Obesity Accelerates Mouse Mammary Tumor Growth in the Absence of Ovarian Hormones

Nomelí P. Núñez and Susan N. Perkins
Laboratory of Biosystems and Cancer, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland; Cancer Prevention Fellowship Program, Division of Cancer Prevention, NCI, NIH, Bethesda, Maryland; and Department of Human Ecology, Division of Nutritional Sciences, University of Texas at Austin

Nicole C. P. Smith
Laboratory of Biosystems and Cancer, NCI, NIH, Bethesda, Maryland; Department of Human Ecology, Division of Nutritional Sciences, University of Texas at Austin; and Basic Research Program, SAIC-Frederick, Inc., NCI Frederick, Frederick, Maryland

David Berrigan
Applied Research Program, Division of Cancer Control and Population Sciences, NCI, NIH, Bethesda, Maryland

David M. Berendes
Laboratory of Biosystems and Cancer, NCI, NIH, Bethesda, Maryland

Lyuba Varticovski
Laboratory of Human Carcinogenesis, Center for Cancer Research, NCI, NIH, Bethesda, Maryland

J. Carl Barrett
Laboratory of Biosystems and Cancer, NCI, NIH, Bethesda, Maryland and Novartis Institute for Biomedical Research, Cambridge, Massachusetts

Stephen D. Hursting
Laboratory of Biosystems and Cancer, NCI, NIH, Bethesda, Maryland; Cancer Prevention Fellowship Program, Division of Cancer Prevention, NCI, NIH, Bethesda, Maryland; and Department of Human Ecology, Division of Nutritional Sciences, University of Texas at Austin

Obesity increases incidence and mortality of breast cancer in postmenopausal women. Mechanisms underlying this association are poorly understood. Suitable animal models are needed to elucidate potential mechanisms for this association. To determine the effects of obesity on mammary tumor growth, nonovariectomized and ovariectomized C57BL/6 mice of various body weights (lean, overweight, and obese) were implanted subcutaneously with mammary tumor cells from syngeneic Wnt-1 transgenic mice. In mice, the lean phenotype was associated with reduced Wnt-1 tumor growth regardless of ovarian hormone status. Ovariectomy delayed Wnt-1 tumor growth consistent with the known hormone responsiveness of these tumors. However, obesity accelerated tumor growth in ovariectomized but not in nonovariectomized animals. Diet-induced obesity in a syngeneic mouse model of breast cancer enhanced tumor growth, specifically in the absence of ovarian hormones. These results support epidemiological evidence that obesity is associated with increased breast cancer incidence and mortality in postmenopausal but not premenopausal women. In contrast, maintaining a lean body weight phenotype was associated with reduced Wnt-1 tumor growth regardless of ovarian hormone status.

INTRODUCTION

Breast cancer is the second leading cause of cancer death among women in the United States (1). In 2006, about 212,920 women were diagnosed with malignant breast cancer, and about
40,970 women die from the disease (2). Epidemiological studies have shown that obesity increases the risk of developing and dying from breast cancer but only in postmenopausal women (3,4,5). The risk of breast cancer is about 50% higher in obese postmenopausal women than in lean postmenopausal women (5). Approximately 30–50% of breast cancer deaths may be attributed to excess body weight (overweight/obesity), which translates to about 11,000–18,000 deaths per year (6). According to the Centers for Disease Control and Prevention, the population of obese adult women has increased dramatically in the United States from approximately 16% in 1962 to >34% today (7). The dramatic increase in obesity, combined with the fact that over 75% of new cases of breast cancer occur in postmenopausal women (2), increases the likelihood that the harmful impact of obesity on breast cancer will continue to rise.

Animal studies have shown that obesity increases mammary tumor development and/or progression (8,9), whereas obesity prevention by calorie restriction (CR) suppresses mammary cancer (9–12). Review of the literature revealed that most of the animal studies that have investigated the obesity-mammary cancer relationship have not been designed to mimic epidemiological findings. Specifically, most previous studies have evaluated the obesity-mammary cancer link in mice or rats with normal ovarian function. Therefore, these studies did not account for the well-established epidemiological observations that the majority of breast cancer cases occur in postmenopausal women and that obesity increases breast cancer development only after menopause. Thus, research on energy balance and/or obesity and mammary cancer in animals with functional ovaries may not reflect the relevant biology in women, suggesting a gap in the existing animal literature regarding the obesity-breast cancer relationship.

Epidemiological data and limited experimental evidence suggest that obesity may alter breast cancer development through alterations in hormonal pathways including estrogen, insulin, insulin-like growth factor-1 (IGF-1), and leptin (5,13–16). This is biologically plausible because many of these growth factors can promote mammary tumor growth and breast cancer tumor metastases (5,15,17,18). In addition, CR, which prevents obesity and protects against mammary cancer in multiple models, is associated with a reduction of many of these growth factors and adipokines (10,11,19–22). In addition, restoring the levels of some of these factors in CR animals (e.g., IGF-1) reverses the protective effects of CR on cancer (22,23).

In these studies, we characterized the impact of obesity on mammary tumor growth, circulating growth factors, and adipokines in mice with or without ovarian function (as a model of the premenopausal and postmenopausal states). Taken together, findings from these studies show that diet-induced obesity enhances tumor growth specifically in the absence of ovarian hormones (consistent with epidemiologic data) and increases several cancer-associated hormones/adipokines. In contrast, remaining lean throughout life, regardless of ovarian hormone status, reduces tumor growth and several cancer-associated hormones.

**MATERIAL AND METHODS**

**Induction of Lean, Overweight, and Obese Body Weight Phenotypes**

Nonovariectomized (NOVX) and ovariectomized (OVX) female mice with a wide spectrum of body weights were generated by manipulating caloric intake. For this purpose, mice were either on CR or given free access to diets of various caloric densities. C57BL/6NCr female mice (Charles River Laboratories, Frederick, MD) were ovariectomized at 5 wk of age; a sham ovariectomized group in which surgery was performed but the ovaries were not removed was included to control for the effects of surgery on the parameters being studied. At 6 wk of age, mice were randomized (12 per group) to receive the following diet treatments (resulting body size phenotypes are indicated in parentheses): 1) 30% CR regimen (lean), 2) 15% CR regimen (normal), 3) fed ad libitum a low-calorie diet providing 3.8 kcal/g (overweight), 4) fed ad libitum a medium-high-calorie diet providing 4.7 kcal/g (very overweight), and 5) fed ad libitum a high-calorie diet providing 5.2 kcal/g (obese). We have observed that when C57BL/6NCr female mice consumed the low-fat diet (3.8 kcal/g) ad libitum, they become overweight and even obese as they get older (unpublished findings); on the other hand, mice on the 15% CR regimen gain weight but do not become overweight or obese. For this reasons, in this article, we refer to the mice in the 15% CR regimen as “normal.” A table with detailed diet formulations has been published previously in Ref. 24. Mice received the various dietary treatments for a period of 10–30 wk. The lean and normal weight mice (15% and 30% CR, respectively) received modified versions of the 3.8 kcal/g formulated diet such that the daily aliquots of diet they received provided 70% or 85% of the mean daily caloric consumption (but 100% of the vitamins, minerals, fatty acids and amino acids) of the respective NOVX or OVX overweight groups. In mammary tumor implantation studies, lean, overweight, and obese mice were used.

In each study, mice were singly housed, either provided their respective diets ad libitum or on CR, and kept on a 12-h light–dark cycle. All diets were purchased from Research Diets, Inc. (New Brunswick, NJ) and stored at ~20°C. Food consumption was recorded twice weekly. Body weights were recorded prior to mice commencing the diet regimens and weekly thereafter. The initial body weights among all groups were similar. Blood samples were drawn from the retro-orbital venous plexus or submandibular region of lightly anesthetized mice (Isoflurane, Mallinckrodt Veterinary Inc., Mundelein, IL) at baseline, 10 wk, 20 wk, and at the end of the study. Serum was collected and frozen in liquid nitrogen and then stored at ~70°C until analyzed. All procedures involving animals were approved and monitored by the NCI Animal Care and Use Committee.
Estrous Cycle in Mice

Estrous status of the mice was determined daily for 12 consecutive days using the EC40 Rat Estrous Cycle Monitor (Fine Science Tools Inc., Foster City, CA) with mouse probe. This method is used because the electrical impedance of the vaginal mucosa is considerably higher during the pro-estrous stage compared to that in the other stages of the estrous cycle (25).

Body Composition Analyses in Mice

Percent body fat and bone mineral density (BMD) was determined by dual energy x-ray absorptiometry using a GE Lunar Piximus II densitometer (Madison, WI) as previously described (26).

Calculation of Percent Body Fat in Premenopausal and Postmenopausal Women

To determine if the body size phenotypes in mice considered lean, overweight, and obese in this study were comparable to adiposity in women also considered lean (BMI less than 25), overweight (BMI between 25 and 30), and obese (BMI greater than 30), we compared percent body fat values from the mice with BMI and percent body fat data from a subset of women aged 30−69 yr in the National Health and Nutrition Examination Survey (NHANES) III study (conducted by the U.S. Department of Health and Human Services in 1996). For this data set, body fat was determined by bioelectrical impedance analysis, and percent body fat was calculated using the formula used by Chumlea et al. (27). Women were considered postmenopausal if they were at least 55 yr old or if they had a uterus and at least 1 ovary, were not breast-feeding, and reported not having had a menstrual period during the past yr (28).

Wnt-1 Transgenic Mouse Model of Breast Tumor Growth

The mammary tumor cells used in these studies were obtained directly from mouse mammary tumor virus (MMTV)-Wnt-1 transgenic mice on a C57BL/NCr background. The genetic defect of MMTV-Wnt-1 transgenic tumors has been extensively described (29−32). Like the majority of human breast tumors in postmenopausal women, the MMTV-Wnt-1 mouse tumors are estrogen-receptor-positive (30), and their growth is inhibited by ovariectomy and tamoxifen (32). The fact that MMTV-Wnt-1 tumors are positive for the estrogen receptor and responsive to endogenous estrogens is relevant because 70% of human breast cancers are positive for the estrogen receptor (33), and there is evidence indicating that obesity may only affect estrogen-positive breast cancers (34). For isolation and transplantation of tumor cells from MMTV-Wnt-1 transgenic female mice, we used a procedure developed for harvesting and transplanting tumors from genetically engineered mice (35). Briefly, mice were euthanized with CO2, and tumors were collected aseptically from donor mice using blunt dissection, trimmed of extraneous tissues, mechanically dissociated by mincing and passage through a 40-micron mesh sterile screen, and suspended in serum-free RPMI 1640 (Quality Biological, Gaithersburg, MD). Cells were further dissociated by serial passage through a syringe with 18−25-gauge needles. The cell suspension was washed twice and resuspended in serum-free RPMI 1640 medium, and viable cell counts were determined by hemocytometer counting following 0.4% trypan blue staining. Cells were resuspended at 2 × 106 cells per ml in 10% dimethyl sulfoxide cell freezing medium and cryopreserved using stepped rate freezing. For subcutaneous implantation, 1 × 105 cells were implanted subcutaneously in 100 µL serum-free RPMI 1640 medium into the right thoracic region. Once tumors became palpable, tumor volume was determined twice weekly with calipers by measuring length, width, and depth of the tumor.

Measurement of Hormones and Adipokines

Serum insulin, leptin, adiponectin, resistin, and tissue plasminogen activator inhibitor (t-PAI) concentrations were determined in 10 µl serum samples from each mouse using a Luminex-based bead array assay kit on a LINCOp lex simultaneously multianalyte detection system (Linco Research, Inc., St. Charles, MO) according to the manufacturer’s instructions. Total serum IGF-1 concentration was measured with a commercially available radioimmunoassay kit (Diagnostic Systems Laboratories, Inc., Webster, TX).

Statistical Analyses

One-way analysis of variance (ANOVA) was used to assess the effects of diet within OVX and NOVX groups. We report the results of ANOVA as well as a posteriori comparison of the means using Tukey’s honestly significant difference procedure (JMP version 5.0; SAS Institute Inc., Cary, NC). Significant effects are reported at a P value of ≤ 0.05.

RESULTS

Ovariectomy to Model the Postmenopausal Phase

Hallmarks of the postmenopausal phase include the discontinuance of estrogen production by the ovaries, the cessation of estrous cycles, and subsequently, the loss of BMD (36,37). To ascertain that ovariectomy was successful in our experiments, the estrous cycle was measured at baseline and at 10 and 20 wk for 12 consecutive days at each time point. The estrous cycle for the NOVX mice was ≈4 days long; however, as expected, no estrous cycle was detected in the ovariectomized mice.

We determined the effects of ovariectomy on BMD. OVX mice had significantly lower BMD than NOVX mice. BMD was 0.051 ± 0.001 in OVX mice and 0.059 ± 0.002 g/cm2 in NOVX mice (±SE; P < 0.05). Thus, we present an experimental mouse model in which the effects of obesity on mammary cancer can be studied in the presence or absence of ovarian hormones, thereby mimicking premenopausal and postmenopausal phases in that ovariectomy discontinues estrous cycles and leads to a decrease in BMD.
OBESITY ACCELERATES MOUSE MAMMARY TUMOR GROWTH

FIG. 1. Induction of lean, overweight, and obese body weight phenotypes. Nonovariectomized (NOVX) or ovariectomized (OVX) female C57BL/6 mice were fed a wide spectrum of calories for 10 wk to induce various body weights. Mice were 6 wk old when they were randomized to the various dietary treatments (12 mice/group). A: This panel shows the body weights for the NOVX mice. B: This panel shows the body weights for the OVX mice. Body weights were recorded weekly. There was a significant diet effect on body weight phenotype, with the CR mice (lean) having the lowest body weights and those consuming the high calorie diets, the highest body weights ($P < 0.05$). The OVX groups, compared with the NOVX groups, gained weight more quickly and achieved a higher final body weight, irrespective of diet regimen ($P < 0.05$). This effect was most apparent in the obese OVX mice. Body weights for the Sham-OVX group were similar to those of the normal NOVX group throughout the study (data not shown).

### Table 1: Body Adiposity in Mice and in Premenopausal and Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6NCr Mice</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOVX</td>
<td>OVX</td>
</tr>
<tr>
<td>Lean</td>
<td>26 ± 6</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Overweight</td>
<td>29 ± 4</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>Obese</td>
<td>46 ± 9</td>
<td>47 ± 2</td>
</tr>
</tbody>
</table>

*Abbreviations are as follows: NOVX, nonovariectomized; OVX, ovariectomized; BMI, body mass index. Comparison of percent body fat in lean, overweight, and obese NOVX and OVX female C57BL/6 mice fed a wide spectrum of calories for 10 wk and premenopausal and postmenopausal women. Body fat was measured by dual energy x-ray absorptiometry in mice and by bioelectrical impedance analysis in premenopausal and postmenopausal women. Percent body fat among the lean, overweight, and obese body weight phenotypes was significantly different in both mice and women ($P < 0.05$). Values represent the mean ± SD.*
TABLE 2
Serum Insulin and IGF-1 Levels\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Nonovariectomized</th>
<th>Ovariectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lean</td>
<td>Overweight</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.22 ± 0.04</td>
<td>0.63 ± 0.05*</td>
</tr>
<tr>
<td>IGF-1</td>
<td>355 ± 36</td>
<td>456 ± 87</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>8.3 ± 0.8</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>Resistin</td>
<td>5.4 ± 0.9</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>t-PAI</td>
<td>2.1 ± 0.2</td>
<td>4 ± 0.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Abbreviations are as follows: IGF-1, insulin-like growth factor-1; t-PAI, tissue plasminogen activator inhibitor. Serum insulin was measured using a Luminex-based bead method. IGF-1 was measured using a radioimmunoassay kit. Serum adiponectin, resistin and t-PAI concentrations were determined using a Luminex-based bead array assay kit on a LINCOplex simultaneously multianalyte detection system. Mice were on the study for 30 wk (12 mice/group). \*significantly different than lean mice ($P \leq 0.05$); **significantly different than overweight mice ($P \leq 0.05$).

In Table 2. The circulating levels of these hormones were proportional to body weight ($P < 0.05$), with obese mice having the highest levels of insulin in both the NOVX and OVX groups. In addition, obese OVX mice had higher insulin levels than the other groups ($P < 0.05$). Serum leptin levels of the lean, overweight, and obese phenotypes increased as body weight increased as shown in Figs. 2A and 2B. The stepwise increase in leptin levels (i.e., from lean to obese) was statistically different ($P < 0.05$), with the increase in leptin levels being more dramatic in the OVX groups. With respect to serum IGF-1 levels, they were lowest in the lean and highest in the obese mice ($P < 0.05$). The difference between IGF-1 serum levels in lean and obese mice was also statistically different ($P < 0.05$).

No associations were observed between body weight, ovarian status, and serum levels of adiponectin or resistin; however, serum t-PAI correlated with body weight (Table 2). Lean mice had the lowest t-PAI, and obese mice the highest t-PAI serum levels.

**Effect of Body Fat and Ovarian Hormones on Growth of Mammary Tumors**

To mimic the observed effects of obesity on human breast cancer in the presence and absence of ovarian estrogens, lean, overweight, and obese mice with or without their ovaries were

FIG. 3. Obesity and tumor growth. A: Tumor volume in nonovariectomized female mice. Tumor growth rate was significantly higher in overweight and in obese mice than in lean mice; however, there were not statistical differences in tumor growth rate between overweight and obese mice. B: Tumor volume in ovariectomized female mice. Tumor growth rate was significantly higher in overweight than in lean mice and higher in obese than in overweight mice ($P < 0.05$). Mice were on the various dietary regimens for 25 wk and then injected subcutaneously into the right thoracic region with $1 \times 10^5$ mammary cancer cells obtained from spontaneous primary mammary tumors mouse mammary tumor virus Wnt-1 transgenic mice. Tumor volume was measured twice weekly using calipers. All groups had 9 mice per group except the lean nonovariectomized group, which had 7 mice.
inoculated subcutaneously with MMTV-Wnt-1 mammary tumor cells. As shown in Fig. 3, ovariectomy delayed Wnt-1 tumor growth, supporting previous observations that these tumors are responsive to endogenous estrogens. For example, final Wnt-1 tumor volume was 1,135 ± 443 in NOVX lean mice and 195 ± 34 SE mm³ in O VX lean mice. The fact that ovariectomy inhibited Wnt-1 tumor growth is important because epidemiological evidence suggests that obesity specifically impacts estrogen-responsive breast cancers (34). As in epidemiological observations, obesity did not promote Wnt-1 tumor growth in the presence of estrogen (“premenopausal” state; Fig. 3). Final Wnt-1 tumor volume in the overweight (1,768 ± 443 mm³) and obese (2,657 ± 494 mm³) NOVX mice was similar (P > 0.05). However, in the absence of the ovaries, obesity promoted Wnt-1 tumor growth. Figure 3 shows the effect of the lean, overweight, and obese body size phenotypes on Wnt-1 tumor growth. There were clear weight-dependent effects on tumor growth in the OVX mice, with lean mice having the slowest tumor growth rate, the overweight mice showing an intermediate response, and the obese mice displaying the fastest tumor growth rate (P < 0.05).

DISCUSSION

We have demonstrated that obesity and estrogen status have a direct impact on breast cancer progression in a mouse model. For this purpose, female mice were OVX to model the female postmenopausal phase. Our data show that ovariectomy indeed mimics the postmenopausal phase in that following ovariectomy, female mice cease to have estrous cycles and have a decrease in BMD. Moreover, as in postmenopausal women, the loss of ovarian estrogens increases susceptibility to gaining weight (38). To mimic the body weight phenotypes found in premenopausal and postmenopausal women (i.e., lean, overweight, and obese), we compared percent body fat in NOVX and OVX female mice that were made lean, overweight, and obese with those of premenopausal and postmenopausal women considered lean (BMI < 25), overweight (BMI between 25–30), and obese (BMI > 30). The data show that percent body fat in female mice considered lean, overweight, and obese was, for the most part, similar to that found in premenopausal and postmenopausal women.

We also observed that body mass and estrogen status determined the rate of tumor growth in a syngeneic mouse MMTV-Wnt-1 mammary cancer model. To determine the effect of body mass on tumor growth rate rather than MMTV-Wnt-1 driven tumor development, we transplanted cells derived from Wnt-1 tumors into naïve syngeneic mice with different body masses. Ovariectomy inhibited growth of MMTV-Wnt-1 mammary tumors, indicating that these tumors respond to the modulation of endogenous estrogen as predicted (32). However, the protective effect of CR on mammary cancer growth was more dramatic in OVX than in NOVX female mice. A dramatic reduction of tumor growth was observed in lean mice, regardless of the presence or absence of ovaries. Because the lean body weight phenotype was induced through CR, our findings are consistent with published reports that CR inhibits tumor growth in animal studies (9–12) and acts as a protective factor for breast cancer in women (19). Thus, this mouse model can be used for further studies on the mechanisms of carcinogenesis and caloric restriction.

Consistent with epidemiologic data (4,5), obesity promoted tumor growth in the absence of ovarian estrogens relative to lean and overweight mice. However, although tumor growth in obese NOVX mice was greater than in lean NOVX mice, there were no apparent differences between the overweight and obese NOVX mice groups. Some epidemiologic studies have suggested that obesity may actually decrease breast cancer risk in premenopausal women (5). This phenomenon was not observed in our studies, although 1 of the limitations of using a transplanted tumor model is that it can only assess differences in tumor growth and not in the early stages of mammary cancer development (e.g., initiation, promotion). Nonetheless, results presented herein are consistent with the well-established epidemiologic data showing that obesity promotes tumor growth in the absence of ovarian hormones.

In these studies, obese mice had higher levels of insulin, IGF-1, and leptin. Evidence from in vitro, animal, and epidemiologic studies supports the notion that IGF-1 can promote cancer development (5,13,39–42). Additionally, leptin promotes proliferation of breast cancer cells (15), and obese individuals have higher circulating levels of leptin (43). Obesity is also associated with chronic hyperinsulinemia, which can lead to Type 2 diabetes, and both are risk factors for breast cancer (14,44,45). Even though there is evidence suggesting that leptin, insulin, and IGF-1 may affect cancer risk, it remains to be determined if their physiological effects differ in the presence or absence of ovarian hormones. Previously, we showed that ovariectomy increases the susceptibility of becoming obese and insulin resistant; in addition, the obesity and insulin resistance associated with ovariectomy was linked with enhanced tumor growth (24). Furthermore, we showed that fatless female mice have higher cancer susceptibility than wild-type mice (46). Similar to obese mice, the A-Zip/F-1 fatless mice are insulin resistant and display elevated levels of factors frequently associated with obesity including insulin, IGF-1, and some pro-inflammatory cytokines as well as the activation of several signaling pathways associated with carcinogenesis (46,47). This suggests that adipose tissue and adipokines are not required for tumor development. Consequently, we postulate that obesity may increase cancer risk through insulin resistance and inflammation rather than through adipose-derived factors.

In brief, we have shown that the lean body weight phenotype, regardless of hormonal status, inhibited mammary tumor growth. In contrast, obesity in the absence of estrogen promotes tumor growth. The contrasting effects of these body weight phenotypes in NOVX and OVX mice provide experimental animal models to identify the biological pathways and gene products to address the mechanisms by which obesity alters mammary
cancer growth in the absence of ovarian hormones. This is important for breast cancer prevention because overweight and obese status in the United States is estimated to contribute to 20–50% of cancer deaths in women (4,6). Even though it is known that overfeeding (which leads to a gain in body fat) and CR (which leads to loss of body fat) modulate mammary carcinogenesis, few studies have focused on the role that body adiposity plays in the carcinogenic processes in both the presence and absence of ovarian estrogens. A better understanding of the biology by which obesity increases cancer risk by using the animal models presented here should accelerate the development of new breast cancer prevention and treatment strategies.

ACKNOWLEDGMENTS

Animal care was provided in accordance with the procedures outlined in the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 86–23, 1985). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This work was supported in part with federal funds from the intramural program for Cancer Research program and the National Cancer Institute contract N01-CO-12400 to SAIC-Frederick Inc. We would like to acknowledge the expertise and help of Lisa Riffle, Craig Driver, Keith Rogers, and Darlene Green. Grant support is from the Breast Cancer Research Foundation (Stephen D. Hursting) and the Department of Defense (Breast Cancer Research Program Concept Award to Stephen D. Hursting). The publisher or recipient acknowledges the right of the U.S. Government to retain an exclusive, royalty-free license in and to any copyright covering this article.

REFERENCES


