Genetic Background Affects Susceptibility to Mammary Hyperplasias and Carcinomas in Apc\textsuperscript{Min/+} Mice\textsuperscript{1}

Amy Rapaich Moser,\textsuperscript{2} Laura F. Hegge, and Robert D. Cardiff

Department of Human Oncology, University of Wisconsin-Madison, Madison, Wisconsin 53792 [A. R. M., L. F. H.], and Center for Comparative Medicine, University of California-Davis, Davis, California 95616 [R. D. C.]

ABSTRACT

Treatment of female C57BL/6J (B6) mice carrying the mutant Min allele of the adenomatous polyposis coli (Apc) gene with ethylnitrosourea (ENU) results in \(\sim 90\%\) of mice developing an average of three mammary tumors within 65 days. As a first step in the identification of loci modifying susceptibility to ENU-induced mammary tumors and hyperplasias, we have tested ENU-treated Apc\textsuperscript{Neo/+} (Min/+ ) mice on several hybrid backgrounds for susceptibility to mammary and intestinal tumors. C57BR/cdJ×B6 (BRB6) Min/+ mice were more sensitive to development of mammary squamous cell carcinomas than B6 Min/+ mice. In contrast, Min/+ hybrids between B6 and FVB/NTac (FVB), 129X1/SvJ (129X1), and 129S6/SvEvTac (129S6) were all significantly more resistant to mammary carcinoma development. However, mice from these three crosses developed more focal mammary hyperplasias than did the B6 or BRB6 Min/+ mice. Susceptibility to intestinal tumors was independent of mammary tumor susceptibility in most hybrids. These results indicate that genetic background can affect independently the phenotypes conferred by the Min allele of Apc.

INTRODUCTION

For most women who develop breast cancer, no predisposing genetic lesion can be identified. Even when a genetic predisposition has been identified, such as in a mutation in BRCA1 or BRCA2, the risk of tumor development is not 100%, and the age at time of development can be quite variable (1, 2). Although some of this variability in phenotype may be attributable to environmental factors, genetic factors also may be involved (3). Identification of genetic modifiers of susceptibility could lead to better risk assessment and, potentially, the development of agents to decrease risk. Given the potentially heterogeneous causes of breast cancer in humans and the genetic variability in the human population, it may be difficult to identify genes that contribute to susceptibility to cancer in humans. However, animal models may provide a means with which to begin to identify and study modifier genes involved in susceptibility to breast cancer.

Strain differences in susceptibility to spontaneous and carcinogen-induced mammary tumors have been demonstrated for both mice and rats (4). The susceptibility of some mouse strains to mammary tumor development is related to the susceptibility to be infected by MMTV\textsuperscript{3} or the presence of endemic MMTV infection within the strain. In rats, several genes have been implicated in the control of susceptibility to mammary tumor development (5). Mammary tumors are not frequent in most mice in the absence of MMTV infection, a predisposing mutation, or targeted expression of an oncogene. The strains of mice commonly used for production and analysis of targeted mutations, 129

\textsuperscript{1}The abbreviations used are: MMTV, mouse mammary tumor virus; ENU, ethylnitrosourea; SCC, squamous cell carcinoma.

\textsuperscript{2}Received 8/18/00; accepted 2/8/01.

\textsuperscript{3}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.

\textsuperscript{4}Supported in part by NIH Grant CA64843 (to W. F. D.) and a UWCCC Pilot Project Grant (to A. R. M.).

\textsuperscript{5}To whom requests for reprints should be addressed, at Department of Human Oncology, K4/330 CSC 3864, 600 Highland Avenue, University of Wisconsin-Madison, Madison, WI 53792. Phone: (608) 265-6520; Fax: (608) 263-9947; E-mail: moser@mail.humonc.wisc.edu.

\textsuperscript{6}Mice. Female C57BL/6J (B6), C57BR/cdJ (BR), and 129X1/SvJ (129X1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Female 129S6/SvEvTac (129S6) and FVB/NTac (FVB) mice were purchased from Taconic Farms (Lake Placid, NY). C57BL/6J Min/+ (B6 Min/+ ) mice were bred at the University of Wisconsin. The B6 Min/+ line is maintained by crossing wild-type B6 females with B6 Min/+ males. All B6 Min/+ mice used and B6, rarely develop mammary tumors either spontaneously or after carcinogen treatment (4). However, the loci that confer resistance are unknown and may differ between the strains. The FVB strain, which is commonly used to generate transgenic mice, is not prone to spontaneous mammary tumors but little is known about the response of the mammary gland to carcinogens (6, 7).

One requirement for the identification of genes that control susceptibility to tumor development in mice is that a characteristic tumor susceptibility. Mice carrying a mutant allele of the adenomatous polyposis coli (Apc) gene are predisposed to intestinal and mammary tumors (8, 9). On the B6 genetic background, all of the mice carrying the Min (multiple intestinal neoplasia) allele of Apc develop \(\sim 100\%\) adenomas throughout the intestinal tract by 100 days of age. B6 Min/+ female mice have a spontaneous mammary tumor incidence of \(\sim 5\%\) by 100 days of age in virgin females (8). Because B6 Min/+ mice rarely survive beyond 120 days because of the effects of intestinal tumors (10), this mammary tumor incidence is perhaps an underestimate of the effect of Min on the mammary gland. Treatment of mice carrying a mutant allele of Apc either with a carcinogen, such as ENU (8), or with irradiation (9), increases the incidence and multiplicity of mammary tumors. In addition, transplanted Min/+ mammary epithelial cells give rise to more mammary tumors than do transplanted wild-type cells when the wild-type hosts are treated with dimethylbenzanthracene (8). These observations indicate that the susceptibility to development of mammary tumors is intrinsic to the mammary tissue.

Min/+ mice have proven very useful in the identification of other genes that can affect intestinal tumor development. Crosses of B6 Min/+ mice with mice from the AKR/J strain led to the identification Mom1, a modifier of intestinal tumor development (11). Min mice have also been used to demonstrate a role in intestinal tumor development for proteins such as Dnmt1 (12), Matriaryl13 (13), and insulino-like growth factor II (14).

As a first step toward identification of genes that control mammary tumor susceptibility, hybrid Min/+ mice were generated and tested for ENU-induced mammary tumors and hyperplasias. B6 Min/+ mice and BRB6 F1 Min/+ mice were very sensitive to the development of squamous cell carcinomas of the mammary gland. Mice from crosses to either of two 129 strains and the FVB strain were very resistant to mammary tumor development. However, these resistant hybrid mice were sensitive to the development of hyperplasias of the mammary gland. In contrast, the B6 and BRB6 F1 Min/+ mice had few hyperplastic lesions. Genetic background also affected intestinal tumor number, but the mammary and intestinal phenotypes were not correlated. This indicates that at least some of the modifier loci may function in a tissue-specific manner.

MATERIALS AND METHODS

Mice. Female C57BL/6J (B6), C57BR/cdJ (BR), and 129X1/SvJ (129X1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Female 129S6/SvEvTac (129S6) and FVB/NTac (FVB) mice were purchased from Taconic Farms (Lake Placid, NY). C57BL/6J Min/+ (B6 Min/+ ) mice were bred at the University of Wisconsin. The B6 Min/+ line is maintained by crossing wild-type B6 females with B6 Min/+ males. All B6 Min/+ mice used

3480
for these experiments were from generations N34 to N38. F1 Min/+ mice were generated by crossing female mice from each strain with B6 Min/+ males. All mice were fed TEEKLAD Breeder chow and had access to acidified water ad libitum. Mice were maintained at the University of Wisconsin Medical School Research Animal Facility, which is approved by the American Association for Accreditation of Laboratory Animal Care and is maintained in accordance with the regulations and standards of the United States Department of Agriculture and the Department of Health and Human Services, NIH.

Genotyping. DNA for genotyping was isolated from 50 µl of blood (11). The genotype at Apc was determined by an allele-specific PCR assay (11).

ENU Treatment and Tumor Collection. Min/+ and +/+ female mice were given a single i.p. injection of 50 mg/kg body weight of ENU between 35 and 45 days of age (8). Mice were palpated weekly to detect mammary tumors, and the date of the first confirmed detectable tumor was recorded. B6 Min/+ mice were killed when moribund or ~70 days after ENU treatment; hybrid mice were killed when moribund or up to 180 days after ENU treatment. For each litter, +/+ mice were killed at the same age as most of their Min/+ siblings. Large mammary tumors present in otherwise healthy mice were surgically resected, and the animals were followed until they developed a second large mammary tumor, became moribund, or reached the end of the study time. No animal underwent more than one survival surgery. Animals were killed by CO2 asphyxiation, and visible mammary tumors were noted and recorded. Mice were killed by CO2 asphyxiation, and visible mammary tumors were noted and collected. Mammary tumors that were present at the site of a resected tumor were considered recurrences and not new tumors. If two closely spaced tumors could not be clearly delineated, they were counted as one tumor. Tumors were fixed in formalin for 24 h and then transferred to 70% ethanol before embedding and sectioning for histological evaluation.

The 1st (cervical), 4th (abdominal), and 5th (inguinal) mammary fat pads were collected, fixed, and stained to allow the enumeration of small lesions within the mammary gland. Glands were spread on glass microscope slides and placed into 70% ethanol for several days. The glands were fixed for 1 h in 1:3 glacial acetic acid:100% ethanol and then dehydrated through 90, 70, and 50% ethanol into dH2O. Glands were then transferred to Carnimine stain [2.5 g Alum potassium sulfate, 1.0 g Carnimine (C-6152; Sigma) in 500 ml dH2O] for 1–3 days. Glands were dehydrated through 70, 90, and 100% ethanol, transferred to xylenes overnight, and then stored in 100% glycerol. All mammary glands were examined by a single observer (A. R. M) without knowledge of the genotype at Apc. Small tumors and other lesions were enumerated and photographed within the whole mount and then collected for embedding and sectioning for histological evaluation. All sections were classified by a single observer (R. D. C) without knowledge of whether the sample was collected at necropsy or from the whole mounts.

Intestines were collected at the time of necropsy and processed as previously described (10). Intestinal tumors in four representative regions of the small intestine and the colon were counted by a single observer (L. F. H.).

Statistical Analysis of Tumor Number. Analyses were done using the MSTAT computer program provided by Dr. Norman Drinkwater, McArdle Laboratory University of Wisconsin, Madison, WI. For comparison of tumor numbers, the Wilcoxon Rank Sum test was used. Fisher’s exact test was used for tests of tumor incidence. The Log rank test was used for comparisons of time to first tumor.

RESULTS

Effect of Genetic Background on Mammary Tumor Development. We crossed B6 Min/+ male mice with female mice from the BR, FVB, 129X1, and 129S6 strains. Female F1 offspring from all of the crosses were treated with a single dose of ENU between 35 and 45 days of age. B6 Min/+ female mice were included in all of the rounds of ENU treatment to establish a baseline of tumor susceptibility of B6 Min/+ mice. Some mice from each cross were not ENU treated and were scored for mammary and intestinal tumors at the time of necropsy. No mammary tumors were seen in a cohort of untreated female Min/+ and +/+ mice from each cross (data not shown).

Mammary tumors were counted and collected as described in “Materials and Methods.” The incidence, mean tumor number, and time to first mammary tumor for the B6 and F1 mice are presented in Table 1 and Fig. 1. B6 Min/+ female mice developed an average of more than three tumors per mouse by 65 days after treatment. BRB6 F1 Min/+ female mice developed significantly more mammary tumors than did the B6 Min/+ mice (P = 0.016), an average of nearly five. The number of mice with tumors and the time to first tumor were not significantly different between these two strains (P = 1 and P = 0.06, respectively). The increased mammary tumor number of the BRB6 F1 mice may be attributable, in part, to the significantly longer survival times of the hybrid BRB6 F1 Min/+ mice relative to the B6 Min/+ mice (P = 2 × 10−7).

In contrast, most of the 129S6B6 F1 Min/+, 129X1B6 F1 Min/+, and FVBB6 F1 Min/+ mice were tumor free, despite surviving for significantly longer than did the B6 Min/+ mice (P < 0.0001 for all of the comparisons). The mammary tumor numbers in these Min/+ mice are significantly different from that of the B6 Min/+ mice (P < 0.0001 for all of the comparisons). The time to first tumor is also significantly longer for the mice of each of these strains than for the B6 Min/+ mice (P < 0.0001 for all of the comparisons).

The FVBB6 F1 mice were very resistant to mammary tumor development. Three of 18 FVBB6 F1 Min/+ mice developed a total of four mammary tumors, a significantly reduced incidence and multiplicity compared with the B6 Min/+ mice (P < 0.0001). The first tumors were noted 110 days after ENU treatment and the others at 114 and 120 days after treatment. One FVBB6 F1 Min/+ mouse was killed at 93 days after ENU because of a rectal prolapse caused by a colon tumor. The rest of the mice survived for at least 110 days, with five surviving for at least 149 days.

The incidence of mammary tumors in both lines of 129 mice was significantly less than that of the B6 mice (P < 1 × 10−7, for both comparisons). Seven of 17 129S6B6 F1 Min/+ mice developed a total of 14 mammary tumors. One 129S6B6 F1 Min/+ mouse developed six tumors, two mice developed two tumors each, and four mice developed one tumor each. The times to first tumor were 62, 87, 110, and 120 days after ENU treatment. Only 1 of 11 129X1B6 F1 Min/+ mice developed tumors.

Table 1 Genetic background affects mammary and intestinal tumor development in Min/+ mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>No. with mammary tumor (%)</th>
<th>Average no. of mammary tumors/mouse</th>
<th>No. with mammary lesions (%)</th>
<th>Average no. of mammary lesions/mouse</th>
<th>Average no. of intestinal tumors/mouse</th>
<th>Average survival in days after ENU (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>45</td>
<td>42 (93)</td>
<td>3.2 ± 0.9</td>
<td>17 (38)</td>
<td>0.6 ± 0.9</td>
<td>34 ± 10</td>
<td>64 (43–78)</td>
</tr>
<tr>
<td>BRB6</td>
<td>18</td>
<td>17 (94)</td>
<td>4.9 ± 2.6</td>
<td>7 (39)</td>
<td>0.5 ± 0.7</td>
<td>14 ± 4</td>
<td>91 (58–118)</td>
</tr>
<tr>
<td>1295S6B6</td>
<td>17</td>
<td>7 (41)</td>
<td>0.8 ± 1.5</td>
<td>11 (85)</td>
<td>2.2 ± 1.6a</td>
<td>36 ± 14</td>
<td>109 (62–120)</td>
</tr>
<tr>
<td>129X1B6</td>
<td>11</td>
<td>1 (9)</td>
<td>0.4 ± 1.2</td>
<td>8 (73)</td>
<td>2.4 ± 2.5</td>
<td>25 ± 14</td>
<td>115 (70–178)</td>
</tr>
<tr>
<td>FVBB6</td>
<td>18</td>
<td>3 (17)</td>
<td>0.2 ± 0.5</td>
<td>18 (100)</td>
<td>4.1 ± 2.4</td>
<td>12 ± 6a</td>
<td>127 (93–178)</td>
</tr>
</tbody>
</table>

_a Information on 13 mice because the glands of 3 mice were lost in processing.
_b Information on 16 mice because the intestines of 2 mice were lost in processing.

3481

Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 2001 American Association for Cancer Research.
mice developed any tumors, with four tumors noted when the mouse was killed 70 days after ENU treatment. No other tumors were seen in the remaining 129X1B6 F1, Min/+ mice, although all of them survived for at least 97 days after ENU treatment and two for 178 days after ENU treatment. Although the 129X1B6 F1 mice seem to be more resistant than the 129S6B6 F1 mice; tumor incidence, tumor number, and small lesions collected from the whole mounts of the mammary glands were analyzed. For the sake of clarity, we will use the term and small lesions collected from the whole mounts of the mammary glands were analyzed. For the sake of clarity, we will use the term "tumor" to apply only to those tumors identified at necropsy as a 5/P lesions was different between the two 129 hybrids ("tumor" to apply only to those tumors identified at necropsy as a 5/P lesions was different between the two 129 hybrids (Fig. 2, A and B), although most had some evidence of squamous metaplasia, usually within central ducts. The squamous nodules were identified in the mammary glands from two FVBB6 F1, Min/+ mice that were not treated with ENU. These were not different in gross or microscopic appearance from the lesions from ENU-treated Min/+ mice of the same strain. In contrast, two lesions noted in the 1st mammary glands of one ENU-treated 129X1B6 F1, +/+ mouse were not similar to the lesions found in the Min/+ mice. In the whole mounts, these lesions appeared to be dense tangles of ducts. No evidence of atypia was noted in the sections from these lesions.

Mammary tumors from ENU-treated B6 Min/+ mice, BRB6 F1, Min/+ mice, and both lines of 129B6 F1, Min/+ mice, and most of the tumors from the FVBB6 F1, Min/+ mice were classified as either well-differentiated keratinizing SCCs (Fig. 2F) or trichoepitheliomas (Fig. 2I). The similarity of these tumors to the squamous nodules and pilar lesions, respectively, raises the possibility that each of these types of hyperplastic lesion gives rise to a specific type of tumor. Only one tumor, from an FVBB6 F1, Min/+ mouse, was classified as a microacinar adenocarcinoma (Fig. 2C). This tumor was first noted at 114 days after ENU treatment. The similarity to the hyperplastic alveolar nodules that were frequent in the FVBB6 F1, Min/+ mice raises the possibility that these hyperplastic lesions could give rise to adenocarcinomas at low frequency.

Effect of Genetic Background on the Development of Hyperplastic Mammary Lesions. The 1st, 4th, and 5th mammary fat pads were collected from all of the mice and scored for small focal lesions not noted at necropsy. Small focal lesions were present in the mammary glands of ENU-treated Min/+ mice from all of the genetic backgrounds, but the number of lesions varied greatly. Fewer than 40% of the B6 or BRB6 F1, Min/+ mice had such lesions, with less than one lesion per mouse. Neither the number of small lesions nor the number of mice with lesions is different between the B6 and BRB6 F1 mice (P = 0.9 for both comparisons). The 129X1B6 F1, 129S6B6 F1, and FVBB6 F1 Min/+ mice all had significantly more lesions than did the B6 Min/+ mice (P = 0.007, P = 0.0003, P = 1 × 10⁻⁶, respectively). Notably, all of the ENU-treated FVBB6 F1 Min/+ mice had small focal lesions, significantly higher incidence than for the B6 mice (P < 2 × 10⁻⁷). More 129S6B6 F1 and 129X1B6 F1, Min/+ mice also had lesions than did the B6 Min/+ mice (P = 0.004 and P = 0.04, respectively). Neither the number nor the incidence of lesions was different between the two 129 hybrids (P = 0.8 and P = 1, respectively). As with tumors, lesions were rare in the glands of untreated Min/+ or +/+ mice or ENU-treated +/+ mice.

Classification of Mammary Tumors and Lesions. Histological sections from representative mammary tumors collected at necropsy and small lesions collected from the whole mounts of the mammary glands were analyzed. For the sake of clarity, we will use the term "tumor" to apply only to those tumors identified at necropsy as a distinct nodule and the term "lesion" will apply to those focal growths identified in the whole mounts. Most of the lesions were <2 mm², although some of the mixed alveolar and squamous nodules from the FVBB6 F1, Min/+ were >2 mm in diameter. In all, 135 lesions were sampled from ENU-treated Min/+ F1 mice (Table 2). No lesions were sampled from B6 Min/+ mice. With a few exceptions, these lesions shared three characteristics: (a) they were focal, (b) they were hyperplastic, and (c) there was no evidence of malignant transformation. The exceptions were the three sampled lesions from BRB6 F1 mice and one from a 129S6B6 F1 mouse. These lesions were classified as SCCs (15). Because these lesions were not noted at necropsy and were <2 mm², they have been included in the lesion category and not the tumor category in Table 1 despite the histological classification.

When viewed in the whole mounts, it was difficult to classify the lesions into clear categories, except for the lesions that were mainly alveolar hyperplasias. However, on sectioning, the lesions could be classified into three general categories. The most common lesions were mixed nodules composed of alveolar hyperplasia and squamous cysts. The alveolar hyperplasia was the predominant feature of these lesions (Fig. 2, I, and J), although most had some evidence of squamous metaplasia, usually within central ducts. The squamous nodule was the second most common type of lesion (Fig. 2, D and E). These nodules were composed mainly of keratinized terminal ductules, often with inflammatory infiltration. In some of these lesions, there was evidence for some alveolar hyperplasia, but this was not the predominant feature of the lesion. The least frequent small lesions were the pilar lesions, which were composed of terminal ductules filled with cells ending in clusters of keratinized termini (Fig. 2, G and H). These lesions resembled developing hair follicles, thus the designation as pilar lesions.

Three nodules, two mainly alveolar and one squamous (Fig. 2D), were identified in the mammary glands from two FVBB6 F1, Min/+ mice that were not treated with ENU. These were not different in gross or microscopic appearance from the lesions from ENU-treated Min/+ mice of the same strain. In contrast, two lesions noted in the 1st mammary glands of one ENU-treated 129X1B6 F1, +/+ mouse were not similar to the lesions found in the Min/+ mice. In the whole mounts, these lesions appeared to be dense tangles of ducts. No evidence of atypia was noted in the sections from these lesions.

Mammary tumors from ENU-treated B6 Min/+ mice, BRB6 F1, Min/+ mice, and both lines of 129B6 F1, Min/+ mice, and most of the tumors from the FVBB6 F1, Min/+ mice were classified as either well-differentiated keratinizing SCCs (Fig. 2F) or trichoepitheliomas (Fig. 2I). The similarity of these tumors to the squamous nodules and pilar lesions, respectively, raises the possibility that each of these types of hyperplastic lesion gives rise to a specific type of tumor. Only one tumor, from an FVBB6 F1, Min/+ mouse, was classified as a microacinar adenocarcinoma (Fig. 2C). This tumor was first noted at 114 days after ENU treatment. The similarity to the hyperplastic alveolar nodules that were frequent in the FVBB6 F1, Min/+ mice raises the possibility that these hyperplastic lesions could give rise to adenocarcinomas at low frequency.

The Effect of Genetic Background on Intestinal Tumor Susceptibility. All of the Min/+ mice, but none of the +/+ mice, developed intestinal tumors. The BRB6 F1, Min/+ mice (P = 2 × 10⁻⁹), FVBB6 F1, Min/+ mice (P = 9 × 10⁻⁹), and 129X1B6 F1, Min/+ mice (P = 0.03) each had significantly fewer intestinal tumors than did the B6 Min/+ mice. However, the 129S6B6 F1, Min/+ mice had as many intestinal tumors as did the B6 Min/+ mice (P = 0.6). The 129S6B6
F1 Min/+ mice also had significantly more intestinal tumors than did the 129X1B6 F2 Min/+ mice (P = 0.04). Thus, the BR, FVB, and 129X1 mice carry dominantly acting modifiers that affect intestinal tumor development. All untreated Min/+ hybrid mice also developed intestinal tumors (data not shown). In all of the cases, ENU treatment resulted in an increase in the number of intestinal tumors.

**DISCUSSION**

Previous studies have indicated a strong role for modifier loci on intestinal tumor development in Min/+ mice (11, 16). An effect of background on mammary and intestinal tumor development in other Apc mutant mice has also been reported (17). Here we demonstrate an effect of genetic background on mammary and intestinal tumor development in Min/+ mice. The effect of genetic background on both carcinomas and hyperplasias was tested. Because these studies were done with F1 hybrid mice, we could only detect modifier alleles that would act in a dominant manner to confer resistance or sensitivity to hyperplasias or tumors relative to the susceptibility of the B6 Min/+ mice. The B6 and BR strains can be considered sensitive backgrounds for Min-induced mammary tumorigenesis as the mice developed a large number of tumors within a short time after ENU treatment. In contrast, the 129X1, 129S6, and FVB strains carry dominant modifier alleles that confer resistance to mammary tumor development in hybrid Min/+ mice. However, the 129X1B6 F1, 129S6B6 F1, and FVBb6 F1 Min/+ mice were all susceptible to the development of hyperplasias, whereas hyperplasias were rare in the B6 and BRB6 F1 Min/+ mice. Thus, the modifier loci that confer resistance to development of mammary tumors do not confer resistance to the development of hyperplasias. The mammary tumor and intestinal tumor susceptibilities were also not correlated in the BRB6 and 129S6B6 F1 mice. The BR, FVB, and 129X1 strains carry dominant resistance alleles at modifier loci affecting intestinal tumor development. Thus, at least some of the modifier loci function in a tissue-specific manner.

The B6 and BR strains of mice were derived from full sibs and ~25% of their genes should be identical by descent (18). B6 and BRB6 F1 Min/+ mice were very similar with respect to the mammary gland phenotype but quite different in susceptibility to intestinal tumors. Thus, these two closely related strains may share alleles at loci affecting mammary tumor susceptibility but differ at some loci affecting intestinal tumor susceptibility. Interestingly, BR mice carry the same mutant allele at Pla2g2a as do B6 mice and would thus be expected to carry a sensitive allele at Mom1 (11, 19, 20). Therefore, the strong resistance to intestinal tumors seen in these F1 mice must map to other loci.

These studies also demonstrate the variability in phenotype between the two 129 strains studied. For each of the 129 strains, crosses have been made at various times to introduce genetic markers such as coat colors or enzyme markers. There is significant heterogeneity between 129 strains based on analysis of DNA markers (21, 22). Thus, it may not be surprising to find a difference in susceptibility to tumor development. Both 129X1B6 F1 Min/+ and 129S6B6 F1 Min/+ mice carry dominantly acting alleles that confer resistance to Min-induced mammary tumors. Although the incidence and multiplicity of mammary tumors in the F1 hybrids are not significantly different between the two strains, only a single 129X1B6 mouse developed any mammary tumors. Analysis of larger numbers of mice followed for a longer time might reveal a significant difference in this phenotype. Although the resulting phenotype may be similar, the resistance to mammary tumors may be attributable to different loci in each of these strains. In addition, our results indicate that 129X1 and 129S6 mice differ with respect to alleles at some loci that modify the intestinal tumor susceptibility of Min/+ mice. Like B6 mice, 129X1 mice carry a mutant allele at Pla2g2a and a sensitive allele of Mom1 (19, 23). Thus, as with the BR mice, 129X1 mice must carry resistance alleles at intestinal tumor modifiers that map elsewhere in the genome. FVB mice, a frequent choice for the production of transgenic mice, do not have a high spontaneous incidence of mammary tumors (6). The FVB strain carries alleles that make it very resistant to...
Min-induced tumor development in both the mammary gland and intestine. However, the FVBB6 F1 Min/+ mice were very sensitive to the development of mammary hyperplasias, especially the mixed alveolar and squamous nodules. In contrast to the resistance to Min-induced mammary and intestinal tumors, FVB mice are more sensitive than B6FVB F1 mice to the induction of mammary tumors by the transgenes MMTV-neu and WAP-ras (24, 25). Additionally, FVB mice are more susceptible than are B6 mice to development of SCCs or trichoepitheliomas, despite being too small to be detected at necropsy by 65 days after ENU. In fact, all of the lesions sampled or the level of transgene expression in the target tissue.

Min/+ mice on all of the genetic backgrounds developed either mammary tumors and/or hyperplasias after ENU treatment. Because ENU is a direct acting mutagen that does not require activation or active transport, there should be no effect of genetic background on ENU activity. If the number of mammary tumors and hyperplasias are combined, all of the hybrids developed approximately as many total mammary lesions as did the B6 mice (P > 0.06 for all of the comparisons). Thus, the mammary modifier loci seem to affect the type of lesion that develops but not initiation by ENU. On the B6 and BRB6 backgrounds, ENU treatment leads to the rapid development of SCCs or trichoepitheliomas with few or no hyperplastic lesions present by 65 days after ENU. In fact, all of the of the lesions sampled from the BRB6 F1, Min/+ mammary glands were classified as SCCs or trichoepitheliomas, despite being too small to be detected at necropsy. Although no lesions from the B6 Min/+ mice were sampled for histology, only a small number of lesions were noted in the mammary glands. None of the lesions from the B6 Min/+ or BRB6 Min/+ mice appeared to be alveolar hyperplasias, indicating that this type of lesion is rare in these mice.

In contrast, both 129 hybrids and the FVBB6 hybrids developed few tumors but many hyperplastic lesions, although these mice survived for much longer than did the B6 mice. Although the most common lesion found in these mice was the hyperplastic alveolar nodule, the tumors that did develop were mainly SCCs or trichoepitheliomas. No new mammary tumors arose in the 129X1B6 and FVBB6 hybrids after 120 days although some mice survived up to 178 days after ENU. This suggests that the progression of the hyperplastic lesions in resistant hybrids is slower or less frequent than in the B6 and BRB6 F1 mice. The hyperplasias, especially the mixed alveolar and squamous hyperplasias, may represent an alternative pathway of neoplasia that only rarely leads to malignancy. This could be tested by maintaining ENU-treated resistant hybrid mice for longer times or through transplantation of the hyperplastic lesions.

One tumor from an FVBB6 F1 Min/+ mouse was classified as a microacinar mammary adenocarcinoma, a Dunn type A tumor. It seems likely that this adenocarcinoma arose from one of the mixed hyperplastic alveolar and squamous nodules that were common in the FVBB6 F1 mice. This may indicate that these lesions can be preneoplastic, although progression may be a rare event. This type of tumor is commonly seen in mice infected with mouse mammary tumor virus or transgenic mice expressing Wnt1 (28, 29). The hyperplastic alveolar and squamous nodules observed in the FVBB6 F1 and 129S6B6 F1 hybrids were also similar to those found in MMTV infected mice or Wnt1 transgenic mice.

A likely explanation for the similarity of the hyperplasias in Min/+ mice to those that develop in MMTV infected mice is that APC plays an essential role in the WNT pathway (30–32). APC acts in concert with a complex of GSK3β, axin, and β-catenin to promote the phosphorylation of β-catenin (33–35). Once phosphorylated, β-catenin is targeted to the proteasome pathway for degradation. Loss of APC function thus can result in the increased accumulation of β-catenin (32). Activation of the WNT pathway causes a decrease in GSK3β activity, also resulting in a decreased degradation of β-catenin. Thus, loss of APC function and activation of the WNT pathway would be expected to have similar consequences in regard to levels of β-catenin in the cell. β-Catenin has been shown to complex with members of the TCF and LEF transcription factor families (36) to regulate the transcription of genes such as c-myc (37) and Cyclin D1 (38), both of which have been implicated in breast cancer (39–44). The tendency of mammary epithelium in the Min/+ mice to undergo metaplasia into squamous epithelium or hair follicle-like structures may be indicative of a role for APC in controlling the developmental fate of this tissue. Several other studies have suggested a role for WNT pathway members in the development and maintenance of hair follicles (45, 46).

It might be more appropriate to ask why more of the tumors from Min/+ mice do not resemble tumors arising in MMTV-infected mice or Wnt1 transgenic mice. One key difference may be the target cells in each of these systems. In Wnt1 transgenic mice, the level of Wnt1 expression in any cell is determined by the promoter driving the transgene. If expression of Wnt1 is restricted to, or highest in, cells committed to alveolar development, tumors will arise from alveolar cells. In MMTV-infected mice, tumors are thought to arise because of the inappropriate activation of genes such as Wnt1 by promoters within MMTV. As with the transgenic mice, the target cells for expression will be determined by the promoter. In the experiments reported here, any cell of the mammary gland could be the target of ENU. The mammary glands were growing rapidly at the time of ENU treatment; thus, many of the cells of the mammary gland would be targets for mutation. The type of tumor that develops might then be a function of which cells are initiated. The modifier loci would then determine the fate of any initiated cells. The B6 and BRB6 Min/+ mice carry modifier alleles that result in the rapid development of squamous metaplasia and tumors, with little or no development of hyperplasias. In the 129 and FVB hybrids, the genetic background may make the development of hyperplastic alveolar lesions with some squamous metaplasia the more common event. If these lesions were less likely to progress to tumors, then fewer tumors would result. Thus, the modifier loci may affect either the progression of the lesions or the type of lesions that develop, or both.

These studies represent a first step toward the identification of genes that modify mammary tumor susceptibility in Min/+ mice. Because both tumors and hyperplasias can be quantified and classified relatively easily, the mapping of the modifiers that control the development of mammary tumors should be possible. Because Min/+ mice develop both mammary and intestinal tumors, the effect of each modifier locus on tumor development in both tissues can be evaluated in the same animals. The independence of the mammary and intestinal susceptibility observed in the crosses described here indicates that at least some modifiers will have tissue-specific effects. The differential susceptibility of strains of mice to different tumor types through different means of induction also indicates the potential for a large number of tissue-specific and pathway-specific modifier loci. Molecular identification of such loci should lead to a better understanding of tumor development.

ACKNOWLEDGMENTS

We thank Dr. Mary Lindstrom for assistance with the statistical analysis; Dr. Norman Drinkwater for the MSTAT statistical program; and the UWCCC histology core for assistance with histological processing and sectioning.
Genetic Background Affects Susceptibility to Mammary Hyperplasias and Carcinomas in $Apc^{Min/+}$ Mice

Amy Rapaich Moser, Laura F. Hegge and Robert D. Cardiff


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/8/3480

Cited articles
This article cites 44 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/8/3480.full.html#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
/content/61/8/3480.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.