Gene for Ovarian Granulosa Cell Tumor Susceptibility, *Gct*, in SWXJ Recombinant Inbred Strains of Mice Revealed by Dehydroepiandrosterone


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ABSTRACT

Spontaneous, malignant ovarian granulosa cell (GC) tumors occur in peripubertal SWR and specific SWXJ recombinant inbred strains of mice. Treatment of these mice with dehydroepiandrosterone (DHEA), an adrenal secretory steroid with anticancer actions against spontaneous and carcinogen-induced tumors of different tissues, gave unexpected results. Diet supplemented with 0.4% DHEA (a) induced significantly more GC tumors in spontaneous tumor-susceptible strains (SWR and SWXJ-1, -4, and -9), (b) induced the first GC tumors observed in five previously tumor-free strains (SWXJ-6, -7, -8, -10, and -12), and (c) failed to induce GC tumors in SJL and in the remaining six SWXJ strains (SWXJ-2, -3, -5, -11, -13, and -14). The strain distribution pattern of DHEA-induced GC tumor susceptibility versus resistance was compared with strain distribution patterns for 35 different loci known to distinguish SWR and SJL progenitor strains. A complete match of DHEA-induced GC tumors with pancreas-2 (Pan-2) on mouse chromosome 4 was found. We have named this new locus GC tumor susceptibility (*Gct*), with the *Gct* (susceptible) allele found in SWR and the *Gct* (resistant) allele found in SJL mice. The *Gct* locus is closely linked to pancreas-2, Pan-2, but the order of genes is not yet confirmed. In addition, data from F1 progeny of matings between SWR and selected inbred strains provide suggestive evidence for a second gene controlling GC tumor incidence that we hypothesize involves steroid metabolism. Differences in GC tumor incidence data from reciprocal F1 progeny of matings between SWR and SJL mice reveal a strong maternal effect that may represent yet a third gene. These data support a heritable basis for GC tumorigenesis in the SWR model involving a small number of genes.

INTRODUCTION

Spontaneous ovarian GC tumors found in young SWR/J and SWR/JBm mice (1) are strikingly similar to a subclass of human ovarian tumors, juvenile granulosa cell tumors (2-5). Both mouse and human tumors often present as multiple foci in an ovary that has intact oocytes and follicles. GC tumors from both species show similar variations in histological patterns. In mice, GC tumors appear in the peripubertal period (4-7 weeks) and only rarely in young adults (10+ weeks). Juvenile GC tumors have been observed in girls from infancy through adolescence. Metastases have been observed in mice ages 5-10 months and in patients after 5 years or more (6). The mouse and human hosts with early onset GC tumors share certain endocrinological features (7).

We have previously reported (1) that spontaneous GC tumors occur: (a) in sublines of SWR but not in the closely related SJL/JBm and ST/BJ inbred strains derived from Swiss stocks; (b) in F1 hybrids of SWR mated to SJL or ST/b; and (c) in 3 of 14 SWR × SJL-derived recombinant inbred (SWXJ) strains; (d) but not in reciprocal F1 hybrids of SWR and A/HeJ, C57BL/6ByJ, CBA/J, or DBA/2J strains. These findings strongly suggest that GC tumor susceptibility in SWR mice is inherited and related inbred Swiss strains share some but not all genes necessary for tumorigenesis. The discovery that administration of DHEA to one of the SWXJ strains (8) significantly increased GC tumor incidence led to a systematic search for new phenotypes in the remaining SWXJ, progenitor, and reciprocal F1 hybrid strains. We report herein data for the first gene controlling GC tumorigenesis and additional evidence for at least one more gene.

MATERIALS AND METHODS

Mice. The SWR/Bm, SJL/Bm, reciprocal F1 hybrids, and SWXJ/Bm recombinant inbred strains of mice utilized in these studies were raised in our Research colonies at The Jackson Laboratory. Mice from the inbred strains AKR/J, MA/Mj, RI/J, F1J, BUB/Bn, PL/J, and ST/BJ were obtained from Animal Resource colonies, The Jackson Laboratory. All mice were maintained under 12-h-light-12-h-dark cycles and were given pasteurized Wayne Lab Blox diet (crude protein, 24%; fat, 4.0%; fiber, 4.5%; ash 5.9%; vitamin and mineral fortified; Wayne Pet Food Div., Continental Grain Co., Chicago, IL) and water (pH 2.8-3.2; 10-20 ppm sodium hypochlorite) *ad libitum*. Environmental conditions included controlled air temperatures of 20-22°C and relative humidity levels of 40-70%. Litters were weaned at 22 to 26 days and pedigreed females were assigned to treatment groups by 28 days of age.

Treatments. The DHEA was administered to experimental mice via their diet. Steroid-supplemented diets, 0.1% or 0.4% DHEA, were prepared by addition of the appropriate amount of DHEA, dissolved in acetone, to finely ground standard Wayne diet. The supplemented diet was mixed thoroughly and then air dried overnight. Supplementation of diet with levels of DHEA in excess of 0.4% is associated with signs of toxicity such as coarse-appearing pelage, substantial loss of weight, and in some strains increased mortality. Control mice were fed the standard diet. Acetone solvent was not added to control diets since our previous finding that estradiol dissolved in acetone and added to diet completely suppressed GC tumor incidence demonstrated that acetone itself was not tumor promotive (8).

Experimental mice were fed DHEA-supplemented diet *ad libitum* until 70 days of age. At the end of the treatment period, mice were killed and ovaries were inspected for the presence of GC tumors that typically range in diameter from 6 to 20 mm. Ovaries of uncertain tumor status were fixed in Bouin's solution and embedded in paraffin, and 7-μm-thick sections were stained with hematoxylin and eosin for evaluation at the light microscope level.

Strain Distribution Patterns for Pan-2 and Lyb-2. The distribution of alleles for the pancreas-2 (Pan-2) locus was determined by high resolution isoelectric focusing of soluble pancreatic proteins. A young adult male and a female of SWR, SJL, and each SWXJ strain were fasted overnight. Mice were killed by cervical dislocation, and pancreases were removed, frozen on dry ice, and individually homogenized in 0.25 ml cold distilled water. Homogenates were transferred to 1.5-ml plastic microfuge tubes and centrifuged at 15,000 × g for 5 min.

The isoelectric focusing gels were run as follows. Polyacrylamide gels (12.5% w/v), containing 3.33% (v/v) pH 3-10 ampholytes and...
1.67% ((+/+) pH 9–11 amphotoles, were prepared according to instruc
tions (LKB Instruments) and aged overnight at 4°C. Prefocusing of gels
was accomplished at 25 mA and 25 W until a potential difference of
350 V was achieved. Fifteen-μl volumes of supernatants were loaded
onto filter paper application squares (LKB Instruments) and laid on the
anodal surface 2 cm from the anodal wick of the prefocused gel.
Isoelectric focusing was resumed for 15 min; then applicator squares
were removed. Focusing was continued until a potential of 1000 V was
attained. The isoelectric focusing apparatus was disassembled and gels
were immediately immersed in a fixative solution of trichloroacetic acid
(11.5%):sulfosalicylate (3.45%):ethanol (30%) for 2 h. Protein bands
were visualized with silver staining using a commercial kit (Bio-Rad).

For determination of isoelectric points, a slice of focusing gel was
removed prior to fixation and cut in 1-cm blocks with a razor block.
Each block was suspended in 1 ml of distilled water overnight and used
for pH determinations to identify approximate isoelectric points of the
pancreatic proteins of interest. The Pan-2 allele of each SWXJ strain
was assigned by comparison with SWR (Pan-2') and SJL (Pan-2')
supernatants simultaneously electrophoresed as standards. These pan-
creatic proteins have isoelectric points of approximately 9.0–9.2.

The distribution of alleles at the Lyb-2 locus on chromosome 4 was
determined by fluorescence-activated cell sorter analysis. SWR mice
express the Lyb 2a allele that determines the specificity Lyb-2.1, while
SJL mice express the Lyb 2e allele that determines the specificity Lyb-
2.3 (9, 10). The Lyb-2 antigen is present on lymphocytes (11). Splenies
from the progenitor and SWXJ strains were teased apart in 4-(2-
hydroxyethyl)-1-piperazineethanesulfonic acid-buffered Hanks' me-
dium containing 5% fetal bovine serum and 0.1% sodium azide. After
passage through Nutex mesh cloth, the cells were washed twice by
centrifugation and treated to remove RBC with ammonium chloride
lysing buffer (12). The treated spleen cell suspensions were washed and
resuspended in Hanks' medium of low IgG2a anti-Lyb-2.3 antibody (New England Nuclear,
Cambridge, MA). The spleen cell suspensions were incubated on ice
for 30 min, washed three times, and incubated on ice for an additional
30 min with affinity-purified fluorescein isothiocyanate-conjugated goat
anti-mouse IgG2a (Southern Biotechnology Associates, Birmingham,
AL). The cell suspensions were then washed three times, fixed in 1%
paraformaldehyde, and analyzed in an Ortho-50H flow cytometer.

Since IgG2a* B-cells comprise a minor cell population in the spleen,
less than 5% of the spleen cells were labeled following incubation with
fluorescein isothiocyanate-conjugated anti-IgG2a alone. Analyses of
progenitor strain mice showed that approximately 40% of SJL/Bm
nucleated spleen cells expressed Lyb-2.3 cell surface antigen, whereas
SWR/Bm spleen cells were negative for this alloantigen. Analyses of
the 14 SWXJ strains showed that six strains expressed Lyb-2.3 and
carry Lyb-2e, while the remaining eight strains were negative and thus
carry Lyb-2a.

Data Analyses. The body and organ mass data were analyzed for
significant differences among means by t test for independent samples.
The GC tumor incidence data were analyzed by χ² test.

RESULTS

The GC tumor incidence data for the progenitor strains, SWR and SJL,
the 14 recombinant SWXJ strains, and the reciprocal F1 hybrid mice fed DHEA are given in Table 1. The various inbred strains are classified as tumor susceptible or
tumor resistant, depending on the occurrence of GC tumors in
control and DHEA-treated groups. Within the tumor-susceptible
class, the “spontaneous” strains SWR/Bm plus SWXJ-1,
-4, and -9 showed significant further increases of GC tumor incidences following 0.4% DHEA treatment. Only SWXJ-4 of the
4 spontaneous GC tumor strains failed to respond to 0.1%
DHEA with a significant increase in GC tumor frequency.
Strains SWXJ-6, -7, -8, -10, and -12 were of particular interest
because 0.4% DHEA induced GC tumors in each of these
previously tumor-free stocks. Two (SWXJ-8 and -12) of these
5 strains also formed tumors when fed 0.1% DHEA. The tumor-
resistant class is composed of SJL/Bm and the 6 remaining
SWXJ (-2, -3, -5, -11, -13, and -14) strains; none developed GC
tumors when fed either dose level of DHEA. Both of the F1
hybrids, derived from reciprocal matings between SWR/Bm and
SJL/Bm, responded to 0.4% DHEA treatment by induction of
GC tumors. Only the SWR × SJL F1 females show sponta-
neous GC tumors and DHEA induced a significant increase.
The 2 tumors found among 114 SJL × SWR F1 mice are of
particular biological importance because they demonstrated
that both F1 hybrids had the genetic predisposition to form GC
tumors.

A strain distribution pattern for the two phenotypic classes,
GC tumor-susceptible (SWR-like) or resistant (SJL-like) was
assigned to the SWXJ strains. Comparison of this GC tumor
susceptibility pattern was made with strain distribution patterns
for 35 polymorphic loci known to differ between SWR and
SJL. These loci represented markers for 12 of the 20 chromo-
some pairs found in the mouse. Table 2 shows the full concord-
ance (14 of 14 matches) found between GC tumor susceptibility
and the locus Pan-2 on the distal portion of chromosome 4.
Therefore, there is a 95% probability that a gene for GC tumor
susceptibility is located within 8.9 cM of Pan-2 (13). We have
named this new locus granulosa cell tumor susceptibility Get,
with the allele for SWR susceptibility designated Get1 and the
allele for SJL resistance designated Get2. Since both reciprocal
F1 hybrids can express GC tumors, we assign the Get1 allele as
resistant.
GENE FOR GRANULOSA CELL TUMORS IN SWR MICE

Evidence for a second gene regulating Get expression derives from the distribution of susceptible SWXJ strains into "spontaneous" and "inducible" subclasses for tumorigenesis. A similar pattern of spontaneous and inducible GC tumors were found in F1 progeny of SWR females mated to males of specific inbred strains. We hypothesize that differences in endogenous steroid metabolism may distinguish between spontaneous and inducible strains. This hypothesis rests upon the fact that steroids which manipulate GC tumor incidence are also endogenously present in the mouse. DHEA is a natural adrenocortical product that is efficiently converted by peripheral tissues to a variety of androgens and, less efficiently, to estrogens (15, 16). We have shown that GC tumor incidence can be increased by DHEA and suppressed by estradiol in SWXJ-9 mice (8). Therefore, we believe that steroid metabolism and this second gene are related.

A third phenotype is observed among F1 progeny of SWR/Bm dams crossed to PL/J or BUB/BnJ sires (Table 3). These F1 progeny have relatively high spontaneous GC tumor incidences that were not further increased by DHEA. This phenomenon may demonstrate that either PL/J and BUB/BnJ carry a third allele of the second gene hypothesized above or there is yet a third gene regulating GC tumorigenesis.

The spontaneous and induced GC tumor incidence data for reciprocal F1 hybrids between SWR and SJL (Table 1) are disparate in that the SJL × SWR F1 females consistently showed fewer tumors. Since both hybrids are identical with respect to the nuclear genome, the difference in tumor incidences suggests that the dam may be able to profoundly influence tumorigenesis in her offspring. Both promotive and suppressive effects on tumorigenesis by dams of specific genotypes have been documented. For example, susceptibility to mammary tumorigenesis in C3H/He mice is conveyed by milk-borne virus (17). Resistance to development of lymphoma is conferred by milk-borne factor(s) that suppress leukemia virus replication in offspring of SJL/J, RF/J, and ST/Bj dams (18, 19). Our current data do not distinguish whether the SWR dam promotes or the SJL dam suppresses GC tumorigenesis. However, we do know that ecotropic leukemia viruses are not involved in GC tumorigenesis because (a) SWR mice do not carry ecotropic viruses (20) and (b) the two SJL ecotropic leukemia viruses do not segregate with susceptibility to either spontaneous or inducible GC tumors in SWXJ strains. Negative evidence for involvement of ecotropic virus with GC tumorigenesis does not exclude a possible maternal interaction involving xenotropic retrovirus or other retrovirus-like elements in the mouse genome maternal. The tentative evidence for a maternal effect on GC tumor incidence requires further experimental verification before any conclusions about mechanism can be drawn.

In summary, we have presented evidence for two or more genes involved with control of GC tumorigenesis in the SWR model. Get, provisionally assigned to chromosome 4, may be an oncogene conveying susceptibility to GC tumors by an as yet unknown mechanism. Another gene, possibly involved with steroid metabolism, appears to regulate Get expression. A third gene may control the observed maternal effect on GC tumor incidence. Elucidation of the genetic and endocrine mechanisms for the control of GC tumorigenesis in the SWR mouse model may provide valuable insight into human ovarian cancer, in the expression of GC tumors in all SWXJ strains that carry Get'. We do not yet know what the nature of Get may be.


table

<table>
<thead>
<tr>
<th>Table 3 Incidence of spontaneous and DHEA-induced ovarian GC tumors in 10-wk-old F1 progeny derived from matings of SWR females with males from selected inbred strains</th>
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<tr>
<td><strong>Data for spontaneous GC tumors in 10- to 26-week-old females of these eight inbred strains are also provided. Fractions represent number of females with GC tumors/total females examined.</strong></td>
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<tr>
<td><strong>Diet</strong></td>
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<td>F1 hybrid</td>
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<td><strong>Group A</strong></td>
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<tr>
<td>SWR/Bm × AKR/J</td>
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<td>SWR/Bm × MA/MjJ</td>
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<td>SWR/Bm × RIILS/J</td>
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<td>SWR/Bm × RF/J</td>
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<td><strong>Group B</strong></td>
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<td>SWR/Bm × SJL/Bm</td>
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<tr>
<td>SWR/Bm × ST/Bj</td>
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<td><strong>Group C</strong></td>
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<td>SWR/Bm × BUB/BnJ</td>
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<td>SWR/Bm × PL/J</td>
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</table>

* The total females examined of the paternal inbred strains are presented as the number of retired breeders (6-7 months) + the number of virgins (6-14 weeks). The inbred colonies (except for SWR/Bm and SJL/Bm) are very small and those data represent the years 1983-1987.

a Data previously published (1) and presented here for comparison.

b Unpublished observations.

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which the importance of genetics has long been recognized (21–
24).

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Gene for Ovarian Granulosa Cell Tumor Susceptibility, Gct, in SWXJ Recombinant Inbred Strains of Mice Revealed by Dehydroepiandrosterone


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