Genetic Susceptibility for C19 Androgen Induction of Ovarian Granulosa Cell Tumorigenesis in SWXJ Strains of Mice

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ABSTRACT

Susceptibility to pubertal onset, malignant granulosa cell (GC) tumors of the ovary is inherited in SWR/Bm and certain SWR-related SWXJ recombinant inbred strains of mice. In some SWXJ strains, GC tumors occur spontaneously (spontaneous strains), and in others GC tumors can only be induced by treatment with dehydroepiandrosterone (DHEA-dependent strains). A gene controlling susceptibility to both spontaneous and DHEA-induced GC tumorigenesis, Gct, has been assigned to Chromosome 4.

Additional research on the role of steroids in GC tumorigenesis has revealed a second gene controlling response to C19 androgenic steroids. Spontaneous strains showed increased tumor frequency after treatment with testosterone (T), whereas DHEA-dependent strains showed no GC tumors following T treatment. Within treatment groups, serum steroid data from DHEA, T, and control treated mice showed no consistent differences between spontaneous and DHEA-dependent strains with respect to progesterone, DHEA, androstenedione, dihydrotestosterone, T, estrone, or estradiol. Thus, observed differences in GC tumor responsiveness to exogenous steroids were not due to different patterns of steroid metabolism among spontaneous and DHEA-dependent strains.

Further studies on the range of effective C19 steroids were conducted using one spontaneous and one DHEA-dependent strain. The spontaneous strain showed increased GC tumor frequency in response to dihydrotestosterone and androstenedione treatment, whereas the DHEA-dependent strain showed no response. This result suggests that spontaneous strains may be sensitive to a broad range of C19 steroids.

To determine whether genetic differences in endogenous steroid levels have a role in spontaneous GC tumorigenesis, serum steroid levels were measured in SWR/Bm and SJL/Bm progenitor strains during the developmental period of risk between 22 and 38 days of age. With the exception of transiently increased DHEA at 22 days, there were no consistent differences in steroid levels analyzed. Thus, serum steroid profiles were not reliably prognostic for GC tumorigenesis.

In conclusion, GC tumor induction in response to T treatment has co-segregated with susceptibility to spontaneous GC tumors in the SWXJ recombinant inbred strains. Thus, the second gene in our ovarian granulosa cell tumor model regulates responsiveness to T. We propose to name this gene spontaneous ovarian tumorigenesis (Sot), with alleles for susceptibility (s) carried by spontaneous strains and resistance (r) carried by DHEA-dependent strains.

INTRODUCTION

Genetically determined, malignant ovarian GC tumors occur spontaneously in 2–3% of SWR inbred mice as females progress through pubertal maturation (1, 2). These pubertal onset GC tumors are controlled by a small number of genes unique to Swiss-related strains. Through development of recombinant inbred strains from mating SWR and SJL (Swiss-related, resistant mice), the SWR genetic alleles for susceptibility or resistance to GC tumors were randomly "recombined" into 14 new strains, designated SWXJ (3). We originally observed that 3 of the 14 SWXJ recombinant inbred strains were also susceptible to spontaneous GC tumors at puberty like their SWR progenitor strain. These 3 SWXJ strains plus SWR hereafter are referred to as spontaneous strains for clarity of discussion. The other progenitor strain, SJL, and the remaining 11 SWXJ strains were resistant to spontaneous tumors. This division of the SWXJ strains into 2 classes of tumor susceptibility in the ratio of about 1:3 indicated that at least 2 genes control spontaneous GC tumorigenesis.

In subsequent studies, mice from all SWXJ recombinant inbred strains were treated with DHEA, a steroid with reported antitumor actions in several rodent models (4–6). In contrast to those reports, DHEA increased the incidence of GC tumors in the spontaneous SWXJ strains and induced GC tumors in 5 additional SWXJ strains that were free of spontaneous GC tumors (2).

The discovery of an additional class of SWXJ strains, those that were resistant to spontaneous GC tumors but susceptible to DHEA-induced tumors (hereafter referred to as DHEA-dependent strains), supported the 2-gene hypothesis for GC tumorigenesis. One gene, designated Gct, conveys susceptibility or resistance for GC tumors. Both DHEA-dependent and spontaneous strains carry the permissive allele, Gct*, whereas the GC tumor-resistant strains carry the resistant allele, Gctr. Although the function of Gct remains unknown, strain distribution patterns for presence or absence of GC tumors and for the polymorphic isozymes coded for by the Pancreas-2 locus, Pan-2, indicated that Gct was linked to Pan-2 in the central region of mouse Chromosome 4. The second gene, as yet uncharacterized, was thought to be involved in steroid metabolism and control susceptibility to spontaneous GC tumorigenesis. This hypothesis stemmed in part from studies of serum steroid metabolites in different strains of mice treated with DHEA showing that one function of this steroid was to serve as a prohormone for further metabolism to distal androgenic hormones (7, 8).

The studies reported herein extend the investigations on the role of androgenic steroids in GC tumorigenesis. Evidence is presented suggesting that the second susceptibility gene regulates responsiveness to androgenic steroids rather than androgen metabolism. The action of this gene, designated spontaneous ovarian tumorigenesis (Sot), combined with Gct*, determines susceptibility to spontaneous GC tumorigenesis among the SWXJ strains.

MATERIALS AND METHODS

Mice. The mice used in these studies were SWR/Bm, SJL/Bm, and 14 recombinant inbred SWXJ strains, originally derived from mating 2 SWR/Bm females to an SJL/Bm male and subsequent inbreeding of separate pairs of F2 progeny (1). All strains reported herein were at least F5–F8 generation mice. Mice were maintained on a 12-h light/12-h dark cycle, housed 3–4 per polycarbonate cage, and fed control or steroid-supplemented pasteurized Wayne Lab Blox 8657 diet (24% crude protein, 4% fat, 4.5% fiber, 5.9% ash, vitamin and mineral fortified; Teklad, Madison, WI) and water ad libitum.

Strain Distribution Pattern of Response to Testosterone. At least 100 mice from progenitor SWR/Bm strain and from the 8 GC tumor-susceptible (spontaneous plus DHEA-dependent) SWXJ strains were fed a diet supplemented with 0.1% T for 7 weeks beginning at 22–24 days. Diet was prepared

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2 To whom requests for reprints should be addressed, at The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609.

3 The abbreviations used are: GC, granulosa cell; DHEA, dehydroepiandrosterone; T, testosterone; P, progesterone; DHT, dihydrotestosterone; AD, androstenedione; ANDROS, androsterone.
by addition of T to powdered Wayne diet as described previously (8). At 10 weeks of age, mice were killed by cervical dislocation and inspected for presence of ovarian tumors. Most tumors were large and easily identified visually. In cases where visual evaluation of ovarian status was inconclusive, ovaries were placed in Bouin’s fixative, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Inspection by light microscopy confirmed the presence or absence of GC tumor foci.

Effectiveness of Other C19 Steroids in Inducing GC Tumors. Studies using mice from the spontaneous strain SWXJ-9 were conducted to determine whether C19 steroids distal to testosterone could also increase GC tumor incidence. Specifically, the biologically potent DHT and its putatively inactive metabolite, ANDROS, were chosen for further study (see Fig. 1). In addition, DHEA-dependent SWXJ-7 strain mice were also tested for ability to respond to ANDROS. Approximately 50 mice were assigned to each treatment group by a split-litter design. Steroids were obtained from Steraloids, Inc. (Wilton, NH). Steroids were administered to 21–25-day-old mice in 1.0-cm capsules made from Silastic tubing (0.078-in inside diameter x 0.125-in outside diameter; Dow-Corning) as described previously (8). Androgens were packed undiluted into the capsules, and empty capsules were used as controls. Capsules were implanted s.c. under tribromoethanol (Avertin) anesthesia (9). After 7 weeks, the mice were killed by decapitation, and then body, uterus, and ovarian weights were recorded and ovarian tumor incidence noted. In 6 mice (4 SWXJ-9; 2 SWXJ-7) treated with ANDROS, visual evaluation of ovarian status was considered inconclusive. Subsequent examination of serial sections from these 6 pairs of ovaries by light microscopy did not reveal neoplastic tissue.

Additional groups of 18–20 mice from spontaneous strains (SWXJ-1, -8, and 9) and DHEA-dependent strains (SWXJ-6 and -7) were fed DHEA (0.4% w/w) or T (0.1% w/w) for 2 weeks to determine the effects of steroid administration on serum steroid levels. Mice were killed by decapitation to obtain serum that was stored at -20°C until assayed by specific RIAs for 7 of 9 steroids (excluding pregnenolone and ANDROS) depicted in Fig. 1 (7, 10).

Progenitor Strain Developmental Serum Steroid Levels. To determine whether progenitor strains had differences in endogenous steroid metabolic patterns, serum was harvested from SWR/Bm and SJL/Bm mice at 22, 29, and 38 days of age. These age groups encompassed the prepubertal period, the pubertal age of tumor initiation, and the age when additional GC tumors are not likely to develop. Serum was stored frozen until specific RIAs were performed.

Role of Adrenal Glands in GC Tumor Induction. To determine whether the adrenal was the source of endogenous steroids that initiate spontaneous GC tumorigenesis, mice were adrenalectomized at different ages from 20 to 33 days. Hybrid (SWR × SWXJ-9)F1 mice were used for this study, since this F1 has the highest incidence of spontaneous GC tumors among our strains of mice (11). Adrenals were removed from 112 mice through bilateral incisions under Avertin anesthesia. Sham operations were performed on 93 control mice by exposing the kidney without touching the adrenal. The ovaries of all mice were inspected visually at the time of surgery for the presence of GC tumors, characterized by clearly visible hemorrhagic follicles (11). Mice were recovered from surgery under extra heat and were supplied with 0.9% NaCl-supplemented water ad libitum. After 6–7 weeks, mice were killed by cervical dislocation, and inspected for presence of functional adrenal cortical rests (12) and GC tumors.

Steroid Radioimmunoassays. Assays for P, T, estradiol, DHEA, and AD were performed as described previously (10). Briefly, samples from 2–3 mice were pooled, and steroids were extracted with ethyl acetate-cyclohexane (2:1) and then partitioned on Celite chromatography by stepwise elution. Eluates were dried, resolubilized in assay buffer, and assayed for 1 of 6 different steroids. Samples for ANDROS determinations were extracted as above and assayed without further chromatographic separation. Antiserum AO-1 to ANDROS was kindly provided by Dr. David Armstrong (13). Titration of serum ANDROS in the presence of added DHEA was performed to determine cross-reactivity. There was no significant cross-reaction detected until 1000 ng DHEA was tested (15% displacement of 3H-labeled ANDROS).

RESULTS

Strain Distribution Pattern of Response to T. Treating all progenitor and GC tumor-susceptible SWXJ strains with 0.1% T-supplemented diet yielded the results presented in Table 1.

The spontaneous strains showed significantly increased GC tumor frequencies in response to T, whereas the DHEA-dependent strains showed no induction of tumors following T treatment. The relative effectiveness of T and DHEA treatment varied among spontaneous strains. In SWR and SWXJ-4 and -9 strain mice, DHEA and T were equally effective. In SWXJ-1 and SWXJ-8 mice, T was significantly less effective than DHEA treatment. The SWXJ-8 strain, previously classified as DHEA-dependent (2), has been reclassified following routine necropsies of all breeding colony mice identified 2 spontaneous GC tumors, one in a nonproductive (5 months) and a second in a retired breeder (6 months).

Serum Steroid Levels following 2-Week Administration of T or DHEA. Serum steroid data following short-term feeding of DHEA or T to representative spontaneous and DHEA-dependent strains are presented in Table 2.

Analysis of variance did not reveal any significant differences between spontaneous and DHEA-dependent strain steroid profiles within treatment groups. Thus, there was no unique pattern of steroid metabolism to which spontaneous tumor susceptibility could be attributed. There were differences between treatment groups in several steroids. As expected, treatment with DHEA- or T-supplemented diet caused a marked increase in serum levels of the administered steroid. DHEA treatment also caused significant increases in AD, DHT, and T, but did not increase estrogen levels. Treatment with T increased serum T in all strains and AD in some strains, but did not elevate serum DHEA. The T treatment did significantly depress serum P and estradiol levels in both spontaneous and DHEA-dependent mice. Two conclusions can be drawn from these data: (a) strain differences in tumor induction by steroid treatment cannot be explained by differences in steroid metabolic patterns detectable in peripheral serum; and (b) T does not exert its GC tumor induction action by increasing DHEA levels.

Representative Strain Response to Other Steroids. Following the discovery of 2 distinct subclasses of GC tumorigenic response to T treatment, SWXJ-9 strain mice were treated with other C19 steroids to determine which steroids could affect tumor incidence. SWXJ-9 mice showed increased GC tumor frequency in response to both DHT (control, 3 of 64 or 4.7%; DHT, 11 of 47 or 23.4%; P < 0.002) and ANDROS treatment (control, 5 of 76 or 6.6%; ANDROS, 17 of 81 or 21.0%; P < 0.01). The frequency of GC tumors induced by both treatments was approximately equal and was very similar to previously reported incidences following treatment with DHEA- or T-filled Silastic capsules (8).

A group of 59 SWXJ-7 strain mice (DHEA-dependent) was also treated with ANDROS to indicate whether DHEA-dependent strains were resistant to C19 steroids other than T. ANDROS was chosen for study, since it is a major metabolite of ovarian androgens (14) and SWXJ-9 spontaneous strain mice responded to this steroid with increased GC tumor incidence. No GC tumors were observed in

Fig. 1. Biogenic pathways for gonadal steroids beginning with pregnenolone (derived from cholesterol). Arrows, direction of enzymatic conversion. Steroids in boldface were administered on serum steroid levels. Mice were killed by decapitation to obtain serum that was stored at -20°C until assayed by specific RIAs for 7 of 9 steroids (excluding pregnenolone and ANDROS) depicted in Fig. 1 (7, 10).
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Table 1 GC tumor incidence data for SWXJ females treated with DHEA or T for 7 weeks

<table>
<thead>
<tr>
<th>Strain</th>
<th>Controls (%)</th>
<th>DHEA* (%)</th>
<th>T (%)</th>
<th>Control</th>
<th>DHEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Spontaneous”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWR</td>
<td>25/1002 (2.5)</td>
<td>26/107 (24.3)</td>
<td>21/121 (17.4)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>SWXJ-1</td>
<td>3/163 (2.5)</td>
<td>21/114 (18.4)</td>
<td>11/127 (8.7)</td>
<td>&lt;0.02</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>SWXJ-4</td>
<td>2/162 (1.2)</td>
<td>9/110 (8.1)</td>
<td>13/110 (11.8)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>SWXJ-8</td>
<td>2/538 (0.4)</td>
<td>49/111 (44.1)</td>
<td>11/102 (10.8)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SWXJ-9</td>
<td>125/121 (10.3)</td>
<td>30/74 (40.5)</td>
<td>36/105 (34.3)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

| “DHEA-dependent”|              |           |       |         |      |
| SWXJ-6          | 0/169 (0)    | 11/103 (10.7) | 0/125 (0) | <0.001 | NS   |
| SWXJ-7          | 0/169 (0)    | 15/111 (13.5) | 0/101 (0) | <0.001 | NS   |
| SWXJ-10         | 0/174 (0)    | 3/109 (2.8) | 0/111 (0) | NS     | NS   |
| SWXJ-12         | 0/160 (0)    | 9/109 (8.3) | 0/111 (0) | <0.02  |      |

* Significance of incidence data tested by χ².
+ DHEA treatment data from Beamer et al. (2) presented for comparison with T treatment.

ANDROS-treated SWXJ-7 strain mice. Thus, from the strains studied, it appears that the gene that conveys responsiveness to T may also extend responsiveness to downstream C19 metabolites of T.

Biological Data. Body and organ weight data for SWXJ-9 mice given implants of various steroid capsules are presented in Table 3. DHT treatment significantly reduced uterine and ovarian weights, and increased salivary gland weights. Ovarian weight was also significantly reduced by ANDROS. These results indicate that biologically effective doses of steroids were released from the capsules.

Progenitor Strain Endogenous Serum Steroid Profiles. To discover whether genetic differences in endogenous steroid metabolism determine susceptibility to spontaneous tumors, serum C19 steroid profiles obtained from the 8 GC tumor-susceptible SWXJ strains at each age did not show significant increases in DHEA at any of these ages. Furthermore, there were no differences in patterns or levels of AD, T, or DHT at other ages that distinguished spontaneous from DHEA-dependent strains or from either progenitor strain (data not shown).

Role of Adrenals in GC Tumor Induction. A total of 24 GC tumor-bearing hosts were found among 112 adrenalectomized (SWR × SWXJ-9)F1 females (21% incidence). In comparison, we found 24 GC tumor-bearing females (26%) among 93 sham-operated littermate controls. When data are partitioned into prepuberal (<25 days; 9 GC tumor hosts/58 mice) and puberal (25–33 days; 15 GC tumor hosts/54 mice) age groups, no significant effect of adrenalectomy was observed. Thus, adrenal glands do not serve as a necessary endogenous source of steroids for GC tumor induction. At necropsy, we observed infrequent examples of adrenocortical rests adjacent to the abdominal aorta and vena cava near the junctions with the renal arteries and veins in both intact and adrenalectomized hosts. Given the low frequency and very small size of these extra-adrenal tissue masses, it is unlikely that they contribute to GC tumorigenesis.

DISCUSSION

It is estimated that up to 60% of human tumors are associated in some way with exposure to steroid hormones (15). The mechanisms through which steroids affect neoplasia are not well understood, and

Table 2 Serum steroid levels in 5 SWXJ strains treated with 0.4% DHEA- or 0.1% T-supplemented diets for 2 weeks

<table>
<thead>
<tr>
<th>Strain and diet</th>
<th>Serum steroid</th>
<th>P</th>
<th>DHEA</th>
<th>AD</th>
<th>T</th>
<th>DHT</th>
<th>E₁estructone</th>
<th>E₂estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA</td>
<td>SWXJ-1</td>
<td>3.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.5 ± 3.0</td>
<td>8.2 ± 1.4</td>
<td>3.4 ± 0.8</td>
<td>1.8 ± 0.1</td>
<td>46 ± 14</td>
<td>62 ± 19</td>
</tr>
<tr>
<td>SWXJ-8</td>
<td>2.4 ± 0.5</td>
<td>27.5 ± 4.5</td>
<td>7.0 ± 0.8</td>
<td>3.1 ± 0.6</td>
<td>1.8 ± 0.4</td>
<td>51 ± 10</td>
<td>26 ± 7</td>
<td></td>
</tr>
<tr>
<td>SWXJ-9</td>
<td>2.9 ± 0.2</td>
<td>21.9 ± 4.4</td>
<td>6.4 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>50 ± 11</td>
<td>34 ± 11</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>SWXJ-1</td>
<td>1.0 ± 0.4</td>
<td>0.8 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td>7.2 ± 1.1</td>
<td>0.2 ± 0.1</td>
<td>65 ± 20</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>SWXJ-8</td>
<td>1.3 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>2.4 ± 1.3</td>
<td>10.0 ± 3.0</td>
<td>0.1 ± 0.03</td>
<td>97 ± 27</td>
<td>6 ± 3</td>
<td></td>
</tr>
<tr>
<td>SWXJ-9</td>
<td>1.4 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>4.5 ± 1.0</td>
<td>9.2 ± 1.8</td>
<td>0.1 ± 0.02</td>
<td>22 ± 14</td>
<td>4 ± 3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>SWXJ-1</td>
<td>2.1 ± 0.7</td>
<td>1.8 ± 1.1</td>
<td>0.2 ± 0.1</td>
<td>1.1 ± 0.5</td>
<td>0.03 ± 0.01</td>
<td>27 ± 4</td>
<td>20 ± 6</td>
</tr>
<tr>
<td>SWXJ-8</td>
<td>2.4 ± 1.1</td>
<td>0.4 ± 0.05</td>
<td>0.3 ± 0.1</td>
<td>0.04 ± 0.01</td>
<td>50 ± 12</td>
<td>67 ± 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWXJ-9</td>
<td>1.8 ± 0.7</td>
<td>0.7 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.4</td>
<td>0.10 ± 0.02</td>
<td>72 ± 11</td>
<td>11 ± 3</td>
<td></td>
</tr>
<tr>
<td>DHEA-dependent</td>
<td>SWXJ-6</td>
<td>5.0 ± 0.4</td>
<td>31.9 ± 3.5</td>
<td>10.0 ± 0.06</td>
<td>2.3 ± 0.8</td>
<td>1.7 ± 0.3</td>
<td>50 ± 10</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>SWXJ-7</td>
<td>2.7 ± 0.9</td>
<td>28.8 ± 7.2</td>
<td>10.5 ± 3.0</td>
<td>4.6 ± 1.6</td>
<td>1.7 ± 0.3</td>
<td>54 ± 14</td>
<td>30 ± 14</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>SWXJ-6</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>10.8 ± 1.7</td>
<td>0.1 ± 0.05</td>
<td>80 ± 16</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>SWXJ-7</td>
<td>1.4 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>5.0 ± 0.4</td>
<td>10.9 ± 3.0</td>
<td>0.2 ± 0.1</td>
<td>77 ± 27</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>SWXJ-6</td>
<td>2.6 ± 0.6</td>
<td>1.8 ± 0.4</td>
<td>0.1 ± 0.01</td>
<td>ND</td>
<td>0.05 ± 0.02</td>
<td>92 ± 9</td>
<td>61 ± 14</td>
</tr>
<tr>
<td>SWXJ-7</td>
<td>2.4 ± 1.0</td>
<td>0.8 ± 0.2</td>
<td>0.1 ± 0.05</td>
<td>0.6 ± 0.3</td>
<td>0.06 ± 0.01</td>
<td>72 ± 14</td>
<td>20 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> E₁, estrone; E₂, estradiol; ND, not determined due to loss of samples during assay.

<sup>b</sup> Mean ± SEM.
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Table 3 Body and organ weight data on SWXJ-9 females treated with DHT or ANDROS

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Body wt (g)</th>
<th>Uterine wt (mg/g body wt)</th>
<th>Ovarian wt (mg/g body wt)</th>
<th>Salivary gland wt (mg/g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19.5 ± 0.2*</td>
<td>4.25 ± 0.26</td>
<td>0.38 ± 0.02</td>
<td>4.06 ± 0.22</td>
</tr>
<tr>
<td>DHT</td>
<td>21.9 ± 0.6*</td>
<td>2.94 ± 0.26</td>
<td>0.17 ± 0.01</td>
<td>5.67 ± 0.46</td>
</tr>
<tr>
<td>ANDROS</td>
<td>19.9 ± 0.2</td>
<td>3.79 ± 0.23</td>
<td>0.28 ± 0.01</td>
<td>4.52 ± 0.24</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

There are probably several types of steroid involvement. Steroids may act to initiate tumors, to support primary growth, or to influence the transition to metaplasia. Investigators have repeatedly sought prognostic or diagnostic relevance of peripheral steroid levels for a number of tumor types. Some correlations between steroid levels and risk of certain tumors have been found. For example, low urinary DHEA metabolite levels (believed to indicate lower synthesis of DHEA) in women are associated with higher risk for breast (16) and bladder (17) cancer. Causal associations and reliable prognostic tools remain elusive, however. One exception is that use of oral contraceptives appears to reliably reduce the risk of developing epithelial carcinomas of the ovary (18–20).

The SWXJ model for malignant ovarian granulosa cell tumors provides an animal model for research on interactions between genes and steroids that control tumorigenesis. In this model, exogenously administered androgens induce tumorigenesis; such induced tumors can progress to cancer without further exogenous steroid support (8). Estrogen treatment prior to tumor initiation effectively suppresses spontaneous tumorigenesis but does not induce regression of established tumors (8, 11).

We have hypothesized that at least 2 genes control susceptibility to spontaneous GC tumorigenesis in SWXJ mice. The Gcf allele is carried by both spontaneous and DHEA-dependent strains. Studies with F1 hybrid mice have determined that mice must carry at least one susceptibility allele at this locus for GC tumorigenesis to occur (1); thus, Gcf may act as an oncogene. Spontaneous strains also carry susceptibility alleles at a second, unlinked locus controlling spontaneous GC tumors. In this report, we have found that DHEA-dependent and spontaneous strains also differ in responsiveness to T treatment. Based on these findings, we have developed the following working model for genetic and steroid control of GC tumorigenesis.

Gcf is presumed to be an oncogene, unique to SWR and SWXJ mice. The Gcf allele is controlled by DHEA. Thus, feeding DHEA activates Gcf in both DHEA-dependent and spontaneous strains, resulting in GC tumorigenesis. In addition, the spontaneous strains carry a separate susceptibility allele at a locus we have named spontaneous ovarian tumorigenesis (Sot). Sot is controlled by C19 androgens, including T, DHT, and ANDROS. We hypothesize that Sot alone is not sufficient for tumorigenesis, but rather acts as a modifier of Gcf activity. According to this model, resistant strains that carried the Sot allele would not develop GC tumors after T treatment, since they also carry the Gcf allele. Indirect evidence supporting the model is derived from rat ovarian granulosa cells (30, 31), but not tested for androgen binding protein presence in the mouse ovary been established. However, these findings require confirmation. DHEA does bind to the androgen binding protein (29) has not been reported, nor has evidence for an abnormal endogenous steroid signal in spontaneous strains. Both progenitor SWR/Bm and SJL/Bm strain mice had significant and similar levels of androgenic steroids in circulation throughout the peripuberal period of risk for GC tumor initiation. This would again suggest that ovarian responsiveness mediated via Sot, rather than steroid metabolism, determines susceptibility to spontaneous GC tumorigenesis.

The adrenalectomy study excluded the adrenal as the source of an endogenous tumor-inducing steroid. By inference then, the ovary is the likely source of androgenic stimulation. Although in humans, the adrenal gland is a major source of circulating androgens reviewed previously (22), the rodent adrenal does not appear to secrete significant amounts of C19 steroids (23). Others report (24, 25) that the rat adrenal does not express the P450c17a enzyme necessary to generate C19 steroids from pregnenolone or P. The spontaneous strain mouse ovary may therefore contain both the necessary stimulus and the susceptible target cell for GC tumorigenesis. A delicate balance of developmental status and steroidogenesis would determine spontaneous tumor rates.

In our model, we propose that Gcf is controlled by DHEA itself, rather than a biologically active androgenic metabolite. We found no evidence for distinct patterns of DHEA metabolism that could account for differing responses to C19 steroids among the SWXJ strains. Although the mechanism(s) through which DHEA could exert biological effects remains unknown, it is possible that a steroid receptor protein could mediate DHEA effects. Evidence for a specific receptor protein (26) or a binding protein (27) for DHEA has been reported, however, these findings require confirmation. DHEA does bind to the androgen receptor, but with a low affinity (28). Interaction of DHEA with androgen binding protein (29) has not been reported, nor has androgen binding protein presence in the mouse ovary been established. However, an androgen binding protein has been partially characterized from rat ovarian granulosa cells (30, 31), but not tested for competition with DHEA. The finding that SWXJ-9 mice respond to a molecular level. Alternatively, Sot and Gcf could control separate aspects of the intricate regulation of granulosa cell proliferation and differentiation.

In spontaneous strains, normal endogenous C19 steroids generated during pubertal development may provide a sufficient stimulus for GC tumor initiation by triggering the modifying action of Sot on Gcf. The steroids used to experimentally induce or suppress GC tumor incidence are all part of the major gonadal steroid metabolism pathway. During pubertal maturation of the ovary, granulosa cells undergo rapid proliferation and acquire differentiated functions as the first group of pre-ovulatory follicles develop. At the same time, the ovary shifts from production primarily of 5α-reduced progestins to synthesis of biologically active sex steroids (21). GC tumor initiation coincides with this transitional pubertal period.

In analyses of serum steroid levels, we did not find consistent evidence for an abnormal endogenous steroid signal in spontaneous strains. Both progenitor SWR/Bm and SJL/Bm strain mice had significant and similar levels of androgenic steroids in circulation throughout the peripuberal period of risk for GC tumor initiation. This would again suggest that ovarian responsiveness mediated via Sot, rather than steroid metabolism, determines susceptibility to spontaneous GC tumorigenesis.

Table 4 Serum steroid levels (ng/ml) during peripuberal ovarian development in SWR and SJL progenitor strain mice

<table>
<thead>
<tr>
<th>Progenitor strain</th>
<th>SHEA</th>
<th>AD</th>
<th>T</th>
<th>DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 days SWR</td>
<td>1.19 ± 0.38*</td>
<td>0.08 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>SJL</td>
<td>0.23 ± 0.06</td>
<td>0.06 ± 0.01</td>
<td>0.22 ± 0.05</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>29 days SWR</td>
<td>0.41 ± 0.09</td>
<td>0.06 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>SJL</td>
<td>0.94 ± 0.16*</td>
<td>0.05 ± 0.01</td>
<td>0.16 ± 0.03</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>38 days SWR</td>
<td>0.50 ± 0.11</td>
<td>0.13 ± 0.04</td>
<td>0.31 ± 0.10</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>SJL</td>
<td>0.41 ± 0.04</td>
<td>0.11 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

b SWR mean significantly different from SJL by t test, P < 0.01.

broad range of C19 steroids supports the hypothesis that specificity of a steroid receptor or binding protein is altered. Experiments to determine the role of the androgen receptor in SWXJ tumorigenesis are in progress.

The model outlined above presents only 2 genes for tumorigenesis, based on the results described in this paper. We recognize that additional genes undoubtedly also affect GC tumorigenesis. The differing tumor frequencies and steroid responsiveness found in the SWXJ strains, and the limitation of GC tumors to Swiss-related progeny (2), suggest that additional genes and "background modifiers" also exert subtle effects on GC tumorigenesis.

Previous research on the hormonal mechanism(s) responsible for the SWXJ mouse model has been hampered by lack of knowledge about precisely which steroids act as tumor inducers in this system. The demonstration that a gene controlling steroid responsiveness has segregated among spontaneous and DHEA-dependent strains provides a new tool for such studies. In addition, these results demonstrate the complexity of gene-hormone interactions in tumorigenesis. Clinical studies correlating cancer risk and hormone exposure or endogenous steroid patterns would do well to consider the genetic susceptibility of patients. Future research clarifying the role of endogenous ovarian steroidogenesis, gene expression, and mechanisms of DHEA induction during GC tumorigenesis is likely to yield insight into mechanisms for both mouse and human steroid-dependent tumors.

REFERENCES


Genetic Susceptibility for C19 Androgen Induction of Ovarian Granulosa Cell Tumorigenesis in SWXJ Strains of Mice

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