Inheritance and Site of Expression of Genes Controlling Susceptibility to Mammary Cancer in an Inbred Rat Model

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ABSTRACT

An inbred rat model for genetically controlled susceptibility to chemically induced mammary cancer has been established. Wistar-Furth (W/Fu) rats were found to be more susceptible to 7,12-dimethylbenz(a)anthracene-induced mammary tumors than were Fischer (F344) rats. The susceptibilities of various F1, F2, and backcross generations of these strains were examined for susceptibility to 7,12-dimethylbenz(a)anthracene-induced mammary tumors. The data suggest that susceptibility is inherited as a dominant trait. Both a single locus autosomal model and an X-linked model have been ruled out. However, the data support the hypothesis that complete susceptibility is controlled by any one of a group of independently segregating genes; i.e., any one gene of this group is both necessary and sufficient to induce maximal susceptibility. It is not known if these genes are identical or different. In order to identify the role of these genes we asked if they were expressed in the mammary epithelial cells themselves or elsewhere in the rat. Chimeric animals were produced by transplanting mammary cells from either W/Fu or F344 rats into the white interscapular fat pad of female W/Fu × F344 F1 rats. One month after transplantation the animals were treated with 7,12-dimethylbenz(a)anthracene and then palpated weekly for tumor development at the graft site. Tumors developed more rapidly and in greater total frequency at sites grafted with W/Fu mammary cells. This result suggests that the genes controlling inherited susceptibility are expressed in the mammary cells. The role of these genes is now under investigation. We have thus far shown that they do not control carcinogen metabolism or activation.

INTRODUCTION

Women are heterogeneous for their propensity to develop breast cancer. Susceptibility to this major form of cancer is controlled by both genetic and physiological factors, both of which have been studied using rodent models (e.g., Refs. 1 and 2). An early model for studying genetic susceptibility was proposed by Huggins (3), who demonstrated that outbred Sprague-Dawley rats were more susceptible to chemically induced mammary cancers than were outbred Long-Evans rats. Inbred strains of rats were also shown to have distinct susceptibilities to chemically induced cancers. For example, Dunning et al. (4) demonstrated that the Copenhagen rat was resistant to hormonally induced breast cancer, while we previously reported that inbred Wistar-Furth rats were as susceptible as outbred Sprague-Dawley rats and inbred Fischer F344 rats were resistant to carcinogen-induced mammary cancer (1).

The pattern of inheritance of susceptibility in the Huggins outbred rat model was one of dominance that seemed to be controlled by a single autosomal gene (1). Using our inbred rat model, we also found susceptibility to be a dominant characteristic (5). Here it is shown that in our inbred model this trait is not controlled by a single locus.

The rat mammary gland has been shown to be a direct target to DMBA2-induced carcinogenesis (6). Furthermore, rat mammary parenchymal cells are capable of activating DMBA (7). However, there are also many data supporting the concept that the direct effects of DMBA on the mammary gland can be modulated by systemic factors such as ovarian and pituitary hormones (8, 9). It is thus important to determine if the gene(s) controlling susceptibility are active at the level of mammary epithelial cell or at the systemic level. Chimeric rats are used here to address this question.

MATERIALS AND METHODS

Animals. W/Fu and F344 rats were purchased from Harlan Sprague-Dawley (Madison, WI). All genetic crosses of these animals were bred in our local animal facility.

Tumor Induction for Genetic Analyses. DMBA (Sigma Chemical Co., St. Louis, MO) of greater than 95% purity as determined by high-performance liquid chromatography was dissolved in sesame oil. Female rats age 55 ± 5 days were intubated with DMBA (130 mg/kg). Starting 3 weeks after DMBA administration each rat was palpated weekly for the detection of mammary tumors. Tumors were confirmed by histopathological examination. Greater than 95% of tumors were mammary carcinomas. The end point used in the genetic analysis experiments was time to the appearance of first carcinoma. This end point was chosen over tumor multiplicity because in previous experiments in our laboratory we found that multiple mammary tumors in rats did not follow a Poisson distribution (10, 11), making the validity of this end point questionable.

Chimeric Rat Construction. Mammary glands were removed from W/Fu or F344 female rats age 30 ± 5 days. After the lymph nodes were removed the glands were minced and enzymatically monodispersed. The monodispersed mammary cells were counted and diluted. Cells were transplanted into the interscapular white fat pads of recipient rats (female W/Fu × F344 F1, rats, age 30 ± 5 days). Each rat received two injections of a cell suspension. Each injection contained 9 × 105 cells. Details of methods of cell preparation and transplantation have been published (12). We have previously shown that when animals are grafted with this number of cells, 100% of the graft sites will develop morphologically and functionally normal mammary tissue (12, 13).

When the grafted rats were 55–60 days old they were intubated with DMBA as described above. All tumors arising in the host mammary gland were resected to preserve the animals’ health. Tumors arising at the graft site were followed by palpation, confirmed by surgical inspection, and counted.
and removed for histopathological examination. Rats were necropsied 11 months after DMBA administration. This time was chosen because of the declining health status of the rats due to multiple and nonresectable tumors in the in situ mammary glands.

RESULTS

Genetic Analysis. Mammary tumors were found to develop more rapidly in W/Fu than in F344 rats (Fig. 1), confirming our previously published findings (1). When W/Fu females were crossed with F344 males, the F1 rats had a susceptibility to DMBA-induced mammary tumors that could not be distinguished from their sensitive W/Fu parent (Fig. 1). The simplest model compatible with these results is a single locus autosomal dominant inheritance pattern (Table 1). This model of inheritance was tested by examining the susceptibility of: the backcross of W/Fu × F344 F1, males with W/Fu females; the backcross of the same F1 males to F344 females; and F2 rats produced by crossing similar F1 females and males. Female rats produced from these three crosses had tumor latencies that were indistinguishable from those of the W/Fu and W/Fu × F344 F1, females (Fig. 2).

While the results obtained with the F344 x Fi cross were backcrossed to F344 females or used to produce F2 rats. This change drastically alters the predictions of an X-linked model (Table 1). The results of 23-week post-DMBA experiments (23) were found not to be compatible with the X-linked single locus model (Table 1).

The total data obtained from testing rats of eight different crosses presented in Figs. 1–3 are compatible with an inheritance pattern in which susceptibility to DMBA-induced breast cancer is controlled by a group of independently segregating dominant genes where the presence of one or more of these genes confers complete susceptibility to the induction of mammary tumors by DMBA (Table 1).

The number of independently segregating genes in this group can only be approximated from the current data. The data in Figs. 1–3 show that only groups of resistant F344 rats, F344 × F1 backcrosses, and the F2 crosses fail to develop mammary tumors in 100% of their rats by 20 weeks post-DMBA feeding.

In fact, at the termination of these backcross and F2 experiments (23 weeks post-DMBA) several rats remain tumor free. Specifically in the F344 × (W/Fu × F344 F1) crosses, two rats of 55 (3.6%) are tumor free while in the F344 × (F344 × W/Fu F1) crosses two rats of 50 (4%) are tumor free. In the two F2 crosses, only a single rat was tumor free at this time (1 of 49, 2%). It should also be noted that in the F344 rats at 23 weeks post-DMBA 13 of 47 (27.7%) of rats were tumor free. For the purposes of analysis, the raw data presented in Table 2, Column 2, can be normalized. Since the inbred F344 strain is defined as resistant we can normalize the data by multiplying 13 of 47 by a correction factor of 3.6 to obtain a value of 100%; i.e., all rats are resistant. If the fraction of tumor-free rats in the F344 × F1 backcross (i.e., 4 of 105) is multiplied by the same correction factor, 13.8% (8–20%, exact binomial 95% confidence intervals) of the offspring of this cross are found to be "resistant." A similar normalization can be done for the F2 rats when the fraction of rats without tumors at 23 weeks, i.e., 1 of 49, is multiplied by the correction factor, and 7.4% (3–11%) are found to be "resistant." These approximations of resistance can be compared to prediction of a mendelian model which assumes: (a) that susceptibility is controlled by a number of independently segregating genes; and (b) that the presence of any of these genes will confer complete susceptibility. As shown in Table 2, the data of these

![Graph](https://example.com/graph.png)

**Fig. 1.** The time to first mammary tumor of rats given DMBA (130 mg/kg) at zero time. Rat strains include F344 (A), W/Fu (B), and W/Fu × F344 F1 (C). See text for details.

<table>
<thead>
<tr>
<th>Cross no. and rat type</th>
<th>Model prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single locus autosomal dominant</td>
</tr>
<tr>
<td></td>
<td>Single locus X-chromosome</td>
</tr>
<tr>
<td></td>
<td>Multiple locus autosomal dominant</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>1. F344</td>
<td>All sensitive</td>
</tr>
<tr>
<td>2. W/Fu</td>
<td>All sensitive</td>
</tr>
<tr>
<td>3. F1 (W/Fu × F344)</td>
<td>All sensitive</td>
</tr>
<tr>
<td>4. Backcross (W/Fu × F1)</td>
<td>½ sensitive, ½ resistant</td>
</tr>
<tr>
<td>5. Backcross (W/F644 × F1)</td>
<td>½ resistant</td>
</tr>
<tr>
<td>6. F1 (F344 × F1)</td>
<td>½ resistant</td>
</tr>
<tr>
<td>7. Backcross (F344 × F1)</td>
<td>½ resistant</td>
</tr>
</tbody>
</table>

Table 1

Rat strains, crosses, and predicted sensitivities to DMBA mammary cancer induction based on several models of inheritance

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crosses are most compatible with a model predicting three independent genes present in the W/Fu rat which can confer susceptibility to chemically induced mammary cancer. It is stressed that this latter analysis is preliminary.

**Chimeric Rats.** Chimeric rats were produced by grafting either mammary cells from F344 (resistant) or W/Fu (susceptible) rats into the mammary-free interscapular white fat pads of W/Fu × F344 F₁ animals. These rats were followed for 11 months for tumor development at the graft site following intubation with DMBA or oil alone. None of the oil-fed control rats developed mammary tumors at the site of the graft irrespective of the strain of origin of the grafted cells. Tumors did develop at the graft site in animals fed DMBA. As shown in Fig. 4, the percentage of carcinomas at the graft sites varied with the strain of origin of the grafted cells. F₁ rats grafted with W/Fu cells developed mammary carcinomas at the graft site in greater frequency and at shorter latency than those receiving F344 cells. At 11 months post-DMBA feeding, 12 of 39 (31%) of the rats with W/Fu grafts had developed carcinomas whereas only 3 of 43 (7%) rats with F344 grafts developed tumors. It should be noted that three of the W/Fu carcinomas were found at final necropsy while one of the F344 carcinomas was found at this time.

In addition to the carcinomas, a few benign mammary tumors (fibroadenomas) were also found. These fibroadenomas were mainly discovered at the final necropsy (11 months post-DMBA). Unlike the findings with carcinomas, no major difference in fibroadenoma frequency could be related to strain of origin of the graft. Four of 39 (10%) rats with W/Fu and 6 of 43 (14%) rats with F344 grafts had fibroadenomas at the transplant site.

**DISCUSSION**

We have shown that susceptibility to DMBA-induced mammary cancer in the W/Fu-F344 rat model is controlled as a dominant trait. Complete susceptibility can be conferred by any of a number of independently segregating genes. A preliminary analysis suggests that approximately three loci may be involved. The pattern of inheritance found in this inbred model is different than that reported for the Huggins Sprague Dawley-Long Evans outbred model in which susceptibility was found to be controlled by a single dominant gene (3). It is most likely that this difference in inheritance is real. However, it should be noted that at least two major differences exist in addition to the strains used: (a) our model uses inbred rats whereas Huggins' model used outbreds, which may complicate genetic analysis; (b) our end point is a latency measurement, i.e., time to first tumor, whereas Huggins used incidence, i.e., frequency of tumors.

The difference between the pattern of inheritance of susceptibility of the SD-LE model and the W/Fu-F344 model may be the result of different susceptibility-conferring genes or a result of different numbers of the same gene. While this question cannot yet be answered certain available information may be relevant. The SD and W/Fu rats have almost identical susceptibilities to DMBA-induced mammary carcinogenesis. This is com-

**Table 2**

Results of tumor induction experiments and predictions of various multigene experiments

<table>
<thead>
<tr>
<th>Rat type</th>
<th>Rats without tumors (23 wk)/total rats</th>
<th>No. of &quot;resistant&quot; rats/total rats*</th>
<th>Observed % of &quot;resistant&quot; rats</th>
<th>Predictions$^{a}$ of % of &quot;resistant&quot; rats based on</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344</td>
<td>13/47</td>
<td>47/47</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>W/Fu</td>
<td>0/48</td>
<td>0/48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F₁</td>
<td>0/48</td>
<td>0/48</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>W/Fu × F₁</td>
<td>0/49</td>
<td>0/49</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F344 × F₁</td>
<td>4/105</td>
<td>14.5/105</td>
<td>13.8 (8–20)$^c$</td>
<td>50</td>
</tr>
<tr>
<td>F₂</td>
<td>1/49</td>
<td>3.6/49</td>
<td>7.4 (3–11)$^c$</td>
<td>25</td>
</tr>
</tbody>
</table>

*a Method of normalization calculations given in text.

*b See text for details.

$^c$ Exact binomial 95% confidence interval.
begins with a hypothesis of susceptibility genes with identical function in these two rat strains. This hypothesis is strengthened by the fact that the SD rat originates from stock that included the Wistar rat (14). Furthermore the possible amplification of this gene in the W/Fu rat might have arisen during its inbreeding by Furth and colleagues, whose aim in breeding this strain was to develop rats with a high leukemia incidence (15). While this aim was not achieved, it is possible that this selective breeding led to multiple copies of the susceptibility gene. This latter hypothesis is being examined by determining if other inbred Wistar strains have single or multiple susceptibility genes.

In an initial attempt to begin to understand the function of these genes, it was asked if they acted at the level of the mammary cells or elsewhere in the rat. Chimeric rats were constructed by grafting either W/Fu or F344 mammary cells into the mammary cell-free interscapular white fat pad of W/Fu × F344 F1 rats. After complete mammary glands developed at these graft sites, DMBA was administered. If the susceptibility genes to DMBA-induced mammary cancer were functioning primarily in the mammary cells themselves, then one would expect a differential response at the graft site. However, if the genes acted abscopally then no differential response would be expected. Our results support the former possibility in that a much greater number of carcinomas was found at sites grafted with W/Fu mammary cells. It is interesting to note that there was no difference in the number of fibroadenomas at the two types of graft sites. It is not yet clear, however, if the genes being studied influence the induction of mammary fibroadenomas.

The action of this gene(s) at the level of the mammary cell is under investigation. Thus far, we have ruled out the possibility that this gene(s) is controlling carcinogen metabolism or activation. Specifically, we have shown that mammary epithelial cells from both strains, when assayed by a mediated mutagenesis assay, activate DMBA to its mutagenic metabolites to an equal extent (1). Other postactivation stages of mammary carcinogenesis are currently under investigation in these rat strains.

Another possibility that must also be explored is that of a larger number of "target" cells in the susceptible mammary gland both in situ and following grafting. We have presented evidence of an inverse correlation of target cell number and latency in radiation-induced mammary carcinomas.3 Evidence in this report also supports this hypothesis for chemically induced cancers. If the latency is compared for in situ cancers (Figs. 1–3) versus cancer in grafted glands (Fig. 4) for W/Fu mammary cells, it is clear that a much longer latency exists at the graft site. It is also clear that the graft site contains far fewer mammary epithelial cells and thus presumably fewer target cells than in situ glands. We plan to directly test this hypothesis of a differential number of target cells in each strain by using a quantitative transplantation model that we have developed that allows us to both measure clonogenic mammary stem cell population and estimate neoplastic transformation per surviving exposed mammary clonogenic cell (12).3

ACKNOWLEDGMENTS

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