutant animals generally recapitulate most of the features of human AT. Heterozygous mutant mice are phenotypically normal and are not susceptible to spontaneous malignancies. However, when exposed to sublethal doses of ionizing radiation, Atm–/– mice show reduced survival and premature graying as compared with wild-type siblings (29). These results suggest that Atm mutations may be haploinsufficient when the mice are stressed by exposure to irradiation. This observation also supports a role for ATM in the response of the cell to DNA damage.

We investigated whether heterozygosity for an Atm or Brca1 mutation would increase breast cancer susceptibility by reducing the ability to maintain genomic stability or repair DNA damage, possibly resulting in an increased mutation rate. Since mice carrying one mutant allele of Brca1 or Atm do not develop spontaneous mammary tumors, we tested our hypothesis using a mouse model that is sensitive to mammary tumor development. The Min mouse carries a dominant mutation in the Apc tumor suppressor gene and is predisposed to intestinal, desmoid and mammary tumors (30,31). After treatment with the alkylating agent ethylnitrosourea (ENU), mammary tumor incidence markedly increases in ApcMin/+ (Min+) female mice (32). Thus, if heterozygosity for a Brca1 or Atm mutation reduces the capacity of a cell to repair DNA damage or maintain genomic integrity, then we would expect an increased mammary tumor incidence in Min+ mice carrying either mutation compared with mice carrying the Min mutation alone.

To test whether heterozygosity for a mutation at Brca1 or Atm increased mammary tumor susceptibility in B6.Min+/+ mice, we crossed 129S6/SvEvTac females heterozygous for Brca1All+/+ (Brca1 +/–) (D.Larson and A.Wynshaw-Boris, unpublished results) or Atm5790neo (Atm +/–) (8) with C57BL/6 J (B6) Min+/+ males. The Brca1 mutant mice have similar phenotypes to several published mutant Brca1 alleles. The targeted mutation was generated by replacement of 1.5 kb of exons 11 with a PGK-neo selectable marker. All mice were produced and maintained at the University of Wisconsin Medical School Research Animal Facility. All female progeny were genotyped for the Min mutation as described (33). Genotyping for the Brca1 mutant allele was performed by PCR using a three primer set with a common 3′ primer (5′-TGC TGA G is specific for the targeted allele-specific 5′ primer (5′-GAC TTC TGT CAG ATG TTG C) and one wild-type allele-specific 5′ primer (5′-TAA TGA GGT GAT CAT GAT A) and one targeted allele-specific 5′ primer (5′-TAA AGC GCA TGC TCC AG). A three primer set was also used to genotype mice for the Atm mutant allele (5′-GAC TTC TGT CAG ATG TTG CTG CC is the common primer, 5′-AGT TGA CCC) and one wild-type allele-specific 5′ primer (5′-TAA TGA GGT GAT CAT GAT A) and one targeted allele-specific 5′ primer (5′-GAC TTC TGT CAG ATG TTG CTG CC). A three primer set was also used to genotype mice for the Brca1 mutant allele (5′-GAC TTC TGT CAG ATG TTG CTG CC is the common primer, 5′-AGT TGA CCC) and one wild-type allele-specific 5′ primer (5′-TAA TGA GGT GAT CAT GAT A) and one targeted allele-specific 5′ primer (5′-GAC TTC TGT CAG ATG TTG CTG CC). The cycling conditions were 2 min at 94°C, 30 cycles of 20 s at 94°C, 30 s at 50°C and 1 min at 72°C, followed by 5 min at 72°C. Brca1 PCR products were visualized on 4% agarose gels and Atm PCR products were visualized on 2% agarose gels.

Mice were injected i.p. with 50 mg ENU/kg body wt when between 35 and 45 days of age (32), then palpated weekly for tumors. Mice were killed when moribund or 100 days after birth. To avoid a delay in palpation caused by tumors in the breast area, we palpated the upper half of the mammary glands. Mice were killed when moribund or 100 days after birth. To avoid a delay in palpation caused by tumors in the breast area, we palpated the upper half of the mammary glands.

### Table I. Tumor incidence and multiplicity in ENU-treated mice from the Brca1+/–×Min+ cross

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total no. of mice</th>
<th>With mammary tumors (%)</th>
<th>No. of mammary tumors (mean ± SD)</th>
<th>No. of intestinal tumors (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brca1+/– Min+</td>
<td>11</td>
<td>45a</td>
<td>0.8 ± 1.3b</td>
<td>23 ± 12a</td>
</tr>
<tr>
<td>Brca1+/– Min+</td>
<td>15</td>
<td>53</td>
<td>0.9 ± 1.1</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>Brca1+/– +/+</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Brca1+/– +/–</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aNot significantly different from Brca1+/– Min+ mice (P = 1.0) by Fischer’s exact test.
bNot significantly different from Brca1+/– Min+ mice (P = 0.67) by Wilcoxon rank sum test.

cNot significantly different from Brca1+/– Min+ mice (P = 0.10) by Wilcoxon rank sum test.

### Table II. Tumor incidence and multiplicity in ENU-treated mice from the Atm+/–×Min+ cross

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total no. of mice</th>
<th>With mammary tumors (%)</th>
<th>No. of mammary tumors (mean ± SD)</th>
<th>No. of intestinal tumors (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atm+/– Min+</td>
<td>19</td>
<td>63a</td>
<td>1.3 ± 1.4b</td>
<td>26 ± 12c</td>
</tr>
<tr>
<td>Atm+/– Min+</td>
<td>14</td>
<td>71</td>
<td>1.4 ± 1.2</td>
<td>24 ± 24</td>
</tr>
<tr>
<td>Atm+/– +/+</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atm+/– +/–</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aNot significantly different from Atm+/– Min+ mice (P = 1.0) by Fischer’s exact test.
bNot significantly different from Atm+/– Min+ mice (P = 0.78) by Wilcoxon rank sum test.
cNot significantly different from Atm+/– Min+ mice (P = 0.94) by Wilcoxon rank sum test.
treatment. Mammary tumors were collected and fixed in formalin. Intestinal tumors were also counted as described (30).

Only the Min+/+ mice from both crosses developed mammary tumors. The number of mammary tumors in Min+/+ /+ mice is significantly different than in the wild-type mice in both crosses. Neither the incidence nor multiplicity of mammary tumors was different for the Brca1+/−/ Min+/+ mice as compared with the Brca1+/−/ Min+/+ mice (Table I) or for the Atm+/−/ Min+/+ mice as compared with the Atm+/−/ Min+/+ mice (Table II). All tumors were classified as squamous carcinomas (34) and no difference in tumor type due to genotype at Brca1 or Atm was observed. No mammary tumors were observed in untreated Min−/− mice.

To determine if there was a difference in tumor susceptibility between Min+/+ mice from each of the crosses, we compared pooled tumor data from Atm+/− and Atm+/+ mice with pooled tumor data from Brca1+/− and Brca1+/+ mice. No difference in mammary tumor number (P = 0.14) or mammary tumor incidence (P = 0.44) was observed.

To test for an effect of heterozygosity for a mutation in Brca1 or Atm on intestinal tumor development in ENU-treated mice we counted intestinal tumors in the mice from both crosses. Only Min+/+ mice developed intestinal tumors. As with the mammary tumors, the number of intestinal tumors was not affected by heterozygosity for a mutation at Brca1 or Atm (Tables I and II). Additionally, there was no difference in the number of intestinal tumors between Min+/+ mice from each line (P = 0.70).

These experiments indicate that heterozygosity for a mutation in either Brca1 or Atm does not result in a greatly increased sensitivity to mammary or intestinal tumor development in Min+/+ mice following ENU treatment. Thus, mice heterozygous for a mutation in either Brca1 or Atm do not demonstrate an increased incidence of mutations that would lead to tumor development after ENU treatment. The proteins encoded by Brca1 and Atm play important roles in the repair of double-strand DNA breaks, recombination and maintenance of genomic stability. ENU is a direct alkylating agent that most likely damages DNA by modifying single bases. Such lesions can be repaired without double-strand break induction. Therefore, the repair of ENU-induced lesions may involve pathways other than those in which BRCA1 and ATM are critical. Although ENU can induce DNA strand breaks and deletions, these events may comprise a minority of the DNA damage. Given what is now known about the function of these two proteins in the response to DNA damage and the observation that irradiation may increase the risk of mammary tumors in women with Atm mutations, it will be important to test for any effect of irradiation on tumor development in these mice.

Acknowledgements

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References


10. Swift,M., Chase,C.L. and Morrell,D. (1990) Cancer predisposition of mice from both crosses. Only the incidence or multiplicity of mammary tumors was different for the Brca1+/−/ Min+/+ mice following ENU treatment. Thus, mice heterozygous for a mutation in Brca1 or Atm (Tables I and II). Additionally, there was no difference in the number of intestinal tumors between Min+/+ mice from each line (P = 0.70).

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