Bin1 Ablation Increases Susceptibility to Cancer during Aging, Particularly Lung Cancer

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Abstract

Age is the major risk factor for cancer, but few genetic pathways that modify cancer incidence during aging have been described. Bin1 is a prototypic member of the BAR adapter gene family that functions in vesicle dynamics and nuclear processes. Bin1 limits oncogenesis and is often attenuated in human cancers, but its role in cancer suppression has yet to be evaluated fully in vivo. In the mouse, homozygous deletion of Bin1 causes developmental lethality, so to assess this role, we examined cancer incidence in mosaic null mice generated by a modified Cre-lox technology. During study of these animals, one notable phenotype was an extended period of female fecundity during aging, with mosaic null animals retaining reproductive capability until the age of 17.3 ± 1.1 months. Through 1 year of age, cancer incidence was unaffected by Bin1 ablation; however, by 18 to 20 months of age, ~50% of mosaic mice presented with lung adenocarcinoma and ~10% with hepatocarcinoma. Aging mosaic mice also displayed a higher incidence of inflammation and/or premalignant lesions, especially in the heart and prostate. In mice where colon tumors were initiated by a ras-activating carcinogen, Bin1 ablation facilitated progression to more aggressive invasive status. In cases of human lung and colon cancers, immunohistochemical analyses evidenced frequent attenuation of Bin1 expression, paralleling observations in other solid tumors. Taken together, our findings highlight an important role for Bin1 as a negative modifier of inflammation and cancer susceptibility during aging. [Cancer Res 2007;67(16):7605-12]

Introduction

Aging is the major risk factor for cancer. Nevertheless, most preclinical models of cancer employ young animals that are unlikely to fully recapitulate the participation of the inflammatory tissue microenvironment, immune senescence, or other age-associated factors. Insights into the cause and treatment of cancer might therefore benefit from studies of genetic pathways that modify cancer incidence during aging. However, few such pathways have been defined.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi:10.1158/0008-5472.CAN-07-1100

Bin1 encodes a nucleocytoplasmic BAR adapter protein that can interact with the c-Myc oncoprotein and inhibit its cell transforming activity (1-3). c-Myc is involved in the development of many human cancers where its overexpression is associated with poor prognosis (4). Multiple splice isoforms of Bin1 exist with diverse patterns of tissue distribution, subcellular localization, and protein interactions (5-8). Although BAR adapter proteins share canonical functions in membrane dynamics (9), certain BAR proteins, such as those encoded by the Bin1 and APPL genes, may also have functions in transcriptional control (2, 3, 10). Notably, only nuclear-localizing isoforms of Bin1 can restrict proliferation, survival, and immune escape of oncogenically transformed cells (1, 2, 11-17). Bin1 is widely inactivated in human cancers by attenuation or mis-splicing (1, 12-14, 18-20). However, the consequences of Bin1 loss to cancer susceptibility in an animal has not been fully evaluated to date.

Homozygous inactivation of Bin1 causes perinatal lethality associated with severe cardiac hypertrophy (21). Therefore, to assess roles of this gene beyond cardiac development, we generated mosaic null mice using a recently constructed “floxed” conditional mutant (22). In mosaic animals, recombination is distributed throughout the animal, offering several inherent advantages for investigating the impact of gene loss on diverse processes including tumorigenesis. First, null cells are distributed throughout every tissue, so the impact of gene loss in different organs can be evaluated without having to generate multiple independent lines harboring different tissue-specific Cre-expressing alleles. Second, each mouse is internally controlled, provided that the gene of interest is haplosufficient, because tissues include cells that are both null (recombined) and expressing (non-recombined). Third, mosaic analysis is useful in mixed genetic backgrounds because the paired control is derived from the same animal rather than from a different littermate with a different distribution of parental alleles. Fourth, Cre expression is restricted to early embryogenesis, alleviating the concern that contemporaneous Cre activity may influence phenotype, a common concern in tissue-specific knock-outs. Lastly, a mosaic model allows one to gauge the impact of field effects by allowing one to vary the extent of gene loss in a tissue. In this study, we employed a mosaic model to evaluate the contribution of Bin1 to cancer suppression in mammals. Our findings suggest that Bin1 limits age-associated inflammation and cancer.

Materials and Methods

Generation and characterization of transgenic mouse strains. We modified the standard design for Cre-mediated gene targeting by introducing a point mutation into the 3’-most loxP site, which we found to (a) confer a selective advantage to Cre-mediated excision of the marker
in vivo while maintaining Cre-mediated excision of the target sequence in vitro; and (b) favor the production of mosaic null animals relative to the standard technology, which was desired for this project. The targeting plasmid has been described previously in a study of tissue-specific gene deletion (22). Briefly, a neo/nosymycin resistance gene (neo) cassette flanked by wild-type (wt) loxP sites was inserted into a genomic targeting vector spanning introns 2 to 5 of the mouse Bin1 gene (23). ES cells with the desired homologous recombination event were infected with a recombinant Cre adenovirus and subcloned to identify cell colonies that had selectively lost the neo marker, leaving intact the targeted exon 3 segment. Correctly targeted ES cell lines were microinjected into C57BL/6j blastocystos, and chimeric mice expressing germ line transmission were interbred to produce strains that included the wt allele (+), floxed knock-out allele (flo), and a previously constructed straight knock-out allele (KO; ref. 21). Bin1 is known to be haplosufficient for viability (21). Therefore, to establish the most efficient system for producing Bin1-expressing or nonexpressing cells by a single Cre-mediated excision event, we crossed the floxed allele (flo) onto a strain with the "straight" knock-out allele (KO; ref. 21). To mediate recombination of the flox allele, Cre alleles were introduced by crosses to the transgenic mouse strains FVB-TgN (EIIa-Cre) C57BL6J (EIIa-Cre mice).

Crossing female rather than male EIIa-Cre mice produced higher rates of mosaic offspring (67% versus 42%)(6). Therefore, female EIIa-Cre mice were used to generate more Bin1 mosaic mice. Tumor formation was monitored in mice up to 18 to 21 months of age, after which animals were euthanized and tissues were isolated and fixed in 10% neutral buffered formalin for histopathologic analysis using standard methods. To monitor fecundity, if no evidence of pregnancy was observed within 4 weeks of mating male and female mice together, the male mice were replaced. Where offspring emerged, litter size was recorded, and the age of the mother was calculated from the date of coitus based on the appearance of a vaginal plug.

Genotype analysis. PCR was used to genotype mice as follows. Mouse tissue samples were digested overnight at 60°C in lysis buffer [50 mmol/L Tris-HCl (pH, 8.0), 100 mmol/L EDTA, 100 mmol/L NaCl 1% SDS, 30 mg/ml proteinase K]. DNA-containing supernatant was diluted 1/50 in 10 mmol/L Tris-Cl (pH, 8.0), and 2 μL of diluted supernatant was used for PCR in a final volume of 20 μL in a PTC-200 Peltier Thermal Cycler (MJ Research). Amplification products were separated by electrophoresis on 2% agarose gels prestained with ethidium bromide using Haelll-digested X174 phage DNA (Fisher) as a molecular size marker. The primers used to monitor the Bin1lox allele were LoxP1 5’-GGAGTTGCGACACCTCTATCC-3’ and LoxP2 5’-GCTCTACACCTCTGAGAAGAC-3’, with expected sizes of 0.9, 1.07, and 0.31 kb for wt, flox, and recombined flox (flox+) alleles, respectively. Following a 4-min denaturation at 94°C, 35 cycles of PCR were done at 94°C for 20 s, 58°C for 1 min, and 72°C for 1 min, with the addition of a 10-min final elongation step at 72°C. The primers and PCR conditions used to monitor the Bin1 KO allele have been described (21). The primers used to monitor the EIIa-Cre gene were Cre1 5’-GCCACCAGCTTGCATGATC-3 and Cre2 5’-GCCACCGCTTGATGATGAC-3’ with allele-positive mice identified by a single 512-bp agarose gel band. PCR conditions for the Cre gene were 2 min denaturation at 94°C followed by 29 cycles of PCR at 94°C for 15 s, 53°C for 30 s, and 72°C for 1 min, with a final 10-min elongation step at 72°C.

Colon carcinogenesis. 1,2-Dimethylhydrazine (DMH) was administered on a traditional protocol as described previously (24). Briefly, mice 6 to 8 weeks old were injected i.p. with DMH each week for 20 weeks at a dose of 30 mg/kg in 10 mmol/L sodium bicarbonate/10 mmol/L EDTA (pH, 8.0). Animals were euthanized 27 weeks after the initial injection, and intestinal tissues that included visible tumors at necropsy were harvested and fixed in 10% neutral buffered formalin for histopathologic analysis using standard methods.

Results

Generation and validation of Bin1 mosaic mice. Homozygous deletion of Bin1 in mice causes perinatal lethality associated with a severe hypertrophic cardiomyopathy (21). Therefore, to bypass lethality, we generated mosaic mice using a conditional floxed knock-out of Bin1 that we have described recently (22). The scheme is illustrated in Fig. 1A. Briefly, deletion of exon 3 leads to exon 2 to 4 splicing that produces out-of-frame stop codon in exon 4. Our design incorporated a mutant loxP site containing a T→C mutation at the 3’-most loxP site in the construct, which confers a selective advantage to in vitro excision of the neo cassette without overly compromising in vivo excision of the target sequences.6 To generate mosaic mice, we employed EIIa-Cre transgenic mice where Cre recombinase expression is controlled by the EIIa promoter. In the absence of adenovirus E1A coactivator, expression driven by the EIIa promoter is restricted to mouse oocytes and preimplantation embryos including the one-cell stage zygote (25, 26). EIIa-Cre and Bin1KO/+ mice were interbred to obtain Bin1KO/+:EIIa-Cre(+/+) offspring. These offspring were then crossed with Bin1lox/flox mice to obtain Bin1KO/loxΔEIIa-Cre(+/+) mice (Bin1 mosaic nulls), Bin1+/loxΔEIIa-Cre(+/+) mice (Bin1 mosaic heterozygotes), and Bin1+/loxEIIa-Cre(+/−) mice (Bin1 non-recombined controls). For simplicity, these strains are referred in the text below as Bin1 mosaic −/−, Bin1 mosaic +/−, and Bin1+/+. Genotype was defined by the wt, flox, and floxΔ alleles, which generated specific PCR products of 0.90, 1.07, and 0.31 kb, respectively (Fig. 1B). Although recombination frequency varied between individual mosaic mice, the proportion of cells harboring recombinated to non-recombined alleles was consistent across multiple tissues (Fig. 1C). Because the proportion of recombinated to non-recombined alleles in all tissues could be predicted by noninvasive genotypic analysis of a standard tail biopsy, to approach the nullizygous state, we selected viable Bin1 mosaic −/− mice with the highest proportion of recombinated alleles as reported by PCR analysis (e.g., as illustrated by animal A in Fig. 1D). In contrast to designs using wt loxP sites, where EIIa-Cre targeting produces ~50% systemic knockouts and ~50% mosaic knock-outs (25), we found that our design using the variant 3’ loxP site produced 63% mosaic knock-outs, 6% systemic knock-outs, and 31% non-recombiant animals among progeny from multiple matings (Fig. 1E). These results showed that using the variant 3’ loxP site increased the efficiency of producing mosaic mice.

To confirm that the floxΔ allele was a true functional knock-out, we determined whether complete systemic recombinants phenocopied the myocardial hypertrophy and perinatal lethality of the straight KO/KO mouse (21). Progeny from five independent litters were examined after crossing Bin1KO/− mice with Bin1loxΔ+/− mice that had been defined as germ line recombinants by genotype analysis and progeny testing. Of the neonates obtained from the litters, 11/52 (21%) were unhealthy, died shortly after birth, and were determined to have inherited the KO/floxΔ genotype. Histologic analysis of the hearts from pups that died confirmed that they had a severe myocardial hypertrophy indistinguishable from that characterized previously in KO/KO neonates (Fig. 1F). We concluded that Cre-mediated recombination of the flox allele could produce a functional knock-out of Bin1.

While breeding heterozygous Bin1+/KO mice over a period of several years, we noticed no apparent phenotypes except that females remained reproductively fertile until well past 1 year of age.

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6 M.Y. Chang, unpublished observations.
Breeders in the mouse colony at our institute are typically retired by 8 months due to poor reproductive capability, so this phenotype was unusual. An examination of the breeding history of 23 female Bin1+/KO mice collected over 2 years indicated that the mean age at the last recorded litter was 14.1 ± 0.5 months (Supplementary Table S1). In contrast, an explicit measurement of the mean age of the last litter in Bin1+/+ females was 11.3 ± 1.2 months, consistent with observations in aged C57BL/10Sn mice that have been

Figure 1. Generation and validation of a conditional allele of the murine Bin1 gene. A, scheme used to produce mosaic mice (see text for details). B, variant loxP site produces complete or mosaic gene knock-out. Tail genomic DNA from offspring of Bin1 flox+ and Ella-Cre transgenic was evaluated by PCR for Cre-mediated recombination. The wt, flox, and floxΔ alleles yield products of 0.90, 1.07, and 0.31 kb, respectively. Both recombined and intact flox alleles are present in tail DNA from mosaic mice. Marker, HaeIII-digested $\phi$X174 phage DNA. C, Cre-mediated recombination occurs with consistent efficiency across different tissues. Tissues from a single animal were examined. D, the extent of cre-mediated recombination varies in individual mosaic mice. Two mice exhibiting a high or low degree of conversion of the flox allele to the floxΔ allele are shown (A and B). In six tissues examined, the proportion of cells with a recombined allele is consistent with prior analysis of tail biopsies. E, the floxΔ allele is a functional knock-out. Histologic analysis of the heart from Bin1(KO)/(floxΔ) pups that expired at birth revealed severe myocardial hypertrophy indistinguishable from that seen in Bin1 null mice (21). dpp, days postpartum.
reported previously (27). Our findings were extended in the Bin1 mosaic model, particularly in null mosaic females whose last litters were at an unusually old age of 17.3 ± 1.1 months (Supplementary Table S1). This striking effect suggested that Bin1 negatively modified some aspect of reproductive physiology during aging.

**Bin1 inhibits inflammation and premalignant lesions in the heart and prostate during aging.** No effects of Bin1 attenuation were seen in any mice through 1 year of age; however, we observed a markedly increased incidence of inflammatory conditions and/or premalignant lesions in more elderly animals (Table 1). By 18 to 20 months of age, 16% to 29% of mosaic mice displayed myocarditis, an inflammatory condition in the heart (Fig. 2A). Additionally, several mosaic mice displayed evidence of pancreatitis (inflammation of the pancreas; Fig. 2B). These conditions are rare in naïve laboratory mice and were not seen in any of the control animals examined. More dramatically, there was evidence of widespread inflammation and/or premalignant lesions in the prostates of mosaic mice (Fig. 2C–F). Prostatitis (inflammation of the prostate) was observed in ~20% of mosaic mice but no animal in the control group. Hyperplasia was evident in 53% to 72% of the mosaic

### Table 1. Inflammation and premalignant lesions in *Bin1* mosaic mice

<table>
<thead>
<tr>
<th>Tissue and abnormality</th>
<th>Bin1 mosaic −/− (N = 19)</th>
<th>Bin1 mosaic +/− (N = 32)</th>
<th>Bin1+/+ (N = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td>3/19 (16%)</td>
<td>6/32 (19%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty metamorphosis</td>
<td>3/19 (16%)</td>
<td>6/32 (19%)</td>
<td>5/12 (42%)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0/19 (0%)</td>
<td>2/32 (6%)</td>
<td>1/12 (8%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>1/19 (5%)</td>
<td>2/32 (6%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostatitis</td>
<td>4/19 (21%)</td>
<td>6/32 (19%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Prostate hyperplasia</td>
<td>10/19 (53%)</td>
<td>23/32 (72%)</td>
<td>1/12 (8%)</td>
</tr>
<tr>
<td>Prostate atypia</td>
<td>4/19 (21%)</td>
<td>5/32 (16%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Prostate intraepithelial neoplasia</td>
<td>1/19 (5%)</td>
<td>1/32 (3%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>6/19 (32%)</td>
<td>9/32 (28%)</td>
<td>3/12 (25%)</td>
</tr>
</tbody>
</table>

NOTE: Major organs were collected at necropsy from mice of 18 to 20 mo of age and processed for histologic examination.

![Figure 2](image_url). Inflammation and premalignant lesions in *Bin1* mosaic mice at 18 to 20 mo of age. Representative histologies are shown.
mice in comparison to only a single case in the control group. Prostate atypia and prostate intraepithelial neoplasia, both frank premalignant lesions, were detected in 16% to 21% and ~4% of mosaic mice, respectively, but not in any control animal. These findings were specific to prostate insofar as we saw a similar incidence of seminal vesicle inflammation in each cohort (Table 1). Furthermore, in mosaic animals, we observed an opposite trend in the incidence of fatty metamorphosis of the liver, a lesion documented in both mosaic and control cohorts (Table 1). Together, these observations argued that Bin1 acted to limit the development of inflammation and premalignant lesions during aging.

**Bin1 inhibits the development of lung adenocarcinoma and hepatocarcinoma during aging.** We previously noted a modest hyperplasia in lungs of Bin1KO/KO embryos harboring a complete gene knock-out (Supplementary Fig. S1). This phenotype was not seen in the Bin1 mosaic knock-out mice, which preserved sufficient Bin1 function to complete development and which lacked any apparent phenotype through ~1 year of age. In contrast, in older mice of 18 to 20 months of age, there was a striking increase in the incidence of lung tumors, with 47% of mosaic mice presenting with tumors at this time compared with a single case seen in the control group (Table 2 and Fig. 3A). This observation suggested that the hyperplasia in KO/KO embryonic lung may represent a premalignant lesion possibly controlled in the mosaic setting until older age. The lung tumors that arose were histopathologically defined as intrabronchial in situ papillary carcinoma (A.P. Soler, data not shown), establishing that they were of lung epithelial origin. Background issues that defeat the use of Bin1 antibodies in staining mouse tissues prevented us from performing an immunohistologic analysis of Bin1 in normal or tumor lung tissues; however, Northern and quantitative reverse transcription-PCR analyses confirmed a relative reduction in Bin1 RNA levels in tumors arising in mosaic animals compared with normal tissues (Supplementary Fig. S2). In human tissues where Bin1 antibodies are fully validated for immunohistochemical analysis (28), we were able to examine immunohistochemical status in lung and lung adenocarcinoma. In normal bronchial epithelia and stage I tumors (localized disease), we documented strong Bin1 expression, whereas in cases of stage II to IV lung adenocarcinoma reduced expression was apparent (Supplementary Fig. S3). The patterns of normal expression and immunohistochemical losses in tumors was reminiscent of that seen in other epithelial tumors such as breast and prostate tumors (13, 14, 28). Taken together, these findings were internally consistent in suggesting a role for Bin1 in the suppression of lung carcinoma during aging.

In mice of 18 to 20 months of age, we also observed a smaller but significant increase in the incidence of hepatocellular carcinoma (HCC), with 6% to 11% of mosaic mice but no control mice exhibiting tumors (Table 2 and Fig. 3B). As noted above, fatty metamorphosis of the liver was documented in all animals but was slightly reduced in mosaic animals (Table 2). Clinical studies indicate that this lesion can be a precursor to cirrhosis and HCC, but typically in association with alcohol abuse or obesity (29). Further studies may reveal greater insight into the relationship between Bin1 ablation and the incidence of fatty metamorphosis as a possible precursor to HCC.

The effects of Bin1 ablation on cancer incidence during aging were selective, insofar as mosaic and control mice displayed a relatively similar incidence of lymphoma, a cancer that arises commonly in old mice (Table 2). Taken together with the above findings, we concluded that Bin1 acted as a negative modifier that suppresses hepatocarcinoma and lung adenocarcinoma during aging.

**Bin1 inhibits colon carcinogenesis.** We reasoned that a modifier effect of Bin1 on spontaneous cancers arising in older mice might also be manifested in carcinoogen-induced cancers in younger animals. To examine this possibility, we compared the response of mosaic and control animals to i.p. administration with DMH, a ras-activating carcinogen that induces gastrointestinal cancers. In the protocol used, weekly treatment with DMH induced mainly colon tumors. All animals in the mosaic group and all but one animal in the control group presented with colon tumors at the experimental endpoint of 27 weeks. However, invasive tumors were displayed in 33% of the mosaic mice but none of the control animals. Moreover, tumor multiplicity was greater, and mouse weight was reduced at the experimental endpoint, consistent with a more progressive status of tumors in the Bin1 mosaic mice (Table 3 and Supplementary Fig. S4). Support for the clinical relevance of these observations were provided by the results of a pilot immunohistochemical study of Bin1 status in 30 cases of human colon carcinoma. In normal colonic epithelia, strong progressive staining of villus cells was observed, similar to that documented previously (28), whereas >50% of the carcinomas examined showed strongly reduced expression of Bin1 (Supplementary Fig. S5). Taken together, these observations reinforced and extended the conclusion that Bin1 acts to suppress the development and/or progression of epithelial cancers of the colon, lung, and liver.

<table>
<thead>
<tr>
<th>Table 2. Tumors arising in aging Bin1 mosaic mice</th>
</tr>
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<tbody>
<tr>
<td><strong>Tissue</strong></td>
</tr>
<tr>
<td>Lung Adenocarcinoma</td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>HCC</td>
</tr>
<tr>
<td>Lymph node</td>
</tr>
<tr>
<td>Lymphoma</td>
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</table>

**NOTE:** Tumors were scored macroscopically in animals of 18 to 20 mo of age at necropsy and subsequently verified by histologic examination. If less than two events of an abnormality were scored in the mosaic cohort, they are not listed, based on a lack of significance; but for abnormalities noted in the table, all the events are noted for the corresponding control group.
Discussion

Genetic modifier pathways dramatically influence rates of cancer initiation and progression (30, 31). Using a genetically mosaic mouse model that bypasses perinatal lethality associated with systemic inactivation of Bin1, we have identified physiologic roles for this gene as a negative modifier of fecundity and cancer susceptibility during aging. Genetic mosaics have been used widely to study otherwise lethal mutations in Drosophila (32), but this approach has been used little in mice despite its ability to successfully rescue embryonic lethal phenotypes (33). Our strategy using a mutated loxP site proved advantageous in several ways. First, it eased production of the desired ES cell line by facilitating selective in vitro deletion of the neo marker while leaving the targeted sequences intact (34). At this stage in strain construction, the desired partial recombination event in a “tri-lox” ES allele usually occurs rarely, sometimes preventing the ability to obtain the desired cell clone for chimera generation. Thus, introducing a point mutation into the 3′-most loxP site conferred a selective advantage to achieve Cre-mediated excision of the marker in vitro within a tri-lox allele without abolishing Cre-mediated excision of the floxed target sequences in vivo. Second, the presence of the mutated loxP site elevated the efficiency of mosaic animal generation by skewing the recombination pattern from complete recombinants occurring at the one-cell stage of development to

Table 3. Bin1 ablation drives progression during colon carcinogenesis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tumor incidence</th>
<th>n*</th>
<th>Mouse weight at endpoint (g)</th>
<th>Early†</th>
<th>Noninvasive‡</th>
<th>Invasive§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bin1+/+</td>
<td>9/10</td>
<td>1.8 ± 0.4</td>
<td>30.2 ± 1.7</td>
<td>1/9</td>
<td>8/9</td>
<td>0/9</td>
</tr>
<tr>
<td>Bin1 mosaic −/−</td>
<td>10/10</td>
<td>2.3 ± 0.5</td>
<td>26.5 ± 0.6</td>
<td>0/9</td>
<td>6/9</td>
<td>3/9</td>
</tr>
</tbody>
</table>

NOTE: Mice in mosaic and control groups (n = 10) were treated with DMH to induce colon tumors as described in the Materials and Methods. Tumors were harvested from euthanized animals at necropsy and processed for histology. Age at euthanasia was 7.8 to 8.0 mo for all DMH-treated mice. Representative examples of tumor histologies are presented in Supplementary Fig. S3.

*Number of tumors per colon scored (multiplicity).
†Submucosal tumors.
‡Mucosal tumors, no muscle invasion apparent.
§Muscle-invasive tumors.
mosaic recombinants occurring after oocyte division had begun. Lastly, using this strategy, we learned that delivering the cre allele through the father recombination occurs with much lower efficiency when than through the mother. Thus, we were able to develop breeding strategies to allow efficient generation of mosaic animals and non-recombinant control animals, despite the common presence of the EIIa transgene.

Although the systemic disruption of Bin1 causes ventricular hypertrophic cardiomyopathy and perinatal lethality (21), mosaic animals readily survived to adulthood even in situations where there was a significant nullizygosity in all organs including the heart. This result indicates that the presence of a subset of wt cells in the animal is sufficient to compensate for the developmental defect associated with Bin1 loss. However, with regard to its role as a suppressor gene, our findings suggested that Bin1 may be haploinsufficient in its ability to fully limit the development of premalignant lesions and certain cancers during aging. This finding may explain why in human cancers, one usually see attenuations rather than homozygous deletions of Bin1, because partial losses may be sufficient to functionally abrogate its tumor suppressor activity.

We found that Bin1 attenuation increased fecundity during aging, but at the cost of elevating age-associated inflammation and cancer. Recent studies suggest that Bin1 helps coordinate normal stress responses, and that it supports mammary gland remodeling during pregnancy (16, 22, 35). These roles are compatible with a tumor suppressor function, but how they may relate to effects on the period of female fecundity during aging is not yet known. Some tumor suppressor genes exhibit antagonistic pleiotropy, supporting fitness early in life but at a later cost to aging that exerts little evolutionary impact because the deficits accrue after reproduction is complete (36). Because the benefits of increased fecundity seemed to accrue in Bin1 mosaic mice before cancers were detected, it is uncertain whether Bin1 fits this model. In any case, it is tempting to speculate that these disparate phenotypes may be linked by previously documented effects of Bin1 loss on elevating expression of the enzyme indoleamine 2,3-dioxygenase (IDO), a potent regulator of T cell immunity (37). In cancer cells, IDO elevation caused by Bin1 attenuation can drive tumoral immune escape and progression (17). In pregnancy, IDO elevation in the placenta limits T cell activation by foreign paternal antigens, stabilizing pregnancies by preventing conceptus rejection (38). In future work, it will be important to determine whether the two phenotypic manifestations associated with Bin1 loss during aging in the mouse are causally related to dysregulation of IDO activity.

We noted an increased incidence of inflammation and/or premalignant lesions in the heart, pancreas, liver, and prostate of Bin1 mosaic mice during aging. Myocarditis was an interesting phenotype given that systemic inactivation Bin1 causes cardiomyopathy during development (21), and that the human Bin1 gene maps to a susceptibility locus for the development of dilated cardiomyopathy (39). Our observations suggest that Bin1 might modify disease in this setting by influencing cardiac inflammation during aging. In Bin1 mosaic mice, we also observed a modest increase in the incidence of pancreatitis, a known risk factor for pancreatic cancer. More dramatically, aging mosaic mice displayed an increase in prostatitis and prostate hyperplasia and in the frank premalignant lesions of prostate atypia and intraepithelial neoplasia. These findings were particularly notable given the evidence that loss of heterozygosity and expression of Bin1 occur often in human cases of metastatic prostate cancer (14). Thus, by promoting inflammation, our findings suggest that Bin1 attenuation might contribute to prostate tumorigenesis during aging or in settings where appropriate initiating lesion(s) are present.

We found that Bin1 ablation greatly increased the incidence of lung adenocarcinoma during aging, with a lesser increase in HCC also evident. Tumor susceptibility varies widely among laboratory mouse strains, but lung and liver cancers occur rarely even in elderly mice. For example, with regard to lung tumors in strains relevant to this study, a lifetime incidence of lung tumors of 1% to 3% has been reported for C57BL/6 (Mouse Genome Informatics) and of 7.7% with a latency of ~21 months has been reported for FVB-N (40). In our work, aged-matched Bin1+/+ control mice on the same mixed strain background exhibited only one case of lung cancer consistent with the published low rate of incidence. Further evidence that Bin1 suppresses cancer was provided by the finding that Bin1 mosaic mice were more susceptible to colon carcinogenesis, where Bin1 ablation heightened the progression status of arising tumors. This finding corroborates and extends a recent study showing that mammary gland-specific deletion of Bin1 is insufficient to initiate tumor formation, but sufficient to drive tumor progression (22). Here, we emphasize that the findings of both studies are consistent: breast cancers were not expected to arise in mosaic mice, because mammary gland-specific deletion of Bin1 is insufficient for development of breast cancer during a similar 2-year period which is sufficient to yield development of lung and liver cancers in mosaic mice where Bin1 was more widely inactivated. Consistent with previous findings (22), we found that mosaic mice exhibit a heightened progression status (more advanced histology) following the induction of 7,12-dimethylbenz(a)anthracene (DMBA)-induced breast cancers. In summary, Bin1 can limit cancer incidence or progression in different settings, perhaps related to the extent to which its role in limiting inflammation may be important at different stages of tumor development in those settings.

Because Bin1 suppresses tumor formation in part by cell nonautonomous mechanisms that support immune surveillance (17), our findings prompt further study of the effects of tissue-specific ablation of Bin1 in lung, liver, and colon epithelial cells. In humans, cancers of the lung, liver, colon, and prostate occur usually in elderly individuals. Given evidence of frequent immunohistochemical losses of Bin1 in human lung and colon cancers, paralleling related findings in breast and prostate cancers (1, 13, 14, 28, 41), the striking age-associated cancer phenotypes in the Bin1 mosaic mice argues that such losses may be clinically relevant. In this regard, further studies of the mosaic model may permit new insights into cancer pathophysiologies associated with immune escape, inflammation, and aging.

Acknowledgments

Received 3/22/2007; revised 5/25/2007; accepted 5/30/2007.

Grant support: G.C. Prendergast is the recipient of NIH R01 grants CA92222, CA100123, and CA10954. Additional support for this project was provided by grants to G.C. Prendergast from the Charlotte Geyer Foundation, the Department of Defense Prostate Cancer Research Program (PC020328), and the Lankenau Hospital Foundation. A.J. Muller is the recipient of grants from the Lance Armstrong Foundation, the Concern Foundation, the Department of Defense Breast Cancer Research Program, and the State of Pennsylvania Department of Health (CURE/Tobacco Settlement Award). 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.

We thank Gwen Guillard for performing the extensive tissue sectioning and histology in this project. James DuHadaway and Erika Sutanto-Ward are acknowledged for technical support at early stages of this project.
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