Characterization of a Carcinogenesis Rat Model of Ovarian Preneoplasia and Neoplasia


1Medical Science Division and 2Department of Biostatistics, Fox Chase Cancer Center, Philadelphia, Pennsylvania

ABSTRACT

Animal models of ovarian cancer are crucial for understanding the pathogenesis of the disease and for testing new treatment strategies. A model of ovarian carcinogenesis in the rat was modified and improved to yield ovarian preneoplastic and neoplastic lesions that pathogenetically resemble human ovarian cancer. A significantly lower dose (2 to 5 µg per ovary) of 7,12-dimethylbenz(a)anthracene (DMBA) was applied to the one ovary to maximally preserve its structural integrity. DMBA-induced mutagenesis was additionally combined with repetitive gonadotropin hormone stimulation to induce multiple cycles of active proliferation of the ovarian surface epithelium. Animals were treated in three arms of different doses of DMBA alone or followed by hormone administration. Comparison of the DMBA-treated ovaries with the contralateral control organs revealed the presence of epithelial cell origin lesions at morphologically distinct stages of preneoplasia and neoplasia. Their histopathology and path of dissemination to other organs are very similar to human ovarian cancer. Hormone cotreatment led to an increased lesion severity, indicating that gonadotropins may promote ovarian cancer progression. Point mutations in the Tp53 and Ki-Ras genes were detected that are also characteristic of human ovarian carcinomas. Additionally, an overexpression of estrogen and progesterone receptors was observed in preneoplastic and early neoplastic lesions, suggesting a role of these receptors in ovarian cancer development. These data indicate that this DMBA animal model gives rise to ovarian lesions that closely resemble human ovarian cancer and it is adequate for additional studies on the mechanisms of the disease and its clinical management.

INTRODUCTION

Ovarian cancer is one of the leading causes of cancer-related deaths among women (1, 2). The understanding of the molecular pathogenesis of ovarian cancer has been hindered by the lack of sufficient numbers of specimens at early-stage disease because of its frequent diagnosis at advanced stages (3, 4). Consequently, the existence of identifiable precursor lesions that ultimately develop into ovarian cancer is still debatable (5, 6).

More than 80% of ovarian cancers originate in the ovarian surface epithelium (7–12). Incessant ovulation, postmenopausal increase of gonadotropin hormone levels, chronic inflammation, and environmental carcinogens are assumed to play key roles in ovarian oncogenesis (13–16).

Animal models that closely recapitulate human ovarian cancer are crucial for understanding its pathogenesis and for testing new treatment strategies. A number of models have been developed to date on the basis of carcinogen treatment, gonadotropin/steroid hormone stimulation, and genetic modeling (for review, see refs. 17, 18). The latter is based on the introduction of genetic alterations through the germ line or conditional inactivation of certain tumor suppressor genes, such as Tp53 and pRb (19), or the ectopic expression of certain oncogenes, or a combination of both (20). Transgenic models, however, depend strongly on the specificity and timing of expression of the used promoter in the ovary and, more specifically, in the ovarian surface epithelium, which until recently was unavailable. Furthermore, most incorporated gene changes thus far are associated with advanced human ovarian cancer, and their role in early-stage disease is unknown. Recently, the MISRII promoter, which exhibits a relatively restricted pattern of expression, was used to drive the expression of the SV40 large T-antigen in the ovarian surface epithelium (21). Approximately 50% of the female mice bearing the MISRII–T-antigen transgene developed bilateral, poorly differentiated ovarian tumors by 6 to 13 weeks of age. Similarly, most genetic models developed to date are unable to reproduce the histopathological diversity of human ovarian cancer and give rise to rapidly developing, advanced-stage disease at very young age. Hence, although very important for understanding the role of discrete genes in ovarian cancer, these models are inadequate for studying the preneoplastic and early neoplastic stages of the disease or for prevention studies. In contrast, the ovarian lesions induced by carcinogens and hormones in general display all three stages of cancer development (initiation, promotion, and progression). The direct implantation of chemical carcinogens, such as 7,12-dimethylbenz(a)anthracene (DMBA) in the rat ovary (22–24), leads to the induction of ovarian tumors at an incidence of ~37%. These include adenocarcinomas, as well as stroma and mesothelial tumors (22, 23, 25). There is, however, lack of information regarding the nature and sequence of events elicited by DMBA and leading to ovarian cancer development.

To improve its usage and physiologic relevance to the human disease, the DMBA model of ovarian cancer was modified (a) by significantly decreasing the DMBA dose, thereby preserving maximally the integrity of the organ and (b) by incorporating multiple gonadotropin hormone treatments, thus introducing an additional risk factor associated with human ovarian cancer, known also to induce hyperovulation and enhanced mitogenesis of the ovarian surface epithelium (26). Characterization of this modified animal model revealed the appearance of early and advanced lesions with a progressive nature that range from nonneoplastic to preneoplastic to malignant. Their histopathology and path of dissemination strongly resemble human ovarian cancer.

MATERIALS AND METHODS

Animals and In vivo Treatments

Six-week-old virgin Sprague Dawley rats (Taconic Farms, Germantown, NY) were used following NIH and Fox Chase Cancer Center animal care guidelines. DMBA mixed with beeswax was directly applied to the right ovary.

Received 5/17/04; revised 9/20/04; accepted 9/17/04.

Grant support: NIH Grant P50-CA83638 (R. Ozols), Liz Tilberis Scholar Award of the Ovarian Cancer Research Fund, Inc., (C. Patriotis), and NIH Training Grant CA-09035-27 (S. Stewart).

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.

Note: S. Stewart is currently at the Division of Cancer Control and Prevention, NCCPHP, Centers for Disease Control and Prevention, Atlanta, GA; T. Querec is currently at Immunology and Molecular Pathogenesis Program, Emory University, Atlanta, GA; and R. Bao is currently at the Novartis Oncology/Pharmacology, Summit, NJ. Supplementary data for this article can be found at Cancer Research Online (http://cancers.aacrjournals.org).

Requests for reprints: Christos Patriotis, Division of Medical Science, 333 Cottman Avenue, W348, Philadelphia, PA 19111. Phone: (215) 728-3656; Fax: (215) 728-2741; E-mail: Christos.Patriotis@FCCC.edu.

8177

Downloaded from cancers.aacrjournals.org on May 3, 2017. © 2004 American Association for Cancer Research.
of 120 animals. The left ovaries were treated with beeswax only. Animals were treated in three study arms (Supplemental Table 1): 60 animals (arm 1) with 2.5 μg of DMBA and 60 animals (arms 2 and 3) with 5 μg of DMBA. The latter was subdivided in 2 × 2 and subjected to six cycles of treatment with pregnant mare’s serum gonadotropin (Sigma, St. Louis, MO) and human chorionic gonadotropin (Ferring Pharmaceuticals, Los Angeles, CA), once every 2 weeks, starting at 2 months after DMBA application (arm 3) or with corresponding vehicle at the same regimen (arm 2). Pregnant mare’s serum gonadotropin (in sterile saline; 0.9% NaCl, Abbott Laboratories, Chicago, IL) and human chorionic gonadotropin (in bacteriostatic water) were administered i.p. and i.m., respectively, each at a dose of 40 IU per animal.

DMBA Suture Preparation

Three or 1.0 g of beeswax (Sigma) was melted in a sterile Petri dish on a sandbath at 135°C in a chemical fume hood under amber light. One gram of DMBA (Sigma) was added to the melted beeswax and mixed until melted. Uncoated silk sutures (7-0 USP; United States Surgical, North Haven, CT) were dipped into the melted mixture for 2 to 3 minutes. Sutures were air-dried and wrapped in a sterilized aluminum sheet. Beeswax-control sutures were prepared similarly. Sutures were stored at 4°C for up to 7 days before surgery. The average DMBA weight per cm suture was ~8 or ~15 μg for a 1.3 or 1.1 mixture of DMBA/beeswax, respectively, corresponding to a dose of ~2.5 and ~5 μg, respectively, for ~3-mm implanted suture.

DMBA Application to the Ovary

Six-week-old virgin rats were anesthetized by inhalation of halothane, followed by i.p. injection of 1 mL/kg body weight xylazine (20 mg/mL), Acepromazine maleate (10 mg/mL) and Ketamine-HCl (100 mg/mL) mixed in a ratio of 1:2:3, respectively. The rat flanks were shaved and washed with iodine solution and 70% etomidate. Sterile conditions were used throughout the surgical procedure. A transverse, ~1.5-cm mid-lumbar incision was made in the right flank of the animal, ~5 mm ventral to the lumbar muscles. The fat pad with the attached ovary was gently pulled out of the cavity with blunt-end forceps, held by the fallopian tube, and, under amber light, a DMBA/beeswax-suture was applied across the ovary, contralaterally to the fallopian tube/fibria. The suture ends were cut flush with the surface of the bursa. The organ was closed with wound clips. Similarly, a beeswax-impregnated suture was implanted into the left ovary. The animals were observed until awaken and daily for the next 10 to 14 days. The wound clips were removed 7 to 10 days after surgery.

Tissue Preparation and Immunohistochemistry

Upon animal sacrifice, the ovaries and other organs (fallopian tubes, uterus, and mammary glands) were harvested, formalin fixed (18 hours), and paraffin embedded. Five-micron serial sections from different areas of each organ were prepared similarly. Sutures were stained with phenol:chloroform:isoamyl alcohol (25:24:1) with the addition of NH4Cl, H2O and once with chloroform. DNA was precipitated with 2 volumes of 100% ice-cold etomidate, 1 μL of glycogen (20 μg/μL) and 2 μL of 4 n NaCl at ~2°C overnight. Pellets were collected by centrifugation at 13,000 × g for 15 minutes, washed with 70% etomidate, recentrifuged, dried, and resuspended in 25 μL of 10 mmol/L Tris-HCl (pH 8.0). DNA concentration was determined spectrophotometrically (ND-1000; NanoDrop Technologies, Inc., Wilmington, DE).

PCR Amplification, Restriction Digest, and Direct Sequencing. Individual gene exons were subjected to PCR amplification with corresponding specific oligonucleotide primers (Supplemental Table 2), followed by diagnostic restriction digest and for Ki-Ras and Tp53 also by direct sequencing at the Fox Chase Cancer Center sequencing facility. Digested and undigested PCR products were resolved in a 4% Tris-acetate agarose gel containing ethidium bromide (5 μg/mL; Sigma) for UV-light detection. In cases where more than one band was visible, the band with the corresponding expected size was purified from the gel with Gel DNA extraction kit (Qiagen, Valencia, CA). Genomic DNA obtained from the ovary of an untreated female rat was used as control. Sequence analysis was carried out with Accelrys SeqWeb V.2.4.1 for the Wisconsin GCG sequence analysis package V.10.

Histopathology and Statistical Analysis

Three 5-μm H&E-stained tissue sections obtained from different areas of each ovary (one section each at 100 μm from the two ends and one from the middle of the organ) were subjected to histopathology evaluation. Calls were made for presence or absence of significant lesions. The latter were subdivided into three groups: nonneoplastic, putative preneoplastic, and tumor (Table 1). Generalized estimating equations in the context of logistic regression were used to model the probability of developing a lesion of a specific severity as a function of treatment and time on study. The outcome measure is a binary indicator of whether a significant lesion was observed in a given ovary at time of sacrifice. The correlation structure was modeled by assuming that two data points were independent if and only if they were obtained from different animals (i.e., the left and right ovary assessments are correlated if they came from the same animal and are independent otherwise). All significance tests were based on two-sided type 3 score statistics. The left and right ovaries of each animal were assigned an ordinal score representing the maximum severity of any lesion observed at time of sacrifice. The lesion score range was as follows: 1 (no significant lesion), 2 (nonneoplastic), 3 (preneoplastic), and 4 (tumor).

Table 1. Incidence and severity of DMBA-induced ovary lesions

<table>
<thead>
<tr>
<th>Severity of lesions</th>
<th>Arm 1</th>
<th>Arm 2</th>
<th>Arm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA (2.5 μg)</td>
<td>35 (59.32)</td>
<td>12 (40.00)</td>
<td>14 (48.28)</td>
</tr>
<tr>
<td>DMBA (5.0 μg)</td>
<td>11 (18.64)</td>
<td>5 (16.66)</td>
<td>3 (4.55)</td>
</tr>
<tr>
<td>DMBA + hormone</td>
<td>12 (20.34)</td>
<td>13 (43.33)</td>
<td>11 (37.93)</td>
</tr>
<tr>
<td>Total ovaries</td>
<td>30 (12.71)</td>
<td>29 (12.29)</td>
<td>59 (25.00)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity of lesions</th>
<th>Arm 1</th>
<th>Arm 2</th>
<th>Arm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lesions cnt. (%)</td>
<td>59 (25.00)</td>
<td>30 (12.71)</td>
<td>29 (12.29)</td>
</tr>
<tr>
<td>Nonneoplastic lesions cnt. (%)</td>
<td>5 (2.12)</td>
<td>0</td>
<td>5 (2.12)</td>
</tr>
<tr>
<td>Neoplastic lesions cnt. (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total animals/Total ovaries cnt. (%)</td>
<td>13,000</td>
<td>13,000</td>
<td>13,000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity of lesions</th>
<th>Arm 1</th>
<th>Arm 2</th>
<th>Arm 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ovaries</td>
<td>23 (76.67)</td>
<td>4 (13.33)</td>
<td>6 (6.99)</td>
</tr>
<tr>
<td>Total ovaries</td>
<td>157 (66.52)</td>
<td>28 (11.86)</td>
<td>46 (19.49)</td>
</tr>
</tbody>
</table>

* Chronic inflammation; foreign body granuloma; prominent corpora lutea; suture granuloma; salpingitis.
† Epithelial hyperplastic lesions: ovarian surface epithelium or bursal flat hyperplasia (either pseudostratification or real stratified hyperplasia); ovarian surface epithelium or bursal papillae or papillomatosis; inclusion cysts; endosalpingiosis. All these lesions can present with or without atypia.

Abbreviation: cnt., number of lesions, ovaries, or animals.
RESULTS

Ovarian Preneoplasia and Neoplasia Induced in Rats with DMBA

Female Sprague Dawley rats were subjected to local application of DMBA/beeswax to their right ovaries in three treatment arms. Their left ovaries were treated as internal controls by application of beeswax alone. To determine the sequence of histologic and molecular changes elicited by DMBA in the ovary, subgroups of animals were sacrificed at various time points, up to 12 months (Supplemental Table 1). Overall, an apparent decrease in volume was evident in the DMBA-treated ovaries in arms 1 and 2. Relative to the control ovaries, the histologic and physiologic integrity of the treated organs was well maintained, with the exception of a small reduction in the rate of follicular development and corpora lutea formation (Fig. 1A). In arm 3, as a result of the stimulatory effect of the administered gonadotropin hormones, the reduction in volume of the DMBA-treated ovaries was less apparent. An average 4 to 5-fold larger number of developing follicles and corpora lutea was observed in both ovaries, as compared with the ovaries of animals in arms 1 and 2 (data not shown). No other histologic changes were observed during the first 4 to 5 months after DMBA treatment in the ovaries. At 5 to 6 months posttreatment and persisting to the end of the experiment, a number of different types of lesions were observed (Table 1): (a) nonneoplastic lesions (chronic inflammation, foreign body granuloma, prominent corpora lutea, suture granuloma, and salpingitis) were found in both DMBA-treated and control ovaries and at a similar frequency; and (b) the appearance of lesions of a putative preneoplastic nature and with a progressive character was observed predominantly in the DMBA-treated ovaries (Fig. 1, B and C). These represent proliferative epithelial lesions, present either along the surface of the organ or in the ovarian cortex. Other preneoplastic lesions represent inclusion cysts or simple serous microcysts; other cortical lesions surrounded by ovarian stroma and characterized by the presence of several gland-like structures, usually covered by a simple serous cuboidal epithelium, and some resembling fallopian tube epithelial differentiation (endosalpingiosis). A few preneoplastic lesions exhibit cellular atypia and are classified as epithelial hyperplastic lesions with dysplasia. None of the hyperplastic epithelial lesions are invasive; they are well circumscribed, small, and with low mitotic rate. These characteristic features separate them easily from either borderline ovarian tumors (also known as serous tumors of low malignant potential) or invasive adenocarcinomas and bona fide ovarian tumors, detected in arms 1 and 3 only. A tumor highly reminiscent of human serous low malignant potential tumor was detected at 12 months after DMBA treatment in arm 1 (Fig. 2A), an invasive serous adenocarcinoma—at 6 months...
in arm 3 (Fig. 2B), a squamous-cell carcinoma—at 9 months, arm 3 (Fig. 2C), and an undifferentiated carcinoma—at 11 months, arm 3 (Fig. 2D).

**Statistics**

The cumulative incidence of preneoplastic lesions and *bona fide* tumors in the DMBA-treated ovaries in arm 1 was 22%, whereas in arms 2 and 3 it was 2-fold higher (43.33 versus 44.82%, respectively; Table 1). However, both the preneoplastic lesions and the *bona fide* tumors in arm 3 displayed a more complex, advanced histology relative to those in arms 1 and 2. When all three types of lesions were considered together in each of the three arms, time to sacrifice was not a significant predictor of lesion severity \((P = 0.356)\). Thus, the probability that an animal bore a lesion of a specific degree of severity was not observed to depend on how long the animal was allowed to survive before sacrifice. The level of DMBA treatment, however, had a significant effect on lesion severity \((P < 0.0001)\). Specifically, the control ovaries had a significantly lower incidence of lesions and at a lower severity than the DMBA ovaries in arms 1, 2 and 3, respectively \((P < 0.05)\). Furthermore, the cumulative incidence of preneoplastic lesions and tumors together was significantly higher in arms 2 and 3 as compared with arm 1 \((P < 0.05)\); however, there was no significant difference in the incidence of these lesions between arms 2 and 3 \((P = 0.73)\).

**Immunohistochemical Characterization of Ovarian Lesions**

**Epithelial Cell Origin.** The epithelial cell origin of the preneoplastic lesions and carcinomas was confirmed by their positive anti-cytokeratin immunostaining, characteristic of most types of epithelial cells (Fig. 3), and the negative anti-vimentin immunostaining that detects a variety of mesenchymal cells (data not shown).

**Expression of Estrogen (ER) and Progestin (PgR) Receptors.** To determine whether ER and PgR play a role during ovarian cancer development in this model, their expression status was examined by immunohistochemistry for ER-\(\alpha\) and PgR (A/B). Although the expression of both receptors is low to undetectable in morphologically normal ovarian surface epithelium cells, all tested preneoplastic lesions and the serous low malignant potential tumor are strongly positive for both ER-\(\alpha\) and PgR (Fig. 4, A and B, left and middle panels, respectively). The expression of both receptors, however, is either markedly decreased or undetected in the invasive carcinomas (Fig. 4, C and D, left and middle panels, respectively).

**Expression of Tp53.** Anti-Tp53 immunostaining was carried out to determine whether Tp53 gene mutations leading to loss of function and accumulation of the protein are also induced during ovarian cancer development by DMBA. A strong positive anti-Tp53 immunostaining was detected in the two invasive and the squamous cell carcinomas (Fig. 4, C and D, right panel, and data not shown) but not in the preneoplastic lesions (Fig. 4A, right panel) or the serous low malignant potential tumor (Fig. 4B, right panel).

**Mutation Analysis**

**Tp53 Gene.** To examine the mutational status of Tp53 during ovarian cancer development in this model, genomic DNA was extracted from microdissected normal-appearing ovarian surface epithelium, preneoplastic lesions, tumors, and a control untreated ovary. Tp53 exons 4 to 8 were PCR-amplified from purified genomic DNA samples with corresponding oligonucleotide primers (Supplemental Table 2). PCR products were subjected to bi-directional sequencing after extraction from agarose gels. Individual Tp53 mutations were detected in four of the examined preneoplastic lesions and in all tumors (Table 2).

**Ki-Ras Gene.** To determine whether activating mutations of Ki-Ras in codons 12, 13, and 61 are associated with ovarian cancer in this model, genomic DNA, purified as for Tp53 analysis, was used for PCR amplification with corresponding oligonucleotide primers (Supplemental Table 2). PCR products were subjected to diagnostic restriction digest with BSS SI (for codon 61) and bi-directional sequencing after purification from agarose gels. Only mutation of codon 61 (CAA→CAC; protein Gln→His) was identified in this rat model and was present in 4 of the 12 examined preneoplastic lesions (Table 2) and in the invasive adenocarcinoma.

**PgR.** The presence or absence of an activating mutation of PgRs at codon 660 was also examined in extracted genomic DNA, with PCR...
amplification with corresponding oligonucleotide primers and diagnostic restriction digest with Tsp R1 (Supplemental Table 2). Such mutation was not detected in any of the examined lesions.

**DISCUSSION**

This study attempted to additionally improve the DMBA-rat model of ovarian oncogenesis and characterize the distinct stages of preneoplasia and neoplasia. The contribution of gonadotropin hormones to this process was also demonstrated. DMBA treatment of the ovary induces putative preneoplastic lesions of epithelial cell origin and with progressive histology that are assumed to represent precursors of ovarian cancer clonal development. Given the difficulties in obtaining a consensus on what human ovarian preneoplastic or precursor lesions are, an attempt was made to classify the putative precursor lesions of the rat ovary with terminology used for human ovarian epithelial lesions. The lesions observed in the rat ovary represent proliferative epithelial lesions of variable degrees of differentiation, without or with dysplasia, and localized along the ovarian surface and cortex. Some of the lesions, especially those seen on the surface, are similar to isolated papillae or diffuse papillomatosis seen in human ovaries. In addition, there are occasionally other ovarian surface epithelium-

![Image](https://example.com/image.png)

**Figure 4.** ER-α, PgR, and Tp53 expression in putative preneoplastic and neoplastic ovarian lesions induced by DMBA. Left panel: anti-ER-α; middle panel: anti-PgR; and right panel: anti-Tp53 immunostaining of (A) DMBA-treated ovaries containing epithelial flat and papillary hyperplasia, (B) serous low malignant potential tumor, (C) invasive serous adenocarcinoma, and (D) undifferentiated carcinoma. Note that the ER-α and PgR immunostains are markedly decreased in C and D and that Tp53 immunostain is markedly decreased or absent in A and B. (Hematoxylin counterstaining; bar scale: 100 μm).

<table>
<thead>
<tr>
<th>Type of lesion (cnt.)</th>
<th>Ki-Ras Codon 61 CAA→CAC (cnt.)</th>
<th>Human codon</th>
<th>Mutation: DNA</th>
<th>Mutation: protein</th>
<th>Prevalence in human ovarian cancer</th>
<th>Protein accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSE/Bursal epithelial papillae (3)</td>
<td>Yes (2)</td>
<td>224 (6)</td>
<td>226</td>
<td>GTG→GGG</td>
<td>Val→Ala</td>
<td>ND</td>
</tr>
<tr>
<td>OSE/Bursal epithelial papillae with dysplasia (2)</td>
<td>Yes (2)</td>
<td>ND</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Papillomatosis (3)</td>
<td>ND</td>
<td>207 (6)</td>
<td>209</td>
<td>AGG→GGG</td>
<td>Silent (Arg)</td>
<td>ND</td>
</tr>
<tr>
<td>Inclusion cysts with papillae (4)</td>
<td>ND</td>
<td>209 (6)</td>
<td>211</td>
<td>ACT→ATT</td>
<td>Thr→Ile</td>
<td>Yes: 0.39%</td>
</tr>
<tr>
<td>Low malignant potential (LMP) tumor</td>
<td>ND</td>
<td>178 (5)</td>
<td>180</td>
<td>GAA→GGA</td>
<td>Glu→Gly</td>
<td>ND</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>ND</td>
<td>255 (7)</td>
<td>257</td>
<td>Deletion ATC</td>
<td>Ile</td>
<td>Yes: 0.39%</td>
</tr>
<tr>
<td>Cystadenoma and invasive adenocarcinoma</td>
<td>Yes</td>
<td>151 (5)</td>
<td>153</td>
<td>CCA→TCA</td>
<td>Pro→Ser</td>
<td>Yes: 0.1%</td>
</tr>
<tr>
<td>Undifferentiated carcinoma (invasive)</td>
<td>ND</td>
<td>218 (6)</td>
<td>220</td>
<td>CAG→CGG</td>
<td>Gln→Arg</td>
<td>Yes: 2.4%</td>
</tr>
</tbody>
</table>

Abbreviations: ND, not detected; N/A, not applicable; GYN, gynecological; cnt., number of lesions from independent ovaries tested for mutation.

[Table 2 Mutations detected in the Ki-Ras and Tp53 genes in DMBA-induced preneoplastic and neoplastic ovarian lesions in the rat]

8181
DMBA-induced ovarian preneoplasia and neoplasia

derived structures that were previously described in humans, i.e., inclusion cysts or simple serous microcysts. None of the observed hyperplastic epithelial lesions are invasive and are quite distinct from either serous low malignant potential ovarian tumors or invasive carcinomas. The development of the putative precursor lesions generally precedes the emergence of bona fide tumors, which also display variable degrees of differentiation and progression, ranging from early tumors to high-grade malignant, invasive carcinomas. In addition to the tumors detected in this study, a bilateral invasive carcinoma with clear-cell histology was detected within 12 months in an animal whose ovaries were treated bilaterally with ~5 μg of DMBA (not part of the three study arms). This advanced tumor displayed widespread dissemination to i.p. organs, production of ascites, and metastatic hemorrhagic foci in the lungs (data not shown).

Statistically, the appearance of lesions of any given severity did not depend significantly on the time of sacrifice after DMBA treatment; however, escalation of carcinogenic dose combined with hormonal stimulation increased significantly the severity of the detected lesions. The cumulative incidence of preneoplastic lesions and tumors was also equivalently increased significantly at the higher DMBA dose in arms 2 and 3. Although the lesion incidence in arms 2 and 3 was similar, the lesions detected in arm 3 were more advanced than those in arm 2, including bona fide tumors that were not observed altogether in arm 2. This data demonstrates the strong contribution of gonadotropin hormones to the neoplastic progression of the ovarian lesions, perhaps due to increased ovarian surface epithelium cell proliferation and their effects on the underlying stroma. As demonstrated earlier, treatment of rats with pregnant mare’s serum gonadotropin and/or human chorionic gonadotropin, in the presence or absence of surgical scarring to the ovary, leads to a 5 to 10-fold increase in the rate of ovarian surface epithelium cell proliferation (26).

The observed DMBA-induced reduction in ovarian volume, accompanied by decreased follicular growth and corpora lutea formation, is in good agreement with previously published data (28). The apparent differences in the observed low-dose response and persistence of ovarian hypoplasia in this study may be due to the slow-release form of DMBA applied directly to the ovary. Although not yet well understood in its full complexity, a suggested mechanism underlying the observed ovarian hypoplasia and cellular destruction is that DNA-adduct formation by DMBA metabolites leads to Tp53-mediated inhibition of DNA synthesis, cell growth arrest, and caspase-dependent or independent apoptosis (29–31). Hence, DMBA-induced mutation(s) that disrupt Tp53 function may allow evasion of affected ovarian surface epithelium cells and contribute to their malignant transformation.

Nonneoplastic and a small number of preneoplastic lesions, as well as a small granulosa cell tumor were also detected in control ovaries. To determine whether such lesions occur spontaneously in this rat strain, 20 nontreated animals were divided in two groups of 10 and maintained to the age of 8 and 14 months, respectively. Examination of their ovaries revealed no significant lesions, which strongly suggests that the lesions observed in the control ovaries may be a consequence of surgical scarring and chronic inflammation, and/or carcinogen carryover from the contralateral ovary. This data indicates that chronic inflammation, a known risk factor of ovarian cancer, may contribute to the DMBA-induced neoplastic process, either directly on epithelial cells through the action of secreted inflammatory cytokines and growth factors or indirectly through their effect on the adjacent stroma.

This study has additionally demonstrated that specific mutations in the Tp53 and Ki-Ras genes, which are among the most frequent mutations found in human ovarian tumors, are also associated with ovarian cancer induced by DMBA. TP53 mutations are found in 35 to 40% of human ovarian tumors (32–34). The identified rat Tp53 mutations of codons 173 and 218 correspond to human codons 175 and 220, respectively, which are among the most frequent in human ovarian cancer (6.8% and 2.4%, respectively).3 Interestingly, both mutations lead to a characteristic accumulation of Tp53 protein. Activating mutations of Ki-Ras, including codon 61 detected in multiple DMBA-induced preneoplastic lesions and in one carcinoma, have been associated with ~20% of human ovarian tumors: of them, ~60% are found in mucinous and ~20% in serous carcinomas (35, 36). The relatively high frequency of Ki-Ras mutations in the preneoplastic lesions and, especially, in the ones with dysplasia provides a strong indication of their clonal (i.e., neoplastic) nature. It additionally argues that Ki-Ras activation, either through mutation or by aberrant upstream signals, is very important during ovarian cancer development. Finally, a significant overexpression of the ER-α and PgR proteins was also demonstrated in the preneoplastic lesions and the serous low malignant potential tumor. However, the expression of the two receptors was markedly decreased or absent in the advanced carcinomas. The importance of this finding, in view of the existing controversy over the expression status of ER-α and PgR in human ovarian cancer (37, 38), mandates additional investigation. Furthermore, the Val66Leu polymorphism that frequently occurs in exon 4 of PgRs has been suggested to have an association with human ovarian cancer characteristics and with overall ovarian cancer risk (39). Population-based studies, however, have demonstrated that no such association exists (40, 41). Lack of this PgR mutation in the examined ovarian lesions is additional evidence to the consistency of the DMBA rat ovarian cancer model with the human disease.

DMBA is a pluripotent carcinogen, which, through the formation of DNA adducts, induces initiating point mutations that alter the expression and/or activity of a number of oncogenes and tumor suppressor genes (42–45). Although DMBA itself is not a known environmental carcinogen associated with ovarian cancer, it shares similar mutagenic mechanisms with other polycyclic aromatic hydrocarbons whose abundance is relatively high in air pollutants and in tobacco smoke and which have been implicated in human cancer development (46, 47). Hence, the observed effect of DMBA in the ovary may be representative of the effect that such carcinogens have in the ovaries of affected women.

Here, we have demonstrated that direct application of a low dose of DMBA in the rat ovary, alone or combined with multiple cycles of gonadotropin administration, elicits a neoplastic process that affects mostly the ovarian surface epithelium and leads to the progressive development of putative epithelial cell preneoplasia, serous low malignant potential tumors, and invasive carcinomas. The similarity in histology and path of dissemination of the DMBA-induced rat ovarian carcinomas with those in the human, as well as the presence of gene mutations that are common in human ovarian cancer, demonstrate the validity of this animal model for additional delineation of the mechanisms underlying ovarian tumorigenesis. Finally, DMBA-induced ovarian oncogenesis in the rat could be used to preclinically test new agents for the prevention and/or therapy of the disease.

ACKNOWLEDGMENTS

We thank C. F. Renner for expert technical assistance and Drs. A. K. Godwin and X-X. Xu for their comments and suggestions.

REFERENCES


3 Internet address: http://www.iarc.fr/P53/index.html.
21. Connolly DC, Bao R, Nikitin AY, et al. Female mice chimera for expression of the mammalian ovary. Mad- 
Characterization of a Carcinogenesis Rat Model of Ovarian Preneoplasia and Neoplasia


Cancer Res 2004;64:8177-8183.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/22/8177

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2004/12/02/64.22.8177.DC1

Cited articles
This article cites 40 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/22/8177.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/64/22/8177.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.