Cancer Susceptibility of Mice with a Homozygous Deletion in the COOH-Terminal Domain of the Brca2 Gene

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Abstract

Inherited mutations of the human BRCA2 gene confer increased risks for developing breast, ovarian, and several other cancers. Unlike previously described Brca2 knockout mice that display predominantly embryonic lethal phenotypes, we developed mice with a homozygous germ-line deletion of Brca2 exon 27 that exhibit a moderate decrease in perinatal viability and are fertile. We deleted this Brca2 COOH-terminal domain because it interacts directly with the Rad51 protein, contains a nuclear localization signal, and is required to maintain genomic stability in response to various types of DNA damage. These homozygous Brca2-mutant mice have a significantly increased overall tumor incidence and decreased survival compared with their heterozygous littermates. Virgin female mice homozygous for this Brca2 mutation also display an inhibition of ductal side branching in the mammary gland at 6 months of age. Given their substantial viability and cancer predisposition, these mutant mice will be useful to further define the role of the COOH-terminal Brca2 domain in tumorigenesis both in vivo and in vitro.

Introduction

Women who inherit germ-line defects of the BRCA2 breast cancer susceptibility gene have up to an 85% risk of breast cancer development by 70 years of age (1, 2). BRCA2 mutation carriers also have increased risks for a variety of cancers including ovarian, pancreatic, colon, prostate, stomach, laryngeal, thyroid, male breast cancer, and ocular melanoma (3). Substantial evidence from studies of human and mouse cells indicates that the BRCA2 protein is an important component in DNA damage response pathways, and loss of this function is considered a major factor in cancer predisposition (4, 5). Brca2-mutant cells and embryos are hypersensitive to ionizing radiation and other DNA-damaging agents and develop numerous spontaneous chromosomal abnormalities (6–10). In addition, several domains of the BRCA2 protein appear to interact directly with RAD51, a protein having distinct roles in normal meiotic and mitotic recombination, DNA damage repair, and chromosome segregation. These BRCA2 domains include the BRCA repeats in exon 11 and a highly conserved RAD51 binding domain in exon 26 (7, 8, 11–13). The interaction of Brca2 with Rad51 is pivotal for the role Brca2 plays in the activation of double-strand break repair and/or homologous recombination, and disruption of these processes may predispose individuals to cancer development.

Functional studies of Brca2 in adult tissues have been hindered by

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2 To whom requests for reprints should be addressed, at Laboratory of Women’s Health, National Institute of Environmental Health Sciences, NIH, MD C4-06, 111 T. W. Alexander Drive, Research Triangle Park, NC 27709. Phone: (919) 541-3229; Fax: (919) 541-3720; E-mail: mcallis2@niehs.nih.gov.
3 The abbreviation used is: ES, embryonic stem.

Advisory in Brief
Cancer susceptibility in homozygous mutant Brca2 mice

Generation of Mice with a Homozygous Germ-Line Deletion of Brca2. ES cells carrying a targeted Brca2 allele with loxP sites flanking exon 27 were generated by homologous recombination (Fig. 1). After transient Cre expression in vitro, ES clones with a Brca2Δ27 allele were identified. PCR products generated with the B2F1 and B2R2 primers that flank the 5′ and 3′ loxP sites (Fig. 1) were sequenced to confirm the expected exon 27 deletion. Germ-line transmission of the Brca2Δ27 allele was identified by standard breeding techniques.

As expected, reverse transcriptase-PCR analysis with primers specific for exon 27 does not yield detectable messages in testes RNA from Brca2Δ27/Δ27 mice, although this reverse transcription-PCR product is easily detectable for Brca2Δ27/+ mice. Assuming a mutant transcript is expressed, it could give rise to a truncated protein product of 3142 amino acids compared with the wild-type murine Brca2 protein that contains 3329 amino acids. Unfortunately, we are unable to confirm the presence or relative levels of this putative mutant Brca2 protein because of the unavailability of specific antibodies directed against the mouse gene product.

Viability of Brca2Δ27/Δ27 Mice. Although the Brca2Δ27/Δ27 animals are viable compared with previous Brca2 germ-line mutants, survival analysis indicates an overall decrease in viability of homozygous mutants compared with heterozygous and wild-type littermates:

<table>
<thead>
<tr>
<th>Group</th>
<th>Brca2Δ27/Δ27</th>
<th>Brca2Δ27/+</th>
<th>Brca2+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin females</td>
<td>30/30 (100%)</td>
<td>43/43 (100%)</td>
<td>44/44 (100%)</td>
</tr>
<tr>
<td>Multiparous females</td>
<td>12/36 (33%)</td>
<td>30/36 (83%)</td>
<td>44/44 (100%)</td>
</tr>
<tr>
<td>Males</td>
<td>10/10 (100%)</td>
<td>12/12 (100%)</td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>52/52 (100%)</td>
<td>65/65 (100%)</td>
<td>80/80 (100%)</td>
</tr>
</tbody>
</table>

*Number of tumor-bearing mice/number of mice at risk (% incidence).

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a subset of Brca2Δ27Δ27 offspring appear to either die during late gestation or shortly after birth, suggesting that loss of Brca2 function impacts overall viability.

Secondly, the overall survival of Brca2Δ27Δ27 compared with Brca2Δ27/+ virgin female mice is significantly decreased (P<0.05) by a life table test, providing additional support that lifetime survival is affected by this Brca2 mutation. Interestingly, test matings of both Brca2Δ27Δ27 males and females with wild-type mice do not reveal apparent infertility or difficulty in raising litters.

Inhibition of Mammary Side Branching in Brca2Δ27Δ27 Mice. Alterations in normal growth and differentiation of mammary tissue from Brca2-mutant virgin females were determined using whole-mount analysis. At 6 months of age, mammary tissue from three Brca2Δ27Δ27 animals exhibited a dramatic lack of side branching and a much lower density of ductules compared with that observed for three heterozygous and wild-type littersmates, which were indistinguishable (Fig. 2). Although the ducts reached the limits of the mammary fat pad in the homozygous mutant animals, a substantial lack of side branching was observed in these homozygous mutant animals, a substantial lack of side branching was observed in these homozygous mutant mice, whereas only a single tumor was observed in the Brca2Δ27/+ animals, and no tumors were observed in Brca2+/+ animals during this time period (Table 2).

Terminal sacrifices of the remaining animals were performed between 17 and 19 months of age. This enabled us to develop a comprehensive tumor spectrum for mice from each genotype (Table 2). With the combined data from all of the moribund and terminal sacrifices, the Brca2Δ27Δ27 mice exhibited a >2-fold increase in overall tumor incidence compared with their Brca2Δ27/+ and Brca2+/+ littersmates. In 41 Brca2Δ27Δ27 mice, 30 tumors were observed, compared with only 11 tumors in 43 Brca2Δ27/+ animals and 4 tumors in 15 Brca2+/+ animals. Overall, 25 tumor-bearing animals were observed among 41 Brca2Δ27Δ27 mice for a tumor incidence of 61% (Table 1). In contrast, only 11 of 43 Brca2Δ27/+ animals were tumor bearing (26% incidence), and 3 of 15 animals (20% incidence) were tumor-bearing in the Brca2+/+ littersmates (Table 1). These data from all mice combined show a highly significant difference (P<0.01) in the overall tumor rates between the Brca2Δ27Δ27 and Brca2Δ27/+ mice. These results were also analyzed separately for virgin females alone, virgin plus multiparous females, and males only. The tumor response patterns and incidences for each of these smaller subgroups are similar to the tumor response of all animals combined. Statistically significant differences (P<0.05) in tumor incidences between the Brca2Δ27Δ27 and Brca2Δ27/+ animals are observed when the data for each subgroup is considered independently. Because a smaller number of Brca2+/+ mice were examined, there was not enough statistical power to distinguish tumor rates between the wild-
type and other genotypic classes. We also did not observe a significant difference in overall tumor incidence between males and females for the Brca2^27/27^ genotype with a 67% (6 of 9) incidence observed in males compared with a 59% (19 of 32) incidence observed for virgin and multiparous females combined (Table 1).

The tumor spectrum is diverse and includes carcinomas, adenomas, lymphomas, and sarcomas (Table 2). Of particular interest is the exclusive appearance of various types of carcinomas in the Brca2^27/27^ mice. These include one mammary adenosquamous carcinoma (Fig. 3), five squamous cell carcinomas (three of gastric origin), two gastric adenocarcinomas, one endometrial carcinoma, and two A/B lung carcinomas. Fig. 3 illustrates representative histological sections for the two types of gastric tumors that were observed. Interestingly, a single mammary adenomyoepithelioma was detected in one Brca2^27/+ mouse. The carcinoma also appears to be increased in the Brca2^27/27^ mice compared with their littermates. Three histiocytic sarcomas, along with a hemangiosarcoma and a leiomyosarcoma, arose in Brca2^27/27^ mice, whereas only two histiocytic sarcomas were identified in their Brca2^27/+ and Brca2^+/+ littermates. We found a wide variety of tumor types in the Brca2^27/27^ mice that were sacrificed before 17 months of age because of morbidity (Table 2). Therefore, no obvious differences in latency between distinct tumor types were observed.

Discussion

Here we describe germ-line Brca2 mutant mice with a predisposition for tumor development. In contrast to previous reports of mice with homozygous germ-line mutations in the Brca2 gene (6–8, 15–17), the Brca2^27/27^ mice exhibit only a 33% decrease in expected viability during perinatal development. Brca2^27/27^ mice of both sexes are fertile and lack gross developmental abnormalities. Mice harboring this germ-line mutation in the COOH-terminal domain of Brca2 have an increased susceptibility for a wide spectrum of solid tumors. The fact that 9 of 10 spontaneous tumors that developed prior to 17 months arose in Brca2^27/27^ mice indicates that not just the incidence but also the survival, because of tumor development, is significantly affected by the presence of this Brca2 mutation. Overall, the tumor spectrum we observed is similar to that reported for knockout mice eliminating the COOH-terminal region of the Brca1 gene, where an increased incidence of a wide variety of carcinomas, sarcomas, and lymphomas was found (24). One unique finding of our study is the exclusive presence of carcinomas in Brca2^27/27^ mice and specifically a substantial number of stomach cancers, both adenocarcinomas and squamous cell carcinomas. We have shown previously that Brca2 expression in adult mouse tissues correlates with cell proliferation and is relatively high in the glandular mucosa of the stomach (25). Interestingly, stomach cancers are among the various tumor types that have been associated with BRCA2 mutations in humans, with a 2.59 relative risk (3).

This report supports a strong correlation between the Brca2 mutation position and the resulting Brca2 mouse knockout phenotype. We and other laboratories have shown previously that mice homozygous for targeted Brca2 mutations 5’ of the BRC repeats in exon 11 exhibit early embryonic lethal phenotypes (6–8, 15–17). These BRC repeats interact with Rad51 (12, 13) and have been highly conserved in evolution (18, 26), which suggests that they are important functional domains of the Brca2 protein. A few viable Brca2-mutant mice have been generated that retain at least some of these BRC repeats, and these animals display multiple severe developmental abnormalities, infertility, and early thymic lymphoma development (7, 8). The Brca2^27/27^ mice lack only the COOH-terminal domain and could produce a truncated Brca2 gene product that preserves all eight BRC repeats. In contrast to all other Brca2-null mice reported to date, these Brca2^27/27^ mice display a modest loss of viability and have no gross developmental abnormalities or obvious infertility. Thus, full retention of the BRC repeats and other functional domains may direct a genotype-phenotype correlation.
This study extends previous findings by several laboratories that have demonstrated the functional significance of the COOH-terminal domain of both human and rodent BRCA2. Morimoto et al. (10) demonstrated that mouse ES cells and embryonic fibroblasts lacking exon 27 are hypersensitive to γ-radiation and undergo premature senescence. Recent reports indicate that the COOH-terminus of Brca2 is required for error-free, homology-directed repair of DNA double strand breaks and the ability to facilitate the induction of Rad51 nuclear foci after ionizing radiation (20, 21). Thus, disruption of the exon 27 Brca2-Rad51 interaction appears to lead to defective repair of DNA damage and generalized chromosomal instability with a subsequent increased risk of neoplastic progression in Brca2-deficient cells (4, 5). The human COOH-terminal region of BRCA2 also appears to be critical because a truncating mutation (9808delCC) at position 3195, which occurs just upstream of the predicted Rad51-interacting domain in human BRCA2 exon 27, is associated with an elevated breast cancer risk (27).

Despite the increased susceptibility of Brca2<sup>27/27</sup> mice to tumorigenesis, these animals are not highly susceptible to mammary tumors. Several factors may account for this observation, but the most likely reason is the fact that the Brca2<sup>27</sup> mutation was examined on a mixed C57BL/6N and 129/SvEv background. C57BL/6N mice and 129/SvEv mice are both extremely resistant to spontaneous as well as radiation-induced mammary carcinogenesis.4 Thus, we are currently using microsatellite marker-assisted breeding techniques to transfer this Brca2<sup>27</sup> mutation onto inbred strains such as BALB/cJ that are susceptible to mammary carcinogenesis.

The reduced ductal branching phenotype seen in Brca2<sup>27/27</sup> virgin female mice may be associated with increased mammary tumor risk in combination with other environmental or genetic factors. We cannot exclude the possibility that the subtle mammary gland phenotype we have observed may be attributable to hormonal differences in the Brca2<sup>27/27</sup> mice compared with their littersmates rather than a more direct Brca2 effect. However, Deng and Brodie (14) and Xu et al. (28) described a blunted ductal branching phenotype in the mammary glands of mice with a mammary-specific targeted Brca1 mutation that was associated with subsequent tumor development after a long latency. In addition, a high incidence of mammary adenocarcinomas was reported recently in mice carrying a mammary tissue-specific mutation that completely disrupts the Brca2 gene (29). Given their substantial viability and a cancer predisposition, Brca2<sup>27/27</sup> mice and cells derived from them will be useful to further define the role of Brca2 in tumorigenesis.

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References


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