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Changes in the Mouse Estrus Cycle in Response to Brca1 Inactivation Suggest a Potential Link between Risk Factors for Familial and Sporadic Ovarian Cancer

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Abstract

Menstrual cycle activity is the most important risk factor for sporadic serous ovarian carcinoma, whereas a germ-line mutation in BRCA1 is the most important risk factor for the familial form. Given the rarity of BRCA1 mutations in sporadic ovarian cancers, we hypothesized that BRCA1 influences the menstrual cycle in a way that mimics the factors underlying sporadic ovarian cancer predisposition, making BRCA1 mutations redundant in such cancers. We compared the length of each phase of the estrus cycle (equivalent to the human menstrual cycle) and of circulating levels of estradiol in control mice and in mice carrying a Brca1 mutation in their ovarian granulosa cells, two thirds of which develop ovarian or uterine epithelial tumors. We also compared the length of the different phases of the cycle in mutants that subsequently developed tumors with those in mutants that remained tumor-free. Mutant mice as well as oophorectomized wild-type mice harboring mutant ovarian grafts showed a relative increase in the average length of the proestrus phase of the estrus cycle, which corresponds to the estrogen-dominated follicular phase of the human menstrual cycle. Total circulating levels of estradiol were also increased in mutant mice injected with pregnant mare serum gonadotropins. The relative increase in proestrus length was highest in mutant mice that subsequently developed reproductive epithelial tumors. We conclude that loss of a functional Brca1 increases murine ovarian epithelial tumor predisposition by increasing estrogen stimulation in the absence of progesterone, recapitulating conditions associated with sporadic ovarian cancer predisposition in humans. *Cancer Res; 70(1); 221–8. ©2010 AACR.*

Introduction

Epithelial ovarian cancer is the most lethal gynecologic malignancy and the fourth leading cause of death from cancer among women. Continuous menstrual cycle activity is the strongest risk factor for the sporadic form of ovarian carcinomas (1, 2), including the serous subtype, the most common histologic subtype of this disease, whereas being a carrier of a mutation in BRCA1 is the most common risk factor for the familial form (3–6). Although BRCA1 expression is reduced in some sporadic ovarian tumors (7, 8), there is no definitive evidence that this protein is important for their development. It is intriguing that mutations in the *BRCA1* gene are relatively rare in the sporadic form of ovarian epithelial tumors given their important role in the familial form. We hypothesized that the reproductive factors that are associated with increased risk of sporadic tumors might mimic the consequences of a BRCA1 mutation, making such mutations irrelevant. This could be analogous to the rarity of P53 mutations in cervical carcinomas because the presence of the human papilloma virus E6 protein, which binds to P53 and is expressed in most of these tumors, eliminates the need for such mutations. The fact that interruption of the menstrual cycle from either pregnancy or oral contraceptive use is not only protective against the sporadic form but also against familial ovarian serous carcinoma (9, 10) is consistent with the idea of an overlap between the genetic and reproductive risk factors.

We sought to determine whether menstrual cycle regulation could be influenced by alterations in BRCA1 expression to test this hypothesis. We used a mouse model based on conditional inactivation of Brca1 in ovarian granulosa cells (11). These cells play a central role in the regulation of the murine estrus cycle, which is comparable with the menstrual cycle in humans. We reported earlier that two thirds of the mutant mice developed serous cystadenomas, benign counterparts of serous ovarian carcinomas, and that these tumors had a distribution similar to that of serous ovarian tumors in humans (11).

We compared the length of the various phases of the estrus cycle in wild-type animals versus mutants lacking a functional *Brca1* gene in their ovarian granulosa cells. Here, we show that the estrogen-dominated preovulatory phase is

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increased relative to the postovulatory phase in mutant mice and that the magnitude of this increase is associated with increased tumor risk. These results suggest the presence of a common link between risk factors for sporadic and familial ovarian cancer and provide insights into the mechanisms underlying ovarian cancer predisposition in human BRCA1 mutation carriers.

Materials and Methods

Experimental animals. The generation and characterization of conditional Brca1 mutant mice was described earlier (11). The mice were housed under standard 12 h of light alternating with 12 h of dark conditions, with automatic lighting changes occurring at 6 a.m. and 6 p.m. Mice were cared for in accordance with institutional guidelines under the protocols approved by the University of Southern California Institutional Animal Care and Use Committee.

Estrus cycle length determinations. Stages of the estrus cycle were determined by cytologic evaluation of vaginal smears. Sterile PBS was gently flushed into the vagina using soft plastic pipettes between 10 a.m. and 12 noon daily over a period spanning at least four consecutive cycles. The lavages were smeared on glass slides, stained with Papanicolaou stain, and examined microscopically to evaluate the cytologic features described in Results. Mice that did not show evidence of continuous cycling activity based on cytologic examination were discarded. The average length of each phase was calculated for each individual mouse and used to calculate the average length ± SD for the entire study group. Accurate measurement of the magnitude of the effects of Brca1 inactivation on the relative duration of the different phases of the cycle was complicated by the fact that samples of vaginal washings were obtained only every 24 h. Thus, the frequency of sample collection was low given that the entire mouse estrus cycle takes only 4 to 6 d. This was unavoidable because higher frequencies would have increased the risk of vaginal irritation, which in turn could have interfered with cycling activity. We compensated for this problem by extending our daily observations over periods of 3 to 4 wk, ensuring that each measurement represented an average of at least four consecutive cycles in each animal.

Ovarian transplantation procedures. Thirty-five-day-old mice were anaesthetized by i.p. injection of ketamine and xylazine. The ovaries and kidneys were exposed through dorsolateral incisions. Bilateral oophorectomies were performed in all recipients. A small incision was made into the capsule of a single kidney in each recipient using spring scissors of appropriate size. A single intact ovary was inserted beneath the capsule through the small incision. Only transplant recipients that had regular estrus cycles based on vaginal cytologic examination by the time they were 8 wk old were included in our studies. The remaining mice, which accounted for 45% to 50% of the total transplant recipients, were discarded.

Serum hormone measurement. Mice that either had been synchronized with pregnant mare serum gonadotropin (PMSG) or had been identified as being in proestrus based on vaginal cytology were anesthetized with ketamine and xylazine. Up to 1 mL of nonhemolyzed blood was collected by cardiac puncture. Serum was collected following overnight incubation at 4°C and stored at −80°C. Estradiol concentrations were measured in the laboratory of one of the authors (F.Z.S.) by RIA with a preceding extraction step using ethyl acetate/hexane (3:2). The assay sensitivity is 3 pg/mL, and the interassay coefficient of variation is 8% to 10% (12).

Statistical analyses. All statistical analyses were performed using the Statistical Analysis System package program (SAS Institute) or the STATA program (Stata Corp.). Statistical significance levels (P values) were calculated with the nonparametric Wilcoxon rank-sum sign test. All P values quoted are two-sided.

Results

Consequences of Brca1 inactivation in ovarian granulosa cells on the estrus cycle. The important landmarks of ovarian follicular development and the hormonal changes associated with progression through the murine estrus cycle are remarkably similar to those seen in the human menstrual cycle (13–16). The estrus cycle is usually subdivided into four phases referred to, respectively, as proestrus, estrus, metestrus, and diestrus. Proestrus corresponds to the human follicular phase of the menstrual cycle and is dominated by elevated levels of estradiol, although residual levels of progesterone may remain in the circulation through the beginning of this phase (15, 16). Estrus corresponds to ovulation. Metestrus, which is often subdivided into metestrus I and metestrus II, is equivalent to the human luteal phase characterized by elevated levels of both progesterone and estradiol. The next phase, diestrus, is best regarded as corresponding to the late luteal phase because circulating levels of progesterone remain significantly elevated during this phase (15, 16).

The cytologic appearance of cells recovered from mouse vaginal washings undergoes profound changes characteristic of each phase of the estrus cycle (Fig. 1). Washings obtained during diestrus consist almost exclusively of scattered inflammatory cells, whereas washings obtained during proestrus show primarily epithelial cells that appear either blue, pink, or orange on staining with Papanicolaou stain depending on their state of maturation (Fig. 1). Only fully mature epithelial cells showing an orange cytoplasm and lacking a nucleus are present at estrus. Metestrus is characterized by an admixture of epithelial cells and inflammatory cells, the former being more abundant during metestrus I than during metestrus II.

We took advantage of these well-defined and highly reproducible changes in vaginal cytology associated with each phase of the cycle to measure and compare the average length of each phase in cohorts of control type mice and mice carrying a Brca1 mutation in their ovarian granulosa cells (11). Daily vaginal lavages were obtained from mutant and wild-type animals and spread on glass slides, stained with Papanicolaou stain, and examined microscopically to determine the phase of the cycle and calculate the average length of each phase. These daily observations were extended

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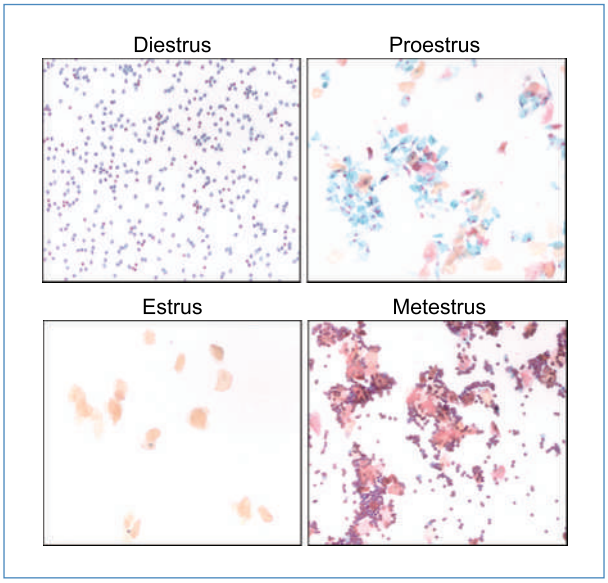

Figure 1. Changes in vaginal cytology associated with the different stages of the estrus cycle. Daily vaginal washings obtained as explained in Materials and Methods from a single mouse were stained with Papanicolaou stain. The illustration shows photographs representative of each stage of the estrus cycle. Diestrus is characterized primarily by inflammatory cells with few, if any, immature epithelial cells. Proestrus shows turquoise epithelial cells of intermediate maturity, which become more mature orange cells toward the end of this phase. Estrus is characterized by fully mature orange cells lacking a nucleus. Metestrus is characterized by intermediate and fully mature epithelial cells admixed with inflammatory leukocytes.

over periods of 3 to 4 weeks, ensuring that each value represented an average of at least four consecutive cycles in each animal. The results showed the average length of the proestrus phase in the 20 mutant 3- to 4-month-old mice examined to be 1.63 ± 0.10 compared with 1.24 ± 0.10 in 18 age-matched wild-type littermates ($P = 0.007$). The difference between wild-type and mutant was even more significant in 7- to 8-month-old mice, as the average length of proestrus in mutants within this age group was 1.33 ± 0.10 compared with 0.89 ± 0.05 in age-matched wild-type littermates ($P = 0.0008$).

Although no statistically significant difference could be shown between the average length of any other phase of the cycle in mutant versus wild-type animals, there was a trend toward a shortening of metestrus in mutant animals (average length of 1.9 days in wild-type compared with 1.3 days in mutant animals). This prompted us to determine whether the average length ratio of the preovulatory to postovulatory phases was different in the two groups of mice. The results (Table 1) showed that, indeed, this ratio was increased in mice harboring a Brca1 mutation in ovarian granulosa cells. This increase was significant whether we used the length of metestrus alone or the combined lengths of metestrus plus diestrus as a measure of the postovulatory phase. We consider the combined length of metestrus plus diestrus to more accurately represent the human luteal phase because circulating progesterone levels, although gradually decreasing, remain significantly elevated during diestrus (13–16). The significance of the difference in the ratio of proestrus to metestrus plus diestrus was more marked in 7- to 8-month-old than in 3- to 4-month-old mice (Table 1), further supporting the notion that the effects of inactivation of Brca1 may be most consequential at more advanced ages.

Potential contribution of the anterior pituitary gland to estrus cycle changes seen in mutant mice. Conditional Brca1 inactivation was achieved in our mouse model by crossing *Brca1*$^{flox/flox}$ mice with a line carrying a *Cre recombinase* transgene driven by a truncated form of the Fshr promoter (11). This promoter had previously been reported to be specific for granulosa cells in females (17) and indeed showed such a high degree of specificity in female pelvic and abdominal organs in studies in which the *Fshr-Cre* transgene was introduced into the R26R reporter strain (11). Given that the full-length form of this promoter is normally expressed in the anterior pituitary and given the important role of this organ in controlling the estrus/menstrual cycle, we further examined the possibility that Brca1 rearrangement had also taken place in this gland in addition to granulosa cells. We first addressed this question by enzymatically amplifying DNA extracted from the pituitary gland of mutant animals using primers specific for either the recombined or the unrecombined alleles of the *Brca1* gene. Indeed, PCR products were obtained with primers specific for the rearranged allele in some mutant animals (Fig. 2). Confirmation that the truncated *Fshr* promoter driving Brca1 inactivation in our mouse model was expressed in a subset of anterior pituitary cells was obtained from the identification of β-galactosidase–positive cells in histologic sections of this organ obtained from R26R reporter mice carrying the *Fshr-Cre* transgene (Fig. 2).

Relative importance of Brca1 inactivation in ovarian granulosa cells compared with anterior pituitary cells in estrus cycle regulation. These results raised the possibility

Table 1. Increase in the length ratio of preovulatory over postovulatory phases of the estrus cycle in mice lacking a functional Brca1 in ovarian granulosa cells

Age	Proestrus/metestrus		Proestrus/metestrus + diestrus	
	3–4 mo	7–8 mo	3–4 mo	7–8 mo
Wild-type mice	0.87 ± 0.10 (n = 18)	0.70 ± 0.011 (n = 14)	0.26 ± 0.02 (n = 18)	0.19 ± 0.02 (n = 14)
Mutant mice	1.37 ± 0.11 (n = 20)	1.57 ± 0.22 (n = 16)	0.38 ± 0.04 (n = 20)	0.39 ± 0.05 (n = 16)
P (wild-type vs mutant)	0.002	0.002	0.02	0.0007

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that the changes in the estrus cycle in mutant mice were influenced by Brca1 inactivation in the pituitary gland or other cell types in which low levels of Brca1 inactivation could also have occurred. We therefore sought to compare the relative importance of intraovarian versus extraovarian tissues in controlling the estrus cycle changes associated with the mutant phenotype. Ovaries of mutant mice were removed and replaced by grafts obtained from wild-type donors, which were placed under the renal capsule of recipients. Reciprocal experiments in which wild-type ovaries were replaced with grafts obtained from mutant donors were also performed. Transplantations of wild-type ovaries into wild-type animals and of mutant ovaries into mutant animals were used as controls. All transplantation procedures were performed using donors and recipients that were 3 weeks old or slightly younger and, thus, had not reached sexual maturity.

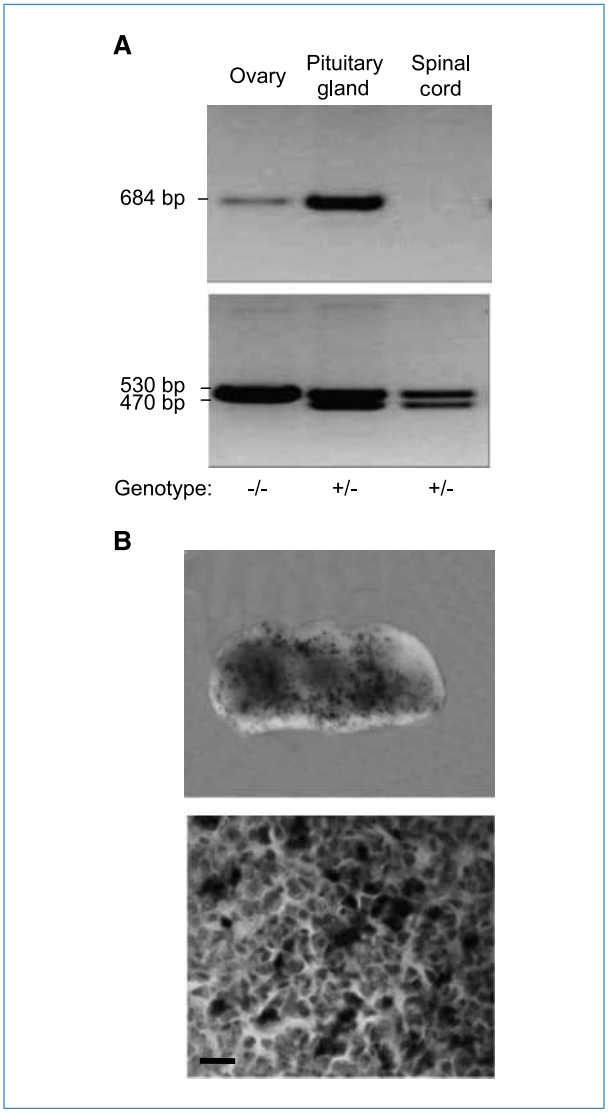

Figure 2. Activity of the *Fshr* promoter in a subset of anterior pituitary cells. *A,* DNA extracted from ovary, pituitary gland, or spinal cord was enzymatically amplified using primers that can distinguish between rearranged and intact forms of the floxed *Brca1* allele (11). The mice carried either one (+/–) or two (–/–) mutant *Brca1* alleles. A 684-bp fragment corresponding to the mutant *Brca1* allele was amplifiable from ovarian and pituitary tissues but could not be amplified from spinal cord. A 530-bp fragment representing the intact (unrearranged) floxed *Brca1* allele was amplified from all tissues. A 470-bp fragment representing the wild-type gene was only seen in mice carrying a single floxed *Brca1* allele. *B, top,* pituitary of a *Fshr-Cre;Brca1* $^{flox/flox}$;R26R mouse stained for *LacZ*. The dark color indicates β-galactosidase activity, which in turn is indicative of *Fshr* promoter activity in the *Fshr-Cre* transgene. *Bottom,* focal promoter activity is also appreciated in the photomicrograph of a similar pituitary. *Scale bar,* 100 μm.

Changes in vaginal cytology characteristic of the various phases of the estrus cycle were readily demonstrable in mice that had undergone ovarian transplantation procedures, showing that the transplanted ovaries were functional (data not shown). Histologic examination of the transplants in representative animals revealed maturing ovarian follicles, confirming this conclusion (Fig. 3).

The lengths of the different phases of the estrus cycle were measured and compared in the transplant recipients at the ages of 7 to 9 months. The results (Table 2) showed that compared with mice harboring wild-type ovaries, the proestrus phase was prolonged in all mutant mice harboring mutant ovaries, including when compared with mutant mice that had received ovarian transplants from wild-type animals. These results not only confirm those obtained earlier with mice not subjected to any transplantation procedure but also show that inactivation of Brca1 in ovaries can prolong the proestrus phase of the cycle even in the presence of a functional Brca1 in the pituitary. Although there was a trend toward an increase in the average length of the proestrus phase in wild-type mice harboring mutant ovaries, this increase was not as marked as in recipients carrying the floxed *Brca1* allele in their germ line and did not reach statistical significance (Table 2). It is possible that the presence of a functional Brca1 in the pituitary can partly overcome the effects of a Brca1 mutation in granulosa cells perhaps through feedback mechanisms controlled by Brca1. The average combined length of proestrus plus estrus, which may be a better measure of the total duration of estrogen stimulation under absent (or markedly reduced) levels of progesterone during the estrus cycle, was significantly longer in all mice harboring mutant ovaries, regardless of whether the ovarian transplant was in wild-type or mutant recipients, further emphasizing the relative importance of the ovary versus the pituitary in driving the mutant phenotype (Table 2).

Consequences of brca1 inactivation on circulating estrogen levels. The mere fact that the average length of proestrus is increased in mice harboring a Brca1 mutation in ovarian granulosa cells implies that such mutant mice are subjected to prolonged estrogen stimulation in the absence (or presence of markedly reduced levels) of progesterone compared with wild-type mice. We sought to determine whether mutant animals were, in addition, subjected to higher absolute levels of circulating estrogens. Given that estradiol levels can fluctuate widely within the proestrus phase depending on whether measurements are made near the beginning or the end of this phase, we synchronized all

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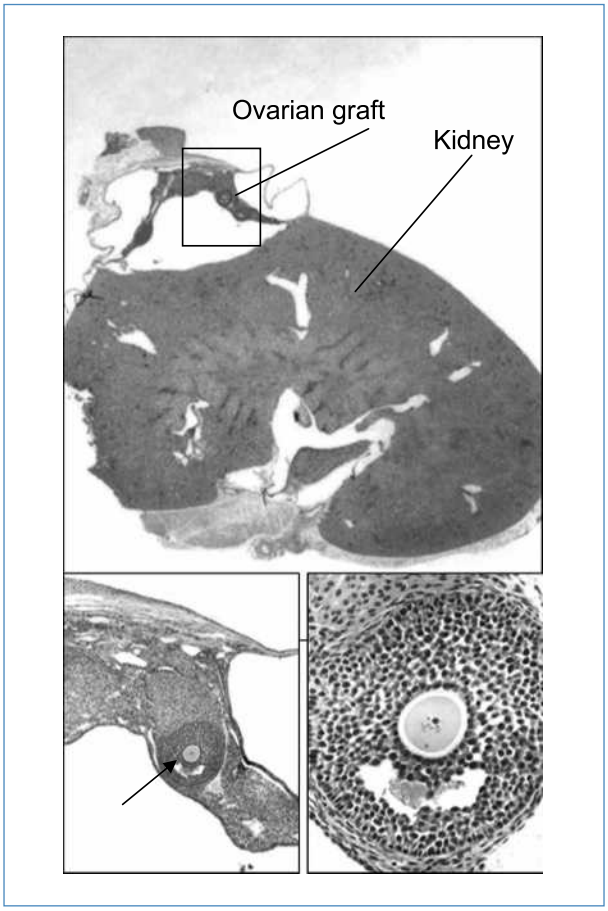

Figure 3. Demonstration of the functionality of transplanted ovaries. The illustration at the top shows a whole-mount photomicrograph of a kidney with an attached subcapsular ovarian transplant. The area within the rectangle is magnified in the bottom left panel, showing a secondary ovarian follicle (*arrow*) that is further magnified in the photograph shown on the right. The presence of such follicles further attests to the functionality of transplanted ovaries.

mice with 5 units PMSG to increase the strength of comparisons of hormonal levels between wild-type and mutants. PMSG, which is analogous to follicle-stimulating hormone, can override endogenous hormones and rapidly induce follicular growth, thus mimicking proestrus. Measurements of circulating hormone levels in blood drawn 48 hours after inoculation with PMSG showed significantly higher estradiol levels in mutant mice, suggesting that such mice were subjected to higher circulating estradiol levels in addition to prolonged estrogen stimulation. The average level of this hormone following PMSG inoculation in 14 wild-type mice was 20 ± 17 pg/mL compared with 45 ± 21 pg/mL in 17 mutant mice ($P_{t\ \text{test}} = 0.002$, Wilcoxon rank-sum test).

Correlation between increased average proestrus to metestrus length ratio and tumor predisposition. We reported earlier that mice carrying a Brca1 mutation in ovarian granulosa cells developed epithelial tumors in their ovaries, oviducts, and uterine wall and argued that the distribution of these tumors, which were morphologically similar to human ovarian cystadenomas, was similar to that seen in the reproductive tract of human BRCA1 mutation carriers (11). We hypothesized that the prolonged unopposed estrogen stimulation present in mutant mice was responsible, at least in part, for predisposition to such tumors. We took advantage of the fact that tumors were detected in approximately two thirds of the mice, implying that a substantial number did not develop any grossly visible tumors, to determine whether mutant mice with higher proestrus to metestrus length ratios were more likely to develop tumors than those with lower ratios. We examined the proestrus to metestrus ratio in mutant mice before the appearance of grossly visible tumors (3–4 months old), at a time coinciding with the earliest appearance of grossly apparent tumors (7–8 months), and following the appearance of such tumors (14–16 months). The mice were sacrificed after the last measurements and divided into two groups: one with grossly visible tumors and one in which no tumors were seen.

A trend toward higher proestrus to metestrus ratios was noted in mice that developed tumors. This trend was statistically significant for measurements performed in the older age group (Table 3). When the ratio of length of proestrus over the combined length of metestrus and diestrus was used, a statistically significant correlation was seen for ratios calculated not only in 14- to 16-month-old mice but also in 7- to 8-month-old mice (Table 3) in support of our hypothesis. This ratio may be a better indicator of the fraction of the cycle that is characterized by unopposed estrogen stimulation (15, 16). The lack of a significant association with ratios observed in younger mice is consistent with the conclusion that the effects of Brca1 mutations on the estrus cycle are not as marked at younger ages.

Discussion

Our results clearly show that mice harboring a *Brca1* gene knockout in their ovarian granulosa cells show a measurable increase in the average length of the proestrus phase of their estrus cycle compared with wild-type mice. This was not driven by Brca1 inactivation in cells other than granulosa cells because mutant mice harboring wild-type ovarian grafts showed average proestrus lengths similar to those in wild-type mice.

We used homozygous Brca1 mutants in our studies to maximize the effects of alterations in Brca1 expression. Our results raise the possibility that human BRCA1 mutation carriers, who are heterozygous for such mutations, may experience similar alterations in their menstrual cycle due to reduction in *BRCA1* gene dosage. This idea still needs to be confirmed, and to this effect, studies focused on examining the consequences of heterozygous deletions of Brca1 on proestrus length and tumor predisposition are ongoing in our laboratory using our mouse model. In addition, the evidence provided in this article is largely correlative and a direct demonstration that loss of a functional Brca1 increases murine ovarian epithelial tumor predisposition by increasing estrogen stimulation in the absence of progesterone is still lacking. Nevertheless, the finding that deletion of mouse *Brca1*,

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Table 2. Differences in the length of individual phases of the estrus cycle based on the genotype of hosts and ovarian grafts

Genotype		No. mice examined	Average length (d)				
Host	Graft		Proestrus	Estrus	Proestrus + estrus	Metestrus	Diestrus
Wild-type	Wild-type	15	1.06 ± 0.08	0.94 ± 0.08	2.00 ± 0.11	1.48 ± 0.19	4.77 ± 0.67
Mutant	Mutant	14	1.35 ± 0.09	1.17 ± 0.17	2.52 ± 0.17	1.63 ± 0.26	4.28 ± 0.64
Mutant	Wild-type	10	1.08 ± 0.08	0.89 ± 0.07	1.97 ± 0.11	1.59 ± 0.15	4.31 ± 0.77
Wild-type	Mutant	9	1.27 ± 0.08	1.21 ± 0.15	2.48 ± 0.16	1.56 ± 0.23	3.68 ± 0.59

Genotypes compared		*P* (top: *t* test; bottom: nonparametric)				
		Proestrus	Estrus	Proestrus + estrus	Metestrus	Diestrus
		0.025	0.211	0.013	0.650	0.600
		0.032	0.250	0.025	0.822	0.526
		0.890	0.720	0.870	0.700	0.660
		0.870	0.980	0.610	0.180	0.600
		0.038	0.197	0.022	0.890	0.970
		0.042	0.312	0.012	0.380	0.860
		0.498	0.850	0.870	0.850	0.530
		0.505	0.630	0.970	0.870	0.610
		0.117	0.094	0.018	0.812	0.276
		0.125	0.112	0.048	0.832	0.310
		0.112	0.068	0.019	0.916	0.528
		0.126	0.139	0.016	0.681	0.513

NOTE:  : normal host with normal ovary  : mutant host with mutant ovary

 : normal host with mutant ovary  : mutant host with normal ovary

the homologue of a gene controlling familial ovarian cancer predisposition in humans, influences both tumor predisposition as well as the ovarian cycle, an important determinant of ovarian cancer risk in humans, is intriguing. The human follicular phase is characterized by elevated levels of circulating estrogens relative to progesterone similarly to murine proestrus. Such hormonal exposure might be an important determinant of the site specificity of the tumors seen in human BRCA1 mutation carriers. This idea is further supported by epidemiologic studies that have consistently shown an association between risk of ovarian cancer and duration of postmenopausal estrogen replacement therapy (18–22). *In vitro* studies showing that estrogen acts as a mitogen for ovarian cancer cell lines, whereas progesterone is growth inhibitory, are also in line with this hypothesis (23). The potential relevance of our observations to tumor predisposition in human BRCA1 mutation carriers is further underscored by our finding that mutant mice that developed tumors had higher average proestrus/metestrus + diestrus length ratios than age-matched mutant littermates that remained tumor-free.

Several mechanisms could account for the changes in the dynamics of the estrus cycle in mutant animals. Estradiol

Table 3. Association between the relative lengths of preovulatory and postovulatory phases of the estrus cycle and tumor predisposition in mutant mice

	Age	3–4 mo	7–8 mo	14–16 mo
Proestrus/metestrus	Mutants with tumors	1.40 ± 0.10 (*n* = 4)	1.67 ± 0.60 (*n* = 5)	1.06 ± 0.12 (*n* = 6)
	Mutants without tumors	1.00 ± 0.20 (*n* = 4)	1.38 ± 0.51 (*n* = 4)	0.60 ± 0.07 (*n* = 8)
	P (with vs without tumors)	0.25	0.62	0.01
Proestrus/metestrus + diestrus	Mutants with tumors	0.55 ± 0.10 (*n* = 4)	0.49 ± 0.05 (*n* = 5)	0.33 ± 0.05 (*n* = 6)
	Mutants without tumors	0.47 ± 0.12 (*n* = 4)	0.29 ± 0.05 (*n* = 4)	0.20 ± 0.02 (*n* = 8)
	P (with vs without tumors)	0.56	0.03	0.02

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biosynthesis, which is an important mediator of cycle progression, can be upregulated by reducing the expression of BRCA1 in human granulosa cells (24). Changes in circulating estradiol levels could, by themselves, contribute to changes in the length of the preovulatory phase of the cycle because levels of this hormone can influence the timing of the luteinizing hormone (LH) surge that triggers ovulation (25, 26). The finding that circulating estradiol levels were higher in mutant compared with wild-type animals synchronized for their estrus cycle stage by exogenous hormonal stimulation strongly suggests that a Brca1 mutation can also influence circulating estrogen levels. It is also possible that secretion of other factors associated with the regulation of the LH surge, such as gonadotrophin surge attenuating factor (27, 28), which is synthesized by granulosa cells (29, 30), is altered in the absence of Brca1 activity. The effect of Brca1 on the level and activity of this factor as well as of hormones such as LH, inhibin, and activin should be investigated.

The effects of Brca1 inactivation on the estrus cycle and on tumor predisposition seemed to be highly influenced by the age of the host animals. Not only was the magnitude of the relative increase in proestrus length higher in 7- to 9-month-old mice than in 3- to 4-month-old mice but also the association between proestrus over metestrus plus diestrus ratio, and tumor predisposition only reached statistical significance for ratios calculated in mice that were at least 9 to 10 months old. This raises the possibility that the consequences of Brca1 inactivation are magnified when the total number of ovarian follicles is decreased due to age.

Our results point to a link between the reproductive risk factors associated with the development of sporadic ovarian cancer and the genetic factors operating in human BRCA1 mutation carriers. It may be that the presence of BRCA1 mutations in sporadic ovarian carcinomas would be redundant in the presence of the strong reproductive risk factors known to be associated with such tumors, accounting for the rarity of BRCA1 mutations in nonhereditary ovarian cancers. These results also underscore the need to understand the mechanisms underlying the association between the menstrual cycle and ovarian cancer risk, which should lead to more effective prevention measures for sporadic as well as familial ovarian cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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