Preneoplastic Lesions in Murine Mammary Cancer

Daniel Medina

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77025

Summary

The current model for murine mammary tumorigenesis indicates that discrete, morphologically identifiable preneoplastic lesions precede and give rise to mammary tumors. The hyperplastic alveolar nodule is the primary lesion that precedes and gives rise to mammary tumors in mice infected with the mammary tumor virus (Bittner) or its variants. In mice fed 7,12-dimethylbenzanthracene or urethan, hyperplastic alveolar nodules are present but infrequent, and the major mammary dysplasias present are ductal hyperplasias. The ductal hyperplasias can be divided into four general morphological types: (a) simple terminal duct hyperplasia; (b) lobular hyperplasia; (c) papillary hyperplasia; (d) end-bud hyperplasia. All types of ductal hyperplasias are characterized by intraductal epithelial hyperplasia and have been shown to give rise to mammary adenocarcinomas in situ or by transplantation into the mammary gland-free fat pads of syngeneic mice. Our current hypothesis is that murine mammary cancer can evolve through one of several intermediate stages. The intermediate stages can be either alveolar or ductal hyperplasias. The latter resemble, in many instances, ductal lesions seen in human breast cancer.

Introduction

The pathogenesis of murine mammary cancer has been extensively studied over the past 20 years. One concept that illustrates the course of tumor progression was proposed and developed by DeOme et al. (6, 7, 9, 11, 12). The concept states that mammary tumors arise from morphologically discrete epithelial lesions that are altered from normal. Considerable evidence has accumulated indicating that HAN are the primary lesions that precede and give rise to mammary tumors, particularly in mice infected with MTV or its variants (5–7, 9). In old retired BALB/c female breeders, free of overt MTV, HAN have been shown to be present infrequently, but when present they give rise to mammary tumors (9, 12). HAN have also been described in DMBA-fed rats and give rise to mammary adenocarcinomas with greater frequency than does the homologous normal mammary gland (3). The main difference in the mammary lesions between MTV-positive mice and DMBA-fed rats is that the latter exhibit a greater morphological variety of mammary lesions (4).

Several reports have indicated that BALB/c and C57BL mice fed chemical carcinogens produce a variety of mammary lesions similar to those of DMBA-fed rats (8, 10). BALB/c and C57BL mice fed DMBA and urethan exhibit a low incidence of HAN and a high incidence of DH with a mammary tumor incidence of 30 to 50% by 10 months of age (10). The DH are characterized by intraductal epithelial hyperplasia and resemble suspected high-risk lesions seen in human breast cancer (1, 2, 15). The data presented herein indicate that murine mammary cancer can evolve through 1 of several precursor lesions. The precursor lesions can be either alveolar hyperplasias or DH.

Materials and Methods

Mice. BALB/cC57Bl, C57BL/K, and DBA/K mice were bred and maintained in a closed mouse colony in the Department of Cell Biology, Baylor College of Medicine, Houston, Texas. All mice were fed Wayne-Lab Blox, given water ad libitum, and housed 4 to 6/cage in a temperature- and light-cycle (14 hr light-10 hr dark)-controlled room.

Carcinogens. Urethan was administered either in the drinking water (0.1% w/v) or injected i.p. (20 mg/mouse) for the desired number of weeks. DMBA, 1.0 mg dissolved in 0.2 ml cottonseed oil, was fed i.g. once weekly for the desired number of weeks.

Hormone Stimulation. Mice received a pituitary isograft under the right kidney capsule at 4 to 6 weeks of age. The pituitaries remained in place for 3 months. Pituitaries free from hypothalamic control secrete, primarily, prolactin which is both luteotropic and mammotropic (13).

Tumors. Mice were examined weekly for the appearance of mammary tumors. All tumors were fixed in formalin, stained in hematoxylin, and examined histologically. Whole mounts of mammary glands were processed as described in Ref. 9.

Transplantation. The mammary glands of some of the carcinogen-treated mice were examined at periodic intervals between 7 and 10 months of age. Samples of suspected ductal and alveolar hyperplasias were transplanted into the mammary gland-free fat pads of syngeneic mice (7, 9). The resulting mammary outgrowths were either serially transplanted in the mammary fat pads at 10- to 12-week intervals or left undisturbed until the appearance of tumors.


2 The abbreviations used are: HAN, hyperplastic alveolar nodules; MTV, mammary tumor virus; DMBA, 7,12-dimethylbenzanthracene; DH, ductal hyperplasias; i.g., intragastrically; KN, keratinized nodules.

Results

Incidence of Mammary Hyperplasia and Neoplasias in Carcinogen-fed Mice. The data presented in Table 1 demonstrate that a variety of alveolar, ductal, and keratinized lesions occur in mice fed either DMBA or urethan. BALB/c mice bearing a pituitary isograft and fed 6.0 mg DMBA produced 68% mammary tumors, primarily adenocarcinomas, by 6.5 to 7.0 months of age. The principal dysplasias seen in the mammary glands were KN. Eighty-five of the mice were positive with a mean of almost 25 KN/positive mouse. HAN and DH were seen infrequently.

BALB/c mice fed 4.0 mg DMBA (at 8, 9, 26, and 28 weeks of age) and maintained as virgins produced 37% tumors, primarily adenocarcinomas, by 10 months of age. The principal dysplasias seen were DH (53% of mice) with HAN and KN being absent.

C57BL mice bearing pituitary isografts and fed urethan in their drinking water produced 24% mammary tumors by 15 months of age. The tumors were both adenocarcinomas and fibroadenomas. The principal dysplasias seen were DH (75% of mice) with HAN and KN being rare.

C57BL × DBA/Fl mice bearing pituitary isografts and given urethan i.p. produced 69% tumors by 12 months. As with C57BL mice, the principal dysplasias were DH with HAN and KN rarely present. The tumors in C57BL mice and their hybrids were both adenocarcinomas (36%) and fibroadenomas (59%).

Morphological Types of Carcinogen-induced Mammary Lesions. KN seen in DMBA-treated, pituitary isograft-bearing BALB/c mice have been described previously (10). These lesions were observed grossly in the mammary glands as alveoli filled with gray-white, keratin-like pearls. In histological sections, alveoli were few and completely filled with desquamated cells and keratin material. Mononuclear cell infiltration and fibrosis were present conspicuously with these lesions. Transplantation of these lesions resulted in ductal outgrowths that exhibited a very low growth potential.

HAN were rarely seen, but when present they appeared as foci of typical alveolar hyperplasias. HAN were recognized subgrossly by both their focal alveolar hyperplasia and by the presence of hemosiderin pigment in their cells which colored them yellowish. The possibility of confusing HAN with minimally keratinized KN at the subgross level was great. Transplantation of HAN resulted in typical hyperplastic alveolar outgrowths as observed subgrossly. Histological sections of these outgrowths revealed some foci (<10%) of alveoli which exhibited squamous metaplasia. The ability to undergo squamous metaplasia was exhibited by 3 of 4 nodule lines examined and persisted through 6 transplant generations.

DH were seen in all 3 strains examined. These lesions were characterized by proliferation of their terminal ductal units and by intraductal epithelial hyperplasia. At least 4 different types of these DH were observed in these mice. The 1st type (Figs. 1 and 2) is classified as a simple terminal duct hyperplasia exhibiting intraductal epithelial hyperplasia. The 2nd type (Figs. 3 and 4) is classified as a lobular hyperplasia. It is characterized histologically by ductal lumina completely filled with uniform-appearing epithelial cells. These lesions were seen predominantly in the urethan-treated C57BL and C57BL × DBA/Fl mice. The 3rd class (Figs. 5 and 6) is classified as an end-bud hyperplasia. It is characterized by proliferation of small or terminal ducts capped by large, abnormal-appearing end-bud-like structures. Histologically, the lumina of the end-bud-like structures are filled completely with uniform-appearing epithelial cells. The end-bud hyperplasias were seen primarily in DMBA-treated BALB/c mice. The 4th type (Figs. 7 to 12) is classified as a ductal papillary hyperplasia. These lesions are the easiest to observe subgrossly because of their dense intraductal appearance. They are characterized histologically by papillary projections into the duct ranging from simple folds involving a few cells to complex structures resembling papillomas (Figs. 9, 10, and 12). In some cases, both papillary hyperplasia and carcinoma in situ appeared in the same lesion but at different regions in the duct (Figs. 9 to 12).

Transplantation of Carcinogen-induced Mammary Ductal Dysplasias. The transplantation of carcinogen-induced ductal dysplasias into the mammary gland-free fat pads resulted in outgrowth of DH which filled the fat pad and produced mammary carcinomas. Figs. 13 to 17 illustrate

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carcinogen (dose)</th>
<th>HAN Incidence</th>
<th>HAN Total</th>
<th>KN Incidence</th>
<th>KN Total</th>
<th>DH Incidence</th>
<th>DH Total</th>
<th>Tumors Incidence</th>
<th>Tumors Total</th>
<th>Means Age at Death (mos.)</th>
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<tbody>
<tr>
<td>BALB/c</td>
<td>DMBA (6 mg/6 wk)</td>
<td>6/20</td>
<td>30</td>
<td>20</td>
<td></td>
<td>2/20</td>
<td>10</td>
<td>2/20</td>
<td>10</td>
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<td>DMBA (4 mg)</td>
<td>0/19</td>
<td>0</td>
<td>0</td>
<td>0/19</td>
<td>0/19</td>
<td>0</td>
<td>10/19</td>
<td>53</td>
<td>10.0</td>
</tr>
<tr>
<td>C57BL</td>
<td>Urethan (0.1% in drinking water)</td>
<td>1/12</td>
<td>8</td>
<td>1</td>
<td>1/12</td>
<td>8</td>
<td>1</td>
<td>9/12</td>
<td>75</td>
<td>9/12</td>
</tr>
<tr>
<td>C57BL × DBA/Fl</td>
<td>Urethan (200 mg)</td>
<td>2/13</td>
<td>15</td>
<td>2</td>
<td></td>
<td>1/13</td>
<td>8</td>
<td>1/13</td>
<td>85</td>
<td>9/13</td>
</tr>
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</table>

a Mice received a pituitary isograft at 4 to 6 weeks of age which remained in place for 12 weeks under the kidney capsule. BALB/c mice received 1.0 mg DMBA per week between Weeks 8 and 13. C57BL and the hybrid mice received urethan between Weeks 8 and 18.

b Mice were maintained as virgin mice and received 1.0 mg DMBA at 8, 9, 26, and 28 weeks of age.

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Tumor-producing Capabilities of Carcinogen-induced Mammary Lesions. The tumor-producing capabilities of the various morphological types of carcinogen-induced mammary dysplasias are shown in Table 2. KN transplanted into the mammary gland-free fat pads of syngeneic mice failed to produce mammary tumors up to 70 weeks after transplantation. Of 90 lesions transplanted, 48 grew and gave rise to "normal" ductal outgrowth and none produced tumors.

Four primary HAN were transplanted to establish nodule outgrowth lines. The 4 HAN lines, C3, C4, C5, and C6, produced mammary tumors at various rates. Lines C3, C4, C5, and C6 produced 78, 70, 86, and 40% tumors, respectively. Both adenocarcinomas and adenoacanthomas were produced from these HAN lines.

Five ductal lines have been established and the preliminary results indicate that 3 of the 4 lines are giving rise to papillary carcinoma, whereas the 4th line produced ductal carcinoma.

Discussion

In murine mammary tumorigenesis, considerable evidence has accumulated indicating that HAN are the primary lesions that give rise to mammary tumors, particularly in mice infected with MTV or its variants (6, 7, 9). The genesis of mammary cancer can be illustrated by the schema in Chart 1 proposed by DeOme et al. (6, 11). In this schema, HAN precede and give rise to mammmary tumors. Additionally, MTV and chemical carcinogens influence both the progression of normal cells to HAN and of HAN cells to tumors. This schema was based on experiments using mice, and a similar course of tumor progression has been postulated to give rise to at least some rat mammary tumors (3). Recently, Singh and Dao (14) have seriously questioned whether the HAN or any preneoplastic population is important in the genesis of rat mammary tumors.

The recent demonstration that both alveolar and ductal mammary dysplasias are present in mice fed DMBA and urethan led to experiments that investigated the tumor-producing capabilities of these lesions. The data presented herein demonstrate that both chemical carcinogen-induced alveolar hyperplasias and DH can give rise to mammary tumors. HAN are relatively rare in DMBA-fed mice, but they have a high tumor potential when they are transplanted into the mammary gland-free fat pads of syngeneic mice.

The DH represent alterations primarily in the terminal ductal units of the mammary gland. They can be tentatively subdivided into 4 categories: simple terminal duct hyperplasia; lobular hyperplasia; end-bud hyperplasia; and papillary hyperplasia. These categories reflect their morphological appearance and are not meant to indicate separate etiological agents or pathways of origin. Thus far, it seems that all of these lesions arise from the terminal duct. All 4 types of ductal lesions have been shown to give rise to tumors in situ, and 2 of the lesions, the end-bud hyperplasias and papillary hyperplasias, have produced tumors after transplantation into the mammary fat pads of syngeneic mice.

The original scheme for mammary tumorigenesis has been revised to account for the recent results in carcinogen-treated mice. The proposed scheme for mammary tumorigenesis is illustrated in Chart 2. The scheme emphasizes the primary production of alveolar hyperplasias by MTV and DH by chemical carcinogens in BALB/c and C57BL mice. It should be clear that chemical carcinogens

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Line</th>
<th>No. of tumors/No. of transplants</th>
<th>%</th>
<th>Mean latent period (wk)</th>
<th>TG_wk (wk)*</th>
<th>Mean age at death (wk)</th>
</tr>
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<tbody>
<tr>
<td>KN</td>
<td></td>
<td>0/48</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>C3</td>
<td>81/104</td>
<td>78</td>
<td>23</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
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<td>C4</td>
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<td>26</td>
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<td>37</td>
</tr>
<tr>
<td>HAN</td>
<td>C5</td>
<td>19/22</td>
<td>86</td>
<td>26</td>
<td>26</td>
<td>40</td>
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<tr>
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<td>C6</td>
<td>7/17</td>
<td>40</td>
<td>38</td>
<td>44</td>
<td></td>
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<tr>
<td>DH</td>
<td>CDH-1</td>
<td>8/13</td>
<td>62</td>
<td>15</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
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<td>CDH-3</td>
<td>4/14</td>
<td>29</td>
<td>10</td>
<td>16</td>
<td></td>
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<tr>
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<td>HDH-1</td>
<td>0/20</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td></td>
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<tr>
<td>DH</td>
<td>HDH-4</td>
<td>4/10</td>
<td>40</td>
<td>14</td>
<td>16</td>
<td></td>
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<tr>
<td>DH</td>
<td>HDH-6</td>
<td>1/5</td>
<td>20</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

* TG_{50}, time for 50% of the transplants to produce tumors.
can give rise to HAN and MTV can give rise to ductal lesions (i.e., plaques, hormone-dependent tumors in GR and DDD mice). The scheme does not rule out 1-step transformation from normal to neoplasia, but it emphasizes the progression of transformed cells through an observable, discrete, intermediate, preneoplastic cell population.

The histological similarity of the intraductal hyperplasias to similar lesions in human breast and the ability to transplant these lesions in their normal anatomical site present a system where the progression of altered cells to neoplasms can be examined for their hormonal and ultrastructural characteristics and which can hopefully be related to the progression of breast cancer as seen in humans.

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References


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