BRIEF COMMUNICATION

Chemical Carcinogen-Induced Tumorigenesis in Parous, Involuted Mouse Mammary Glands

Daniel Medina, Gilbert H. Smith

Breast cancer remains the major cancer among women in the United States in terms of noncutaneous cancer incidence and the second leading cause of cancer deaths (1). Among the many risk factors for breast cancer, reproductive history, genetic background, and age are the strongest and most consistent (2,3). However, the strongest protective factor is also related to reproductive history, i.e., early age at first pregnancy (<20 years of age), which confers a 50% reduction in lifetime risk compared with the lifetime risk of breast cancer in nulliparous women. The protective effect of early first pregnancy has been demonstrated repeatedly in numerous epidemiologic studies and provides a physiologically operative model to achieve practical and affordable prevention of breast cancer in humans.

Chemical carcinogen-induced rat mammary tumorigenesis has been the model of choice to demonstrate pregnancy-related cancer prevention. Pregnancy with or without lactation (4–7), treatment with estrogen and progesterone (8,9), and treatment with human chorionic gonadotropin (10) exert protective effects against mammary tumorigenesis in rats treated with either 7,12-dimethylbenz[a]anthracene (DMBA) or nitrosomethylurea (NMU). The protective effect is manifest in outbred and inbred rats (4,9,11). The molecular mechanisms that form the basis for the protective effect have not been elucidated, although different hypotheses to explain the protective effects have been proposed (5–7,9).

It is of interest that comparable experiments have not been reported in mouse models of mammary tumorigenesis. In a series of articles, Marchant (12–14) reported that chemical carcinogen-induced tumorigenesis was inhibited when the carcinogen was administered to actively lactating mice. However, the response of the involuted gland was not reported. Swanson et al. (15) reported that the involuted mammary glands of 5- to 6-month-old parous BALB/c mice exhibited a weak tumorigenic response to NMU. The weak tumorigenic response of the mammary glands of 6-month parous mice may simply reflect the weak tumorigenic response of the aged mammary gland in general, since tumorigenesis in the virgin gland was not examined (15). Therefore, it has not been firmly established whether the mouse is a suitable model for hormone-induced refractoriness to chemical carcinogenesis. In this brief communication, we report the response of the involuted mammary gland of two different mouse strains to chemical carcinogen-induced tumorigenesis.

C57BL and DBA/2f mice were bred and maintained in a closed conventional mouse colony at the Baylor College of Medicine, and the F1 hybrid mice (BD2f) were used in the experiments. C3H/Sm mice were maintained at Biocon Inc. (Rockville, MD), bred, and shipped to the Baylor College of Medicine when mice were 1 week postpartum. All mice were cared for in accordance with the guidelines established by the Department of Health and Human Services in the “Guide for the Care and Use of Laboratory Animals.” These two strains were chosen because previous results had shown that they were susceptible to DMBA-induced mammary tumorigenesis at the doses of chemical carcinogen used (16–18). The BD2f1 and C3H/Sm mice were allowed to lactate for 7 days, then the pups were removed and the glands were allowed to regress for 3 and 4 weeks, respectively, prior to commencement of carcinogen treatment at 15 and 12 weeks of age, respectively. An equal number of littermates were simultaneously maintained as virgins. Mice were followed for tumor appearance by weekly palpation for 1 year or longer. DMBA was administered at a dose of 1 mg by intragastric gavage once weekly for either 6 weeks (BD2f1) or 2 weeks (C3H/Sm), as described previously (16). All mammary tumors were collected, fixed in 10% neutral buffered formalin, processed for routine hematoxylin-eosin staining, and examined for histopathology. The tumor incidences were evaluated statistically by Fisher’s exact test. All P values are two-sided and, when less than .05, are statistically significant.

The responsiveness of the parous and age-matched virgin glands of BD2f1 and C3H/Sm mice to DMBA-induced mammary tumorigenesis is shown in Table 1. In the BD2f1 mice, mammary tumor incidence was statistically significantly decreased (P = .01) from 70% (14 of 20) to 25% (five of 20). There was a delay of 10 weeks in the initial tumor latency in the parous mice. Both the age-matched virgin and parous mice were followed until they were 52 weeks of age. In the C3H/Sm mice, mammary tumor incidence was decreased from 57% (eight of 14) to 21% (three of 14), but the decrease did not reach statistical significance (P = .1) because of the smaller group sizes. There was no significant delay of initial tumor latency. Statistical analysis of the pooled results for the two strains shows that the decrease in mammary tumor incidence was highly statistically significant (P = .001). The histopathology of mammary tumors arising in the parous versus age-matched virgin mice was similar and included both type B adenocarcinomas and squamous adenocarcinomas.

The data presented herein demonstrate that the involuted mammary gland of parous mice exhibits a marked refractoriness to chemical carcinogenesis compared with that of age-matched virgin animals. To our knowledge, this is the first documented example of refractoriness of the involuted mouse mammary gland to chemical carcinogen-induced mammary tumorigenesis under conditions that are similar to those reported in the rat and presumed to be operative in the human. In both mouse

Affiliations of authors: D. Medina, Department of Cell Biology, Baylor College of Medicine, Houston, TX; G. H. Smith, Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD.

Correspondence to: Daniel Medina, Department of Cell Biology, Texas Medical Center, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 (e-mail, dmedina@bcm.tmc.edu).

See “Note” following “References.”

© Oxford University Press
strains, the mice nursed for 7 days prior to removal of the pups. The experimental results demonstrate that the mouse is a suitable model to examine the mechanisms of hormone-induced refractoriness to carcinogen-induced mammary tumorigenesis.

The issue of the suitability of the mouse as a model to examine the relationship of hormone-induced differentiation of the mammary gland and onset of cancer has become important for several reasons. Marquis et al. (19) and Rajan et al. (20) reported that the expression of BRCA1 and BRCA2 messenger RNA (mRNA) increased during pregnancy and remained elevated in the involuting mammary gland of FVB mice. They speculated that, if this were true also in humans and rats, it might be a significant factor in the well-documented refractoriness of the involuted gland to carcinogenesis. To date, expression levels for BRCA1 or BRCA2 mRNA have not been reported for parous mammary gland in rats and humans. The demonstration that the parous mouse mammary gland is refractory to chemical carcinogen-induced tumorigenesis provides a model to examine the possible role of BRCA1 and BRCA2 in mammary tumor suppression.

Another important aspect of these results is the availability of well-documented transgenic and gene-deletion models in the mouse that allow researchers to study the molecular and genetic mechanisms that underlie the basis for refractoriness of the parous mammary gland to tumorigenesis. Currently, there exist mouse knockout models for numerous hormone receptors, cell cycle genes, and signal transduction genes that may be used to answer such questions. For example, do the mammary glands of the progesterone-receptor or the cyclin-D1 knockout mice, which do not experience pregnancy-induced differentiation, become refractory to carcinogen-induced tumorigenesis following pregnancy?

Finally, studies on normal and tumorigenic development of the mouse mammary gland are greatly facilitated by the ease of transplanting into the mammary fat pad. Such studies have provided the opportunity to distinguish between host and tissue effects and between stroma and epithelial-based determinants (21–23). In summary, demonstration that paracytologically induced resistance to tumorigenesis exists in the mouse mammary gland will provide fresh and varied opportunities to study the mechanisms underlying the basis for hormone-induced refractoriness.

Table 1. Mammary tumorigenesis in age-matched virgin and parous mice*

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Group</th>
<th>DMBA dose, mg</th>
<th>No. of mice with tumors/total No. of mice (%)</th>
<th>Initial latent period, wk†</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD2fF1</td>
<td>Virgin</td>
<td>6‡</td>
<td>14/20 (70)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Parous</td>
<td>6‡</td>
<td>5/20 (25)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>C3H/Sm</td>
<td>Virgin</td>
<td>2§</td>
<td>8/14 (57)</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Parous</td>
<td>2§</td>
<td>3/14 (21)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1†</td>
</tr>
</tbody>
</table>

*DMBA = 7,12-dimethylbenz(a)anthracene; BD2fF1 = C57BL × DBA/2fF1 mice.†Refers to host age.‡DMBA administered between 15 and 20 weeks of age.§DMBA administered between 12 and 13 weeks of age.||Tumor incidence of virgin compared with that of parous mice by Fisher's exact test. All P values are two-sided and statistically significant for P < 0.05. Comparing pooled virgin versus pooled parous mice, P = 0.001.

REFERENCES


968  BRIEF COMMUNICATION  Journal of the National Cancer Institute, Vol. 91, No. 11, June 2, 1999


**NOTE**

Manuscript received October 7, 1998; revised March 12, 1999; accepted March 30, 1999.