Multigenic and Imprinting Control of Ovarian Granulosa Cell Tumorigenesis in Mice

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ABSTRACT

Spontaneous juvenile ovarian granulosa cell (GC) tumors that occur in young girls are similar to GC carcinomas that develop in SWR-derived inbred mice. We analyzed female offspring from a series of matings among SWR and SJL inbred mice for chromosomal loci underlying tumor susceptibility. Intercross F₂ female mice were produced by reciprocal matings of (SWR × SJL)F₁ and (SJL × SWR)F₁ parents. Tumorigenesis in these F₂ mice as well as in SWXJ recombinant inbred and congenic strains of mice derived from SWR and SJL showed significant (P < 0.001) association with Gct₁, a dominant susceptibility locus on chromosome (CHR) 4 and with Gct₂ on CHR 12. Suggestive (P < 0.01) association was found with Gct₃ on CHR 15. A fourth susceptibility locus, Gct₄ on CHR X, was demonstrated with a strong parent-of-origin effect associated with the paternal genotype. Imprinting and complex interactions among these four loci combine to establish the probability for GC tumorigenesis in this mouse model.

INTRODUCTION

Juvenile ovarian GC³ tumors occur spontaneously in young girls and in pubertal SWR inbred strain mice (1-4). The developmental age of onset, endocrine features, and malignant potential of GC tumors in the SWR model bear remarkable resemblance to those of juvenile GC tumors described in prepubertal girls (5-7). Histological characteristics of mouse and human GC tumors are similar, although the grooved nuclei observed in adult-onset human GC tumors (8) are not observed in the mouse tumors. Progression to malignant disease is generally a less frequent occurrence in humans, whereas in mice, metastases are often found in the renal node, liver, pancreas, spleen, and lung after 7 months of age.

In addition to SWR mice, ovarian GC tumors also occur in (SWR × SJL)F₁ hybrids and in a subset of SWXJ RI strains of mice, establishing the heritability of this ovarian tumor (1). The 14 SWXJ RI strains were derived from intercrossing tumor-susceptible SWR with the closely related tumor-resistant SJL inbred strain and then inbreeding the second familial (F₂) and subsequent generation progeny for 20 generations.) Spontaneous tumor incidences among SWR and SWXJ-1, -4, -8, and -9 strains vary from 0.5-15%. In studies of steroid hormone effects, we found that the precursor, DHEA, increased the incidence of GC tumors in the spontaneous tumor strains (SWR and SWXJ-1, -4, -8, and -9) and induced GC tumors in 4 of the 10 spontaneous tumor-free SWXJ strains (SWXJ-6, -7, -10, and -12; Refs. 9 and 10). Furthermore, a downstream metabolite of DHEA, testosterone, significantly increased tumor incidence in females from the spontaneous tumor-susceptible SWXJ RI strains but not in females of the spontaneous tumor-free SWXJ strains that responded to DHEA (11). These findings demonstrate that steroid hormones exert key roles in ovarian tumorigenesis through distinct genetic mechanisms. Because each SWXJ RI strain carries a unique combination of genes from SWR and SJL, those strains that develop ovarian GC tumors can be analyzed for genes contributing to spontaneous and steroid-induced neoplastic processes.

The genetically complex trait of ovarian GC tumorigenesis is exemplified in the SWR model. Previous investigations revealed two loci controlling susceptibility to GC tumors in the SWR tumor model: (a) GC tumorigenesis (Gct₁), identified by DHEA treatment and located on the distal third of CHR 4; and (b) spontaneous ovarian tumorigenesis (Sot), identified by testosterone treatment, map position unknown. In this report, additional genetic analyses of F₂, congenic, and SWXJ RI strain mice reveal: (a) two additional loci, Gct₂ and Gct₃, on CHRs 12 and 15, respectively; (b) Gct₄ (formerly Sot) on CHR X; and (c) a strong parent-of-origin effect associated with transmission of CHR X through the male parent.

MATERIALS AND METHODS

Mice. Mice were produced and maintained in our research colony under 14 h:10 h light/dark cycles. Pasteurized diet NIH-31 with 6% fat (Agway, Inc., Syracuse, NY) and HCl-acidified water (pH 2.8-3.2) were available ad libitum. Female offspring from all crosses were weaned at 21-24 days of age and housed in groups of four or five within 51-square-inch polycarbonate boxes containing sterilized white pine shavings.

Matings to produce progeny for genetic analyses of tumor susceptibility were of several types: (a) reciprocal F₁ crosses were made between strains SWR and SJL. All four possible F₁ × F₁ crosses (designated as female × male) were made [cross A, (SJL × SWR)F₁ × (SJL × SWR)F₁]; cross B, (SJL × SWR)F₁ × (SWR × SJL)F₁]; cross C, (SWR × SJL)F₁ × (SJL × SWR)F₁; and cross D, (SWR × SJL)F₁ × (SJL × SWR)F₁; and (b) standard sibling matings for each SWXJ RI strain; (c) reciprocal crosses between SWR and selected SWXJ RI strains to produce F₂ hybrids; and (d) X CHR congenic strains prepared by transferring SJL chromosomal segments into the SWR strain background through repeated backcrossing.

Mice were necropsied at 8 weeks of age, and ovaries were visually checked for GC tumors. These GC tumors are typically 5-10 mm in diameter, with irregular nodules of proliferating tissue plus hemorrhagic and occasional necrotic foci. Some modestly enlarged ovaries were fixed in Bouin’s solution for histological assessment; however, none proved to contain preneoplastic follicles or microscopic tumors. When a GC tumor-bearing mouse was found, the kidneys, spleen, and liver were collected, frozen in liquid nitrogen, and stored at −70°C. The same tissues were similarly collected and stored from normal littermates and from age-matched normal controls from all other matings in each experiment.

Genetic Mapping. DNA was prepared from the kidneys of tumor-bearing as well as tumor-free control F₂ mice from all crosses using a standard chloroform-phenol method. PCR products were generated and analyzed on agarose gels as described previously (12, 13). The genetic markers used were selected from more than 400 anonymous DNA SSLPs and polymorphic genes distinguishing SWR and SJL genomes. The SSLPs representing the proximal,

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middle, and distal regions of each CHR were initially typed, and then additional SSLP markers were used to analyze chromosomal regions with suggestive evidence of linkage to GC tumorigenesis. Some CHRs, especially CHRs 8 and 19, could not be thoroughly analyzed, because SWR and SJL, which are closely related Swiss strains, proved polymorphic for only ~30% of more than 1400 markers tested. In addition to the markers presented for CHRs 4, 9, 12, 15, and X in Table 1, markers for other CHRs tested were: (a) D1Mit68, D1Mit104, D1Mit105, and D1Mit17; (b) D2Mit1, D2Mit21, D2Mit22, D2Mit48, and D2Nds1; (c) D3Mit203, D3Mit39, D3Mit82, and D3Mit19; (d) D5Mit76, D5Mit10, and D5Mit122; (e) D6Mit139, D6Mit29, and D6Mit15; (f) D7Mit76, D7Mit159, D7Mit326, and D7Mit12; (g) D8Mit80 and D8Mit92; (h) D10Mit80, D10Mit130, D10Mit12, and D10Mit14; (i) D11Mit77, D11Mit164, D11Mit170, D11Mit126, D11Mit61, and D11Mit59; (j) D13Mit253, D13Mit27, and D13Mit292; (k) D14Mit54, D14Mit160, and D14Mit107; (l) D16Mit57, D16Mit64, and D16Mit71; (m) D17Mit197, D17Mit6, and D17Mit205; (n) D18Mit116, D18Mit205, D18Mit3, and D18Mit6; and (o) D19Mit32 and D19Mit10.

Statistics. Genotyping data for 83 individual markers were assessed for association with tumorigenesis by comparing tumor-bearing mice and non-tumor-bearing control mice using $\chi^2$ contingency tests. For the genome-wide analyses, the probability level for declaring significant association was set at $P < 0.001$ to account for multiple testing. A locus was considered as suggestive of association if a $\chi^2$ equivalent to $P < 0.01$ was achieved. The allelic distributions for all 83 loci in the controls were tested against the expected distribution of 1:2:1, and no significant deviations were found.

Two autosomal markers showed significant association with GC tumorigenesis. D4Mit232 yielded the highest significance level of association ($\chi^2 = 41.18; P < 0.0001$). This marker is closely linked to the Gct1 locus first identified by strain distribution pattern analyses in the SWXJ RI strains (10). We have not yet defined the distal boundary of the region of significant association with Gct1 because of insufficient polymorphisms between SWR and SJL for loci distal to D4Mit190. Significant association with GC tumorigenesis also was detected for D12Mit172 located on the proximal region of CHR 12 ($\chi^2 = 13.16; P < 0.001$).

Other chromosomal regions that showed suggestive association with GC tumorigenesis were identified by D15Mit133 located on CHR 15 in the region from 16–28 cm ($\chi^2 = 11.38; P < 0.003$) and by three markers (D9Mit21, D9Mit353, and D9Mit17) located on...
Table 2  Estimated RR for GC tumorigenesis assessed for markers on autosomes 4, 12, and 15 as derived from the logistic regression modela

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<th>Mit marker</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
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<tr>
<td>D4Mit232</td>
<td>20.45</td>
<td>6.42-65.17</td>
<td>0.0001</td>
</tr>
<tr>
<td>D12Mit172</td>
<td>2.47</td>
<td>1.16-5.24</td>
<td>0.019</td>
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<tr>
<td>D15Mit133</td>
<td>3.83</td>
<td>1.76-8.32</td>
<td>0.001</td>
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a CI, confidence interval; an odds ratio of 1.00 implies no increase in tumor risk.

CHR 9 between 29.5 and 61 cM ($x^2 > 9; P = 0.011-0.006$). We also observed that D11Mit69 ($P < 0.027$) located on distal CHR 11 and D13Mit253 ($P < 0.015$) located on proximal CHR 13 may be associated with GC tumors. However, the level of significance attained by CHR 11 and 13 markers did not reach the $P < 0.010$ level established for suggestive association with tumorigenesis.

Six markers for CHR X also were typed, and the data are shown in Table 1. Following the original experimental design, when a GC tumor mouse was found, controls were collected from all four F1 X F1 mating (crosses A–D). The distribution of alleles for genetic markers in these controls showed the expected 1:2:1 ratio of genotypes. Surprisingly, only F1 female progeny of (S/J X SWR)F1, sires developed tumors (crosses A and C; see Table 6), thus GC tumor-bearing mice could only be S/J or J/J for the CHR X markers. There were no significant deviations from the expected 1:1 ratio of these two genotypes in the GC tumor-bearing mice. Whereas these data show that there is no effect of the maternal CHR X on tumorigenesis, the surprising finding of a paternal effect was further explored and described under “Parent-of-Origin Effect.”

Distribution of Autosomal Alleles

F1 Progeny. Data for CHR 4 in Table 1 yielded the strongest association with GC tumorigenesis: (a) 47 of 56 (84%) tumor-bearing mice were homozygous (S/S) for the SWR allele D4Mit232; (b) 9 of 56 (16%) mice were heterozygous (S/J) for the SJL allele. In comparison, 15 of 59 (25%) non-tumor-bearing littersmate controls were S/S, 33 of 59 (56%) were S/J, and 11 of 59 (19%) were J/J, indicating random distribution of the alleles. Thus, at least one SWR-derived Gct1 allele linked to D4Mit232 on CHR 4 is required for tumorigenesis. In contrast, a few tumor-bearing mice were homozygous for SJL alleles at markers for CHRs 9, 12, and 15, indicating that tumorigenesis could occur without the requirement of at least one SWR allele at these chromosomal loci.

Given that homozygosity for D4Mit232 SJL alleles was not observed in GC tumor-bearing mice, we genotyped the remainder of the available controls from the A and C matings to identify those that were either S/S or S/J at D4Mit232 and thus potentially able to develop GC tumors. We identified 54 such controls for comparison with the 56 GC tumor-bearing mice. We then genotyped these controls for D9Mit17, D12Mit172, and D15Mit133. We found that, when comparing GC tumor-bearing mice with these selected controls, distributions of alleles for CHR 12 ($\chi^2 = 14.76; P = 0.0006$) and for CHR 15 ($\chi^2 = 9.72; P = 0.0078$) were again nonrandomly associated with GC tumors, whereas the distribution of CHR 9 alleles was not significantly different ($\chi^2 = 2.50; P = 0.29$) from that of the controls.

A multiple logistic regression model was fitted to the markers for D4Mit232, D12Mit172, and D15Mit133 to obtain estimates of the adjusted RR for ovarian GC tumors due to allelic substitutions at these three loci. The control sample was restricted to mice from crosses A and C with S/S or S/J alleles at D4Mit232, because these were the only mice at risk for GC tumorigenesis. All three allelic combinations at D12Mit172 and D15Mit133 were present in cases and controls. The effects of allelic substitution (on the logit scale) were additive for both D12Mit172 and D15Mit133. There was no evidence for interaction among the loci, nor was there any evidence for a main effect on interaction with the type of cross (cross A versus cross C). The estimated odds ratios and 95% confidence intervals for the logistic regression model are shown in Table 2.

SWXJ RI Strains. To further examine the importance of tumor susceptibility alleles for D4Mit232, D12Mit172, D15Mit133, and D9Mit17, we determined their distributions among the 14 SWXJ RI strains (Table 3). The strain distribution pattern for D4Mit232 is concordant with that of susceptible and resistant alleles for Gct1, the first susceptibility locus identified by DHEA induction in the SWXJ RI strains (10). Two (SWXJ-8 and -9) of the four spontaneous tumor strains carry SJL alleles for D12Mit172, D15Mit133, and D9Mit17. Based on our SWXJ RI typing data for CHR 12 in SWXJ-8 and -9 (13), we propose that the second susceptibility locus linked to D12Mit172 lies within a region of approximately 11 cM. Similarly, the third susceptibility locus linked to D15Mit133 lies within a region marked proximally by D15Mit98 at the Massachusetts Institute of Technology map distance of 10.9 cM. However, the distal boundary of this region cannot yet be determined, because the entire CHR distal to D15Mit133 for both SWXJ-8 and -9 is SJL-like. It is unlikely that both of these strains carry SWR susceptibility alleles near D12Mit172 or D15Mit133. Another susceptibility locus linked to D9Mit21 and to D9Mit17 lies within a large region of 31.7 cM for which we have not yet identified polymorphisms. For both SWXJ-8 and -9 RI strains, there is a recombination event between D9Mit21 and D9Mit17. This large region could very well contain a SWR Gct1 susceptibility locus. Taken together, these data suggest that the CHR 12 and CHR 15 loci

Table 3 Genotype of SWXJ RI strains for alleles on CHRs 4, 12, 15, 9, and X (S, SWR (S/S); J, SJL (J/J))

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<tr>
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<tr>
<td>D9Mit16</td>
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<td>S</td>
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<td>D8Mit38</td>
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Strains are classified as DHEA inducible and as spontaneous with respect to the presence (+) or absence (−) of ovarian GC tumors.

SWXJ RI strain

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The nonspontaneous tumor strain SWXJ-13 also carries the DXMitló susceptibility. Tumor incidence ($x^2 = 0.26; P = not significant$). We conclude that at proximal DXMM6 and distal DXMit38 showed no relationship with SWR.SJL-X4 ($x^2 = 9.22; P = 0.002$). Furthermore, the SJL allele combined tumor incidence than do females of sublines SWR.SJL-X1 to -X4. Females of SWR.SJL-X2 and SWR.SJL-X3 carry separated at backcross 7. Table 4 identifies the markers used for the DXMitSS to DXMH182. Four sublines (SWR.SJL-X1, -X2, -X3, and a SWR congenic strain that contained most of the SJL CHR X from DXMitló and ovarian tumorigenesis, we initiated the construction of the SWR susceptibility allele linked to D4Mit232. Data suggest that DXMitló is not sufficient for tumorigenesis without an involvement of an X-linked locus in GC tumor susceptibility. However, SWXJ-13 does not carry the obligate susceptibility allele for GC tumors at Get!, as marked by D4MH232. These SJL allele. However, SWXJ RI strains was contributed from the ovarian tumor-free SJL progenitor on CHR 4, the strong susceptibility allele at DXMitló in the SWXJ RI strains with respect to DXMitló and spontaneous tumors. In contrast to Get!, these results indicate a parent-of-origin effect. The mating system and tumor incidence in progeny at each cross were significantly more frequent when the SJL DXMit6 allele was inherited from the sire. In contrast, tumor incidences were equivalent in progeny from two sets of reciprocal matings between the SWR and SWXJ RI strains carrying the SWR DXMit6 allele. Collectively, these data suggest that: (a) susceptibility alleles act differently when inherited from the X CHR Alleles

**SWXJ RI Strains.** Table 3 presents CHR X genotyping for the SWXJ RI strains (13), where it is evident that the DXMit61 SJL allele is associated with spontaneous GC tumors. Markers on either side of DXMit61, i.e., DXMit22 (8.7 cm proximal) and DXMit38 (14.2 cm distal), showed recombination with DXMit61, whereas more distant markers (data in Ref. 13) showed a random strain distribution pattern with respect to DXMit61 and spontaneous tumors. In contrast to Get! on CHR 4, the strong susceptibility allele at DXMit61 in the SWXJ RI strains was contributed from the ovarian tumor-free SJL progenitor strain! Specifically, the 4 spontaneous tumor RI strains (SWXJ-1, -4, -8, and -9) carry DXMit61 SJL alleles, whereas 9 of 10 spontaneous tumor-free SWXJ RI strains carry the SWR allele. This strain distribution pattern for DXMit61 in 13 of 14 SWXJ RI strains is consistent with an involvement of an X-linked locus in GC tumor susceptibility. The nonspontaneous tumor strain SWXJ-13 also carries the DXMit61 SJL allele. However, SWXJ-13 does not carry the obligate susceptibility allele for GC tumors at Get!, as marked by D4Mit232. These data suggest that DXMit61 is not sufficient for tumorigenesis without the SWR susceptibility allele linked to D4Mit232.

**Congenetic Strains.** To further test for a relationship between DXMit61 and ovarian tumorigenesis, we initiated the construction of a SWR congenic strain that contained most of the SJL CHR X from DXMit55 to DXMit182. Four sublines (SWR.SJL-X1, -X2, -X3, and -X4), each carrying overlapping segments of the SJL CHR X, were separated at cross 7. Table 4 identifies the markers used for the four sublines and shows the paternally transmitted genotype of the N7 females examined. Females of SWR.SJL-X2 and SWR.SJL-X3 carry the paternal DXMit61 SJL allele, and they have a significantly higher combined tumor incidence than do females of sublines SWR.SJL-X1 and SWR.SJL-X4 ($x^2 = 9.22; P = 0.002$). Furthermore, the SJL allele at proximal DXMit46 and distal DXMit38 showed no relationship with tumor incidence ($x^2 = 0.26; P = not significant$). We conclude that the DXMit61 marker is linked to a CHR X locus important for GC susceptibility.

**Parent-of-Origin Effect**

**Congenetic Strains.** We previously reported that 2.7% of (SWR × SJL)F1 females develop tumors, whereas the (SJL × SWR)F1 females are tumor free (1). While constructing a SWR.SJL-X congenic strain, we observed a similar parent-of-origin effect. The mating system and tumor incidence in progeny at each generation are shown in Table 5. Females (N3, N5, and N7) that inherited the SJL X chromosomal segment DXMit55 to DXMit182 from their sire have higher tumor incidences than those that inherit this segment from their dams. These results indicate a parent-of-origin effect and suggest that imprinting is involved.

**F2 Progeny.** A parent-of-origin effect was also observed in the F2 females. Table 6 shows that F2 progeny from crosses B and D did not develop GC tumors, whereas F2 females from crosses A and C did (0.9 and 1.2% incidence, respectively). Offspring of both (SJL × SWR)F1 and (SWR × SJL)F1 females developed GC tumors, whereas only the offspring of (SJL × SWR)F1 males developed GC tumors. The simplest explanation for these results is that the SJL X CHR carries a potent GC tumor susceptibility allele. When this allele is inherited from the sire, GC tumorigenesis can occur. When the SJL-derived allele is inherited from the dam, as found in half of the female offspring of crosses B and D, tumorigenesis does not occur.

**SWXJ RI Strains.** Reciprocal crosses were made between SWR and five of the SWXJ RI strains carrying different alleles at DXMit61. Further evidence for a parent-of-origin effect is shown in Table 7, in which tumor incidence differs depending on which allele is inherited and which parent contributes the allele. GC tumors were significantly more frequent when the SJL DXMit6 allele was inherited from the sire. In contrast, tumor incidences were equivalent in progeny from two sets of reciprocal matings between the SWR and SWXJ RI strains carrying the SWR DXMit6 allele. Collectively, these data suggest that: (a) susceptibility alleles act differently when inherited from the
male parent; and (b) a locus linked with DXMit16 on the SJL X CHR has a profound effect on the expression of tumor susceptibility alleles.

**DISCUSSION**

We have presented evidence that juvenile-onset GC tumors are inherited as a complex trait in the SWR strain and in a subset of SWXJ RI strains. The genetic analyses of GC tumorigenesis in SWR and SWXJ RI mice are complicated by its polygenic nature, qualitative expression, infrequent occurrence (~1–2% in the SWR strain), and the similarity of genomes between SWR and SJL strains (13). Nevertheless, we found evidence that as many as three autosomal genes and one X chromosomal gene are associated with GC tumors. Lander and Kruglyak (15) have proposed rigorous criteria for the assessment of linkage in complex trait analyses. These authors defined stringent Ps for categories of significant and suggestive linkage for quantitative trait loci derived from genome-wide searches to minimize spurious linkage, due in part to failure to account for the number of tests conducted during the genome scans. We chose the conservative levels of $P < 0.001$ (significant) and $P < 0.01$ (suggestive) for defining the association of chromosomal regions with tumorigenesis. Such Ps reduce the likelihood of falsely declaring linkage.

In recognition of the relationship between the four loci and GC tumorigenesis, we propose to name the series of GC tumor susceptibility loci: (a) Gct1 on CHR 4; (b) Gct2 on CHR 12; (c) Gct3 on CHR 15; and (d) Gct4 on CHR X. We conclude that these loci interact to influence the probability of GC tumorigenesis. Our data indicate that at least one SWR-derived Gct1 allele is required for tumorigenesis, and that this locus behaves like an oncogene, with the SWR allele acting in a dominant fashion. Furthermore, we found that a paternally inherited SJL-derived Gct4 allele on CHR X is strongly promotive of tumorigenesis. With respect to the other autosomal loci, the F _2_ data suggest that SWR-derived alleles at Gct2 and Gct3 participate in an interactive fashion in tumorigenesis, whereas the SWXJ RI genotypes for these two loci suggest that they are not necessary for tumorigenesis. This apparent discrepancy in the effects of these loci could result from: (a) not enough RI strains to evaluate the multiple loci underlying tumorigenesis (16); or (b) another susceptibility locus that has yet to be identified that lies within a region lacking SSLPs such as described for CHR 9.

Two models could account for the polygenic control of GC tumorigenesis: (a) in model one, tumors could occur primarily because of one of several combinations of genotypes; and (b) in model two, tumors could occur in any of a large number of genetic combinations. In the first model, particular alleles are required for tumor formation, whereas in the second model, only a few alleles at a larger number of loci are required. If the first model applies, we would expect to find a limited number of genotype combinations in tumor-bearing mice. If the second model applies, many more genotype combinations are expected, and the distribution of genotype combinations in tumor-bearing and tumor-free mice is expected to be very similar. The data indicated that GC tumorigenesis is most likely to occur when SWR alleles are present at each of the three autosomal loci and the DXMit16 SJL allele is inherited from the sire, an outcome that supports the first model.

The fourth locus, Gct4, on CHR X merits further consideration, because it may be the Sot locus we reported previously (11). Initially, Sot was defined as a gene that influences spontaneous tumorigenesis enhanced by testosterone treatment, with the susceptible allele contributed to the SWXJ RI strains by SWR. However, we were unable to map this locus by strain distribution analyses, because we could not reliably determine which strains were testosterone-resistant strains. After the identification of a SJL-derived susceptibility allele on CHR X that was associated with spontaneous tumorigenesis, we repeated the treatment of SWR mice with testosterone and found no increase in GC tumor frequency [GC tumors occurred in 1 of 108 (0.9%) testosterone-treated mice and in 1 of 110 (0.9%) control mice]. We interpreted this to mean that SJL is the source of a strong susceptible allele at Sot. Furthermore, because the SWXJ RI strain distribution pattern for DXMit16 assigns the SJL DXMit16 allele to the 4 spontaneous tumor SWXJ RI strains and the SWR DXMit16 allele to 9 of 10 spontaneous tumor-free strains, it is likely that Sot is Gct4. However, our current data do not enable us to say whether the Gct4 locus and the parent-of-origin effect represent the same or different phenomena. Finally, given the important role of androgens in GC tumor initiation and growth, it is worth noting that the androgen receptor (Ar) locus maps less than 1 cM proximal to DXMit16 and is a candidate gene for Gct4.

The loss of alleles for specific tumor suppressor genes is associated with many different cancers (17). The remaining allele is often mutated and, when expressed, contributes to uncontrolled proliferation associated with neoplasia. Because, by definition, an inbred strain of mice is homozygous at essentially all genetic loci, LOH is difficult to study as a mechanism for pathological change in SWR mice. Nevertheless, LOH could be addressed in the DNA from GC tumors of F _2_ mice in which loci are heterozygous for the chromosomal regions associated with tumorigenesis (CHRS 4, 12, 15, 9, and X). A sample of five tumors from mice whose genomic DNA was heterozygous at D4Mit232 was examined for LOH at D4Mit232 as well as for markers on CHRS 9, 12, 15 and X associated with GC tumor development. Although admittedly not a rigorous test of LOH in tumors, we found no differences between genomic and tumor DNA, suggesting that LOH is not the cause of GC tumor formation.

Loss of imprinting is also associated with a variety of human cancers (18). The data gathered on the SWR GC tumor model clearly implicates imprinting. In the absence of biochemical data, we cannot determine whether the X CHR region associated with the DXMit16 SJL marker fails to imprint a gene or inappropriately imprints a gene. The X-inactivation-specific transcript gene, Xist (19), is located within 6 cM of DXMit16, and the SJL Xist allele is likely to be present within the incipient congenic SWR.SJL-X2 and -X3 strains shown in Table 5. The Xist gene has been reported to be imprinted, and its function is affected by the parent of origin (20). Other genes in this region that have regulatory roles in developmental processes and are associated with genomic imprinting (21) include: (a) Ddx2 [distortion controlling, CHR X, locus 2 (22)]; (b) Meep2 [methyl CpG-binding protein 2 (23)]; and (c) Xce [X-inactivation-controlling element (24, 25)].

Identification of tumor susceptibility genes in the mouse can be used to predict the possible location of relevant human genes via homologous relationships among conserved gene clusters (26). At least nine loci associated with cancer of various tissues have been located on mouse distal CHR 4, and eight cancer loci are located on the homologous region of human CHR 1p34–36. Thus, these conserved regions of the mouse and human genomes carry numerous genes with roles in the regulation of cell proliferation. On the other hand, the susceptibility locus on mouse CHRS 12 and 15 share homology with human CHRS 7q22–31 and 8q23–24, respectively. There are as yet no obvious relationships with cancer-related genes in these regions of the mouse or human genome, respectively.

The SWR mouse model is the first polygenic model for spontaneous carcinoma and offers a unique opportunity to analyze genetic susceptibility and resistance in the absence of carcinogen induction. Previously, polygenic susceptibility to carcinogen-induced tumors has been identified in other mouse models. For example, 11 loci are associated with carcinogen-induced liver tumorigenesis. These loci
include seven susceptibility genes (27–29), two resistance genes (30), and two susceptibility genes in females (31). Fijneman et al. (32) have shown that four genes interact in susceptibility to carcinogen-induced lung cancer. Carcinogen-induced colon tumors in mice are associated with as many as five different loci (33). Tumors induced by mouse mammary tumor virus or murine leukemia virus represent different types of polygenic models. In these systems, multiple proto-oncogenes activated by proviral insertions may cooperate in the induction of neoplasia (34). In the SWR model, we have found no biochemical evidence of reverse transcriptase activity, cytogenetic evidence of gross chromosomal aberration, or ultrastructural evidence of viral particles associated with GC tumors (1). For each of the model systems noted above, including SWR and SJL, other genes are likely to participate, but modest allelic variation between these strains has made the genetic mapping of additional loci difficult.

In summary, spontaneous GC tumorigenesis in the SWR inbred mouse is the consequence of complex genetic regulation that includes critical genes on CHRs 4 and X plus modifier genes on CHRs 12 and 15. In addition, the locus on CHR X is intimately involved with the parent-of-origin regulation of tumor susceptibility gene expression in female offspring, i.e., genomic imprinting, a mechanism not previously associated with ovarian cancer.

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Multigenic and Imprinting Control of Ovarian Granulosa Cell Tumorigenesis in Mice


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