

Black Cohosh Increases Metastatic Mammary Cancer in Transgenic Mice Expressing *c-erbB2*

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Abstract

Black cohosh is an herbal extract that is often used as an alternative to estrogen-based replacement therapies to treat hot flushes that frequently accompany the transition to menopause. Although cancer-free women as well as breast cancer patients and survivors use black cohosh to relieve vasomotor symptoms, there is limited information on its potential to influence breast cancer development or progression. Therefore, in this study, the effects of black cohosh on mammary tumorigenesis were investigated in the MMTV-*neu* mouse model due to its similarities to HER2⁺ breast cancer, including stochastic development of mammary tumors, which frequently progress to metastatic disease. Using an adjusted dose for the mice to correlate to the recommended dose in women (40 mg/d), no differences were detected in the incidence or onset of mammary tumors in black cohosh-treated versus control females. The lack of effect on mammary tumor development suggests that black cohosh would not influence breast cancer risk if given to women before tumor formation. In contrast, black cohosh significantly increased the incidence of lung metastases in tumor-bearing animals compared with mice fed the isoflavone-free control diet. Additional studies will be needed to correlate these findings to women taking different black cohosh products at various times during breast cancer development; however, these results suggest caution for women using black cohosh, especially for extended periods of time. As metastatic progression is linked to patient survival, these data stress the importance of investigating how women's therapies influence all stages of mammary tumorigenesis, particularly for assessing their safety. [Cancer Res 2008;68(20):8377–83]

Introduction

Black cohosh (*Actaea racemosa* L., syn. *Cimicifuga racemosa* [L.] Nutt.) is an herbal preparation used to treat gynecologic disorders and menopausal symptoms. The black cohosh extract (BCE) is prepared from the root and rhizomes from this member of the buttercup family using ethanol, methanol, or 2-propanol (isopropanol). BCE contains a mixture of chemicals that shows considerable qualitative and quantitative variation between commercial products, extracts obtained from the different

alcohols, and related species (1, 2). Many components have been identified in BCE, but no active agent(s) for reducing vasomotor symptoms have yet been identified.

Not all reports indicate that black cohosh is effective for reducing climacteric symptoms, but most randomized, controlled clinical trials show some efficacy for menopausal symptom relief (3–7). Unlike traditional hormone replacement therapies (HRT), most reports indicate that BCE does not possess estrogenic activity in mammary or uterine tissues in rodents and women (7–12) or bind to either estrogen receptor (ER α and ER β ; refs. 13, 14). Other central activities have been suggested to account for its ability to relieve vasomotor symptoms and to improve mood, including as serotonin, dopamine, and μ opiod receptor partial agonists (15–17). After the Women's Health Initiative (WHI) study associated HRT use with increased breast cancer risk (18), BCE has been recommended as a replacement for HRT for symptomatic, but breast cancer-free, perimenopausal women (19). However, despite scant knowledge about its effects on breast carcinogenesis, BCE is also used by breast cancer patients and may ameliorate hot flushes due to age, tamoxifen, or other cancer therapies as an alternative for the contraindicated estrogens in HRT (5, 6, 20).

Owing to the profound influence menopausal symptoms have on the quality of life in some aging women, safe alternative therapies are needed when estrogens are contraindicated or undesirable, especially because the ages surrounding menopause correspond with a time of increasing breast cancer risk (21, 22). Use of HRT diminished after the WHI report identified increased risk for breast cancer and cardiovascular disease (23), resulting in some women switching to natural therapies, such as black cohosh, for relief from vasomotor symptoms. A study published in 2006, after the WHI report (18), ranked black cohosh eighth among herbal supplements sold in retail outlets in the United States (24). Although the public perception may be that natural therapies are safer than pharmaceutical agents, in truth, generally less is known about their potential risks. Most studies evaluating the safety of BCE have used cultured human breast cancer cell lines to determine its influence on malignant cells. Several studies have shown that BCE inhibits proliferation of both estrogen-dependent and estrogen-independent human breast cancer cell lines (25–28); however, *in vivo* animal studies that assess its influence on mammary tumorigenesis are limited (11). In addition, little is known about its potential influence on tumor metastasis. Therefore, this preclinical study was designed to determine whether BCE influences mammary tumor development and progression.

In this study, BCE effects on mammary carcinogenesis were investigated using female MMTV-*neu* mice. Although no animal models exactly replicate human breast cancer, their use is advantageous for controlling variables that are prevalent in human

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studies to allow direct cause-effect correlations. The MMTV-*neu* model expresses the unactivated rat *neu* (*c-erbB2*) gene and mimics many features of HER2⁺ breast cancer, including stochastic, multistep carcinogenesis; overexpression of *neu*, the rodent homologue of HER2 (29); tumor pathology (30); and estrogen dependence for tumor development (31).⁷ An important aspect of this mouse model is the propensity to progress to metastatic disease (29), which requires all steps from intravasation through extravasation, as with human breast cancer metastasis.

Materials and Methods

Tumor study. All animal work was approved by the Cedars-Sinai Institutional Animal Care and Use Committee in accordance with NIH guidelines. Hemizygous female MMTV-*neu* mice harboring the *neu* proto-oncogene [FVB/N-Tg(MMTVneu)202Mul/J; ref. 29] were used for the tumor and pretumor studies. MMTV-*neu* and FVB/N breeders (The Jackson Laboratory) and progeny were maintained on a semipurified isoflavone-free diet to prevent exposure to these phytoestrogens during all developmental stages of the study mice. The control diet is a modification of AIN-93G using corn oil with 20% protein, 16% fat, 64% carbohydrates, and 3,713 kcal/kg (Harlan-Teklad). For analysis of tumor outcomes, 300 hemizygous MMTV-*neu* female mice were randomized at weaning into the two treatment groups and maintained on the control diet. At 2 mo of age, the 150 mice in the black cohosh treatment group were transferred to the BCE diet (control diet containing the isopropanolic BCE equivalent to 40 mg of root and rhizome in 1,800 kcal of diet, with ~0.3 mg consumed per day for a mouse consuming 4 g of diet/d). This dose is based on the recommended 40 mg/d for women and an average woman's diet of 1,800 kcal (Calories). As black cohosh is generally used by premenopausal and perimenopausal women, who are still cycling and producing ovarian hormones to treat gynecologic disorders and vasomotor symptoms, intact female mice were used for this study. Due to the stochastic nature of tumorigenesis in this model, long-term BCE treatment was required to ensure exposure during all stages of tumor development and progression. Tumor onset was determined by weekly palpations of the mammary glands starting at 4 mo of age until the maximum age of 16 mo. Tumor-free mice were euthanized at the maximum age. Mice with mammary tumors over 2 cm in diameter, multiple large tumors, tumors affecting mobility, or tumor ulceration or animals showing other symptoms of ill health were euthanized before the maximum age.

Tumor volume and histopathology. Postmortem, mammary tumors were measured on three dimensions after resection, and tumor volume was calculated using the formula for an ellipsoid, $\text{cm}^3 = 4/3 \pi (a/2 \times b/2 \times c/2)$. Excised lungs were examined for grossly detectable lesions. Four percent paraformaldehyde-fixed mammary tumors and lungs were embedded in paraffin, cut into 6- μm sections, stained with H&E. Sectioning and staining were performed by Mass Histology Service. The sections were examined by a board-certified veterinary pathologist (M.J.J.). As the majority of metastatic lesions in this mouse model are detected by histopathologic analysis, both micrometastases and lung lesions detected by gross inspection that were confirmed by the pathologist to be metastatic lesions were assessed for determining metastatic incidence.

Pretumor study. An additional 25 hemizygous MMTV-*neu* mice per group were randomized and treated as above for the tumor study. The mice were tested daily for stage of estrus using morning vaginal smears starting at 2 months of age, at the time of the diet change for the BCE group. Vaginal smears were stained with Dif-stain kit (IMEB, Inc.) for assessment. The mouse was euthanized on the first day in estrus after the end of the 30-day monitoring period and stage of cycle confirmed again at necropsy. Serum E₂ and P₄ were determined with the Double Antibody Estradiol and Coat-a-Count Progesterone kits (Diagnostic Products Corporation, Siemens); only mice in estrus at necropsy with sufficient volume of serum for assaying levels of both hormones were examined.

⁷ Unpublished data.

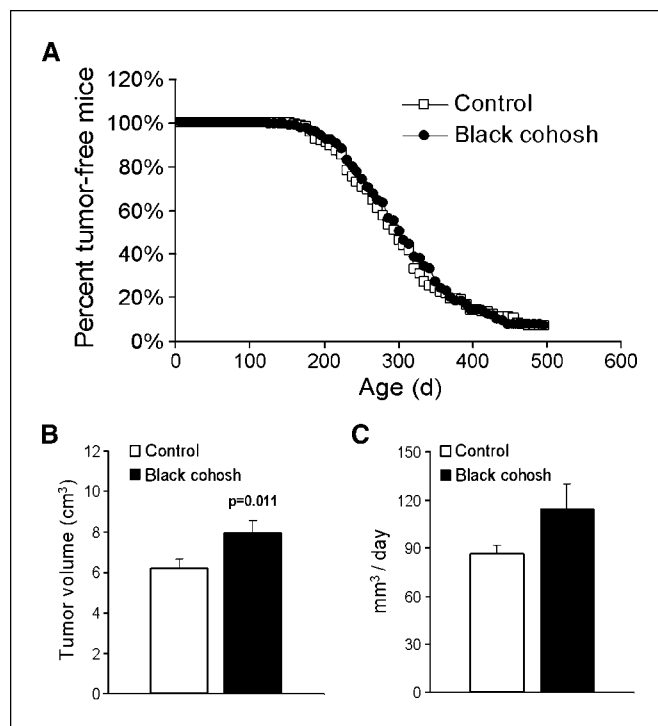


Figure 1. Development of primary mammary tumors is not modified by black cohosh. Mammary tumor outcomes were assessed in MMTV-*neu* mice fed the control diet from conception to death (*Control*) or until 2 mo of age followed by the BCE diet (40 mg/1,800 kcal) until death (*Black cohosh*). **A**, survival curves for the percentage of animals that are tumor-free at the designated ages is depicted for the control and black cohosh groups up to the maximum age of 16 mo. **B**, the tumor volumes for the total tumor burden for each mouse were significantly higher in black cohosh-treated ($n = 95$) versus control animals ($n = 107$). The median tumor volume values for the total tumor burden were 4.7 versus 6.5 cm^3 for the control versus BCE groups. **C**, adjusting for the length of time that each mouse had the tumor after detection (mm^3/d) resulted in no significant differences between the treatment groups for total tumor burden with median values of 74.7 versus 91.6 mm^3/d for the control versus BCE-treated groups. **Columns (B and C)**, mean; **bars**, SE; **P values**, compared with the control group, were determined by the Mann-Whitney test.

***neu* RNA levels.** RNA from mammary tissue was prepared using the Absolutely RNA miniprep kit (Stratagene), reverse transcribed with oligo dT using the Reverse-iT kit (ABgene). Reverse transcription reactions were analyzed by real-time reverse transcription-PCR (RT-PCR) in the iCycler (Bio-Rad) using iQ SYBR Green Supermix (Bio-Rad) with the primer sequences below for 50 cycles at 95°C for 30 s, 60°C for 60 s. The primer sequences are as follows: rat *neu* forward primer TGGATGTACCTG-TATGAGACG, reverse primer GGATTCAAGCAGCAAGGAAAAG; mouse *neu* forward primer CATTACCTACCACCCTCT, reverse primer GCTTCCTTAGCTCTGTCTCC; and cyclophilin A (*ppia*) forward primer AGGTGAAAGAAGGCATGAAC, reverse primer ACAGTCGGAATGGT-GATCT. *neu* gene expression was normalized to *ppia* expression from the same reverse transcription reaction.

Statistical analyses. For hypothesis testing between the groups, χ^2 was used for categorical variables (Fisher's exact test was used when the frequencies were small), *t* test was used to assess the difference in the means when distributions were approximately symmetric, whereas the Mann-Whitney test was used to compare medians when the distributions were skewed. Analyses were performed using GraphPad Prism 5.0 software.

Results

Mammary tumor development was assessed in adult MMTV-*neu* mice treated with an isopropanolic BCE starting at 2 months of age

versus animals maintained on the control, isoflavone-free diet since conception. The dose tested is based on the recommended 40 mg daily dose for women, but adjusted to account for the different metabolic rate and size of the mice. Mammary tumor incidence at the maximum age of 16 months was similar in the two treatment groups (123 of 131 mice, 93.9%, control and 118 of 127 mice, 92.9%, BCE). Tumor latency also showed little variation between the groups, 290.2 ± 6.5 days for control versus 294.3 ± 6.4 days for BCE-treated mice. Comparing primary mammary tumor onset with age showed that mammary tumors in the BCE-treated *neu* mice developed at nearly an identical rate to mice maintained on the control diet (Fig. 1A). Mammary tumor multiplicity was also equivalent with 1.88 ± 0.09 versus 1.86 ± 0.08 mammary tumors per tumor-bearing mouse for the control versus BCE-treated animals. Collectively, these data indicate that mammary tumor development was not influenced by the herbal therapy and are in agreement with a previous study evaluating BCE effects on chemically induced mammary tumors (11).

Total tumor volume at death for the entire tumor burden was significantly greater with BCE treatment than for the control group (Fig. 1B; $P = 0.011$). Although, on average, the mammary tumors in mice treated with the BCE are larger in volume than in the control group, many tumors in both groups were cystic, resulting in large variations in the amount of tumor tissue versus fluid. As tumor volume does not account for the fluid to tumor mass variations, these data do not necessarily suggest that BCE-induced tumors to grow at a faster rate and could alternatively suggest that BCE results in tumors that produce more cystic fluid. In addition, timing for euthanizing the mice was scheduled based on many factors besides size of the first tumor detected, such as tumor location affecting mobility, number of tumors, tumor ulceration, and general health; therefore, the length of time tumors had to grow before euthanasia varied. Total tumor volume per day was determined to adjust for length of time between tumor detection and death. Although the number of days with tumor was not significantly different between the treatment groups (data not shown), statistical significance did not remain when accounting for time with tumor, although the values remained higher in the BCE group (Fig. 1C).

Due to the propensity of metastatic progression in the MMTV-*neu* model, the incidence of lung metastases was also determined for mammary tumor-bearing mice fed the control versus BCE diet.

Examples of metastatic lesions before and after extravasation are shown in Fig. 2. Mice treated with BCE since age 2 months had a higher incidence of metastatic lesions that were grossly visible at necropsy and later confirmed by histopathology (Fig. 3A; $P = 0.0019$). Additionally, more BCE-treated mice had multiple, visible lung lesions compared with the control mice, suggesting further disease progression with BCE, although statistical significance was not reached (Fig. 3B). The incidence of metastatic lung lesions that were identified or confirmed by histopathology was also increased in mice treated with BCE (Fig. 3A; $P = 0.0004$). These data show that exposure to BCE during mammary tumor development resulted in a higher risk of tumors progressing to this potentially deadly form of cancer.

The time from tumor detection to death was assessed for all mice with metastatic lung lesions to verify that the increased incidence was not due to the tumors having more time to progress to metastatic disease. No differences were evident for mice with lung metastases (Fig. 3C), demonstrating that the increased metastatic incidence was not related to the length of time the tumors were allowed to grow. Grossly detectable lung lesions were observed (and confirmed by histopathology) in mice as early as 28 and 38 days after tumor detection for the control and BCE groups, respectively. In contrast, mice with no metastatic lung lesions had detectable mammary tumors for 142 days for the control and 171 days for the BCE-treated animals. These data suggest that the propensity to develop metastatic cancer is inherent to the specific tumor versus growth time in this mouse model.

Although unexpected due to the lack of effect on primary mammary tumor development, we examined expression of the *neu* transgene in BCE-treated versus control female mice to ensure that the increased incidence of metastatic lesions was not due to up-regulation of the *neu* transgene by BCE. Levels of rat *neu* were examined in normal mammary tissue to evaluate BCE-induced effects on transgene expression because tumor formation in the MMTV-*neu* model results in profound elevations in *neu* expression (29), which could be unrelated to BCE. Therefore, *neu* expression was evaluated in mammary tissue of 3-month-old MMTV-*neu* mice on the control diet or treated with BCE for 1 month. Although the levels of the *neu* transgene were lower in the BCE-treated mice compared with control (0.34-fold), the difference was not significant (Fig. 3D). The expression of the endogenous mouse *neu* gene was also similar in both treatment groups (Fig. 3D),

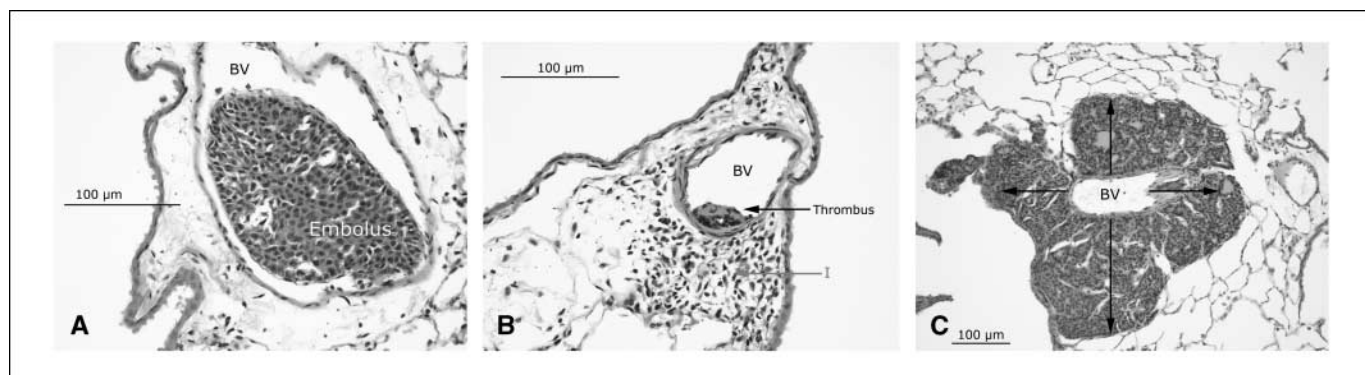


Figure 2. Metastatic lung lesions in the MMTV-*neu* mice originate in the pulmonary arteries as emboli that have or have not invaded into the surrounding lung tissue. BV, blood vessel; I, invasion of tumor cells into lung parenchyma. Metastatic lesions are defined as the presence of (A) an embolus entirely in blood vessel; (B) a thrombus with invasion into adjacent lung tissue; and (C) invasion of tumor into lung tissue surrounding blood vessel, without a thrombus or embolus. Eighty-one percent of female mice with metastatic cancer had foci with evidence of extravasation (invasion into the lung parenchyma) for both treatment groups.

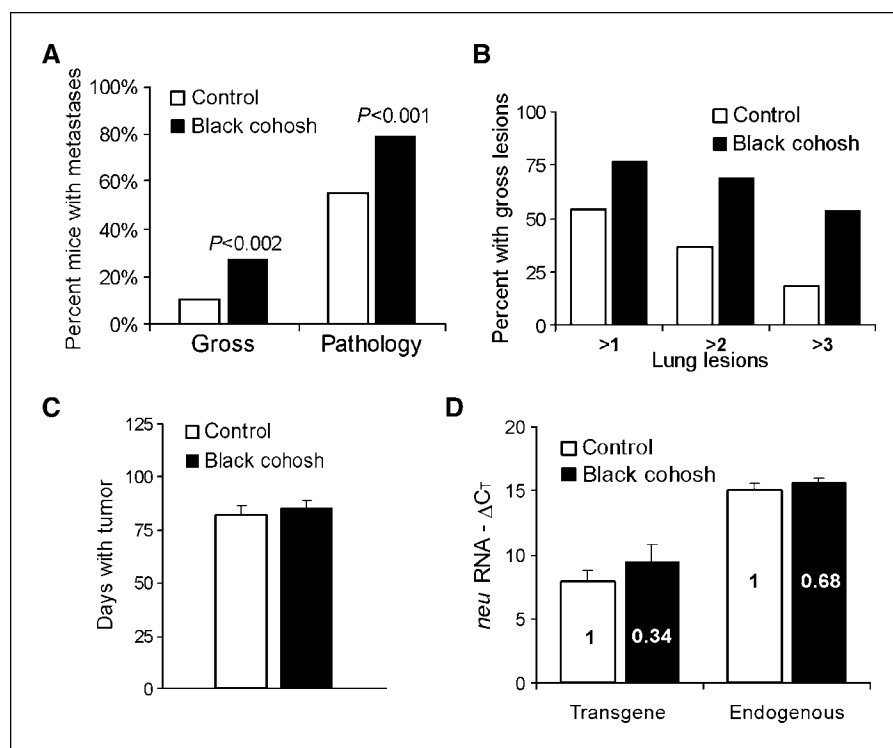


Figure 3. Metastatic incidence is increased in black cohosh-treated mice. *A*, for all female mice with mammary tumors (with lungs examined by histopathology), the incidence of grossly detected metastatic lung lesions (*Gross*) discovered at necropsy and the total percentage of mice with lung metastases detected by histopathology (*Pathology*), including gross lesions plus micrometastases, is also shown. $n = 107$ for control and $n = 95$ for black cohosh-treated mice; P values versus the respective control group were determined by the Fisher's exact test. *B*, for mice with grossly detectable lung lesions (confirmed as metastases by histopathology), the percentage that have multiple lung lesions, with >1 , >2 , and >3 lung lesions per mouse ($P = 0.244$, 0.080 , and 0.071 , respectively; Fisher's exact test), are shown for both treatment groups, $n = 11$ for control and $n = 26$ for black cohosh. P values were unadjusted for the multiple comparisons. *C*, the number of days from tumor detection to death for animals with metastatic lesions (grossly detected lesions and micrometastases detected by histopathology) are shown for control ($n = 59$) and black cohosh-treated mice ($n = 75$). Columns, mean; bars, SE; analyzed by the t test. *D*, levels of rat *neu* transgene and endogenous mouse *neu* transcripts were measured by real-time RT-PCR in normal mammary tissue from 3-mo-old mice, before mammary tumors were detected in either group; $n = 9$, control and $n = 10$, black cohosh. Threshold values (C_T) of *neu* were normalized to the housekeeping gene, *ppia* (cyclophilin A) to determine ΔC_T ; higher ΔC_T values indicate a lower abundance message. The fold change versus the control group calculated by the $2^{-\Delta\Delta C_T}$ method is indicated within each column. Median ΔC_T values were 9.0 versus 8.7 for the *neu* transgene in the control versus BCE groups. The transgene was expressed at higher levels than the endogenous *neu* gene in mammary tissue from the control (142-fold) and BCE-treated mice (70-fold). The median ΔC_T values for the endogenous mouse *neu* gene were 15.3 versus 15.1 for the control versus BCE groups, with $n = 9$ for each group. Columns, mean; bars, SE; P values for the ΔC_T values were determined by the Mann-Whitney test.

suggesting that black cohosh would not increase HER2 expression in a woman's breast before cancer development. As *neu* levels were not up-regulated by BCE, the increased rate of metastatic cancer was not related to nonnatural influences by the herbal therapy on transgene expression in this mouse model. Thus, the increased metastatic incidence may reflect the potential of BCE to induce similar influences on human breast cancer.

To verify that BCE does not modify estrogen-responsive endpoints, several reproductive outcomes were also examined in 3-month-old MMTV-*neu* mice before tumor development. During estrus, the circulating levels of 17β -estradiol and progesterone were comparable for both treatment groups, suggesting that ovarian hormone production and/or their hypothalamic/pituitary regulators were not influenced by the herbal extract (Fig. 4). In addition, estrous cycling was not modified by BCE treatment because the number of days in estrus (Fig. 5A) and mean length of cycle (Fig. 5B) within the 30-continuous-day period were similar for both groups. The mean number of complete cycles (estrus-estrus) was also similar for the control (3.0 ± 0.3) and BCE-treated mice (2.9 ± 0.2). No uterotrophic activity was evident for BCE as uterine wet weight (data not shown) and uterine weight per body weight (Fig. 5C) were similar for both groups

of young mice. In addition, uterine weight/body weight was also comparable with the control group after long-term treatment with BCE for mice in the tumor study that were euthanized with tumors or at maximum age (Fig. 5C). Based on these endpoints, there was no evidence that the tested dose of BCE modifies the hormonal environment in the mice. Additionally, body weight was unaffected after 1 month on the BCE diet for the 3-month-old animals in the pretumor study or after long-term treatment for the aged tumor study mice (Fig. 5D), suggesting equal consumption of diet by both the BCE-treated and control groups. Thus, the resulting influences of BCE on the tumor outcomes could not be due to differences in caloric intake.

Discussion

In this large, preclinical study, an additional 1 in 4 (24%) female mice with mammary cancer developed metastatic disease after treatment with BCE. This increased metastatic incidence after long-term treatment with BCE occurred without corresponding effects on primary tumor development or tumor growth. These results indicate the potential for agents to influence restricted

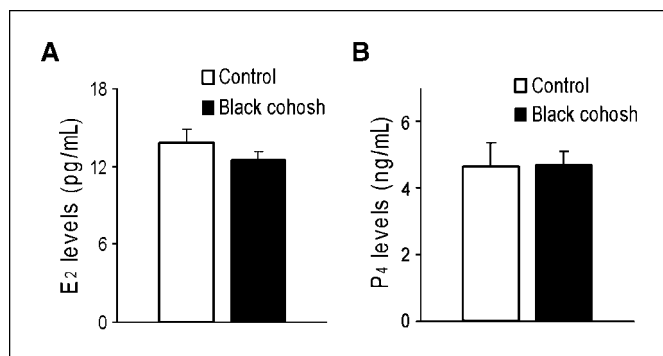


Figure 4. Circulating ovarian hormone levels are unaffected by black cohosh treatment. A, serum 17 β -estradiol levels (E_2) for control ($n = 14$) and black cohosh-treated females ($n = 15$) were determined for 3-mo-old mice in estrus. B, serum progesterone (P_4) levels for control ($n = 14$) and black cohosh-treated mice ($n = 15$) were measured in the same animals examined for E_2 levels. Columns, mean; bars, SE; analyzed by the t test.

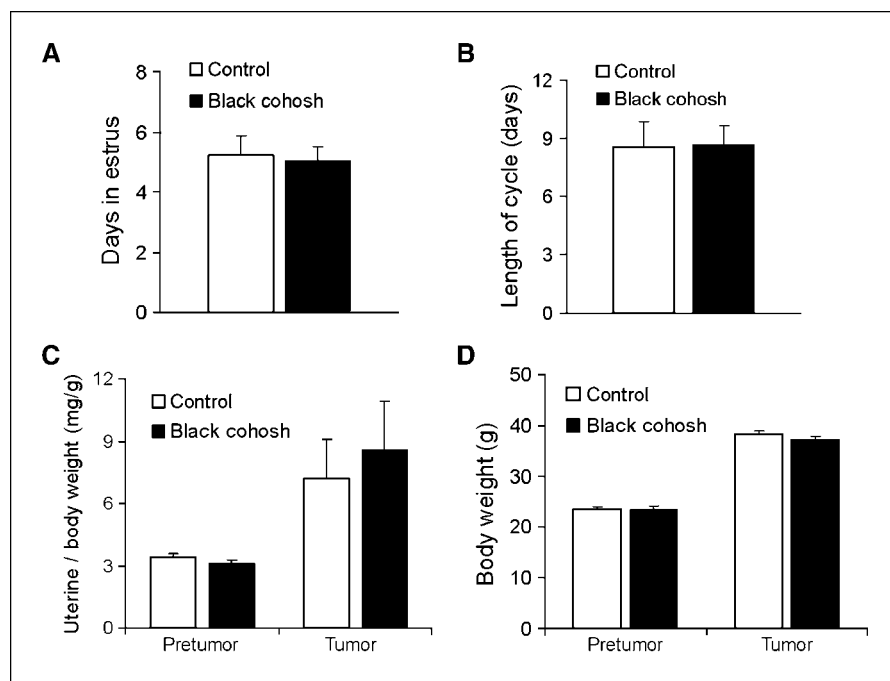
stages of carcinogenesis and suggest that studies which only examine tumor development or growth may not accurately reflect the potential cancer-related risks of a therapy or chemical in question.

Although mammary tumor growth was not increased in the BCE-treated mice, growth was not inhibited as has been reported for both estrogen-dependent and estrogen-independent human breast cancer cell lines grown in culture (25–28). Although the dissimilar effects of BCE on the growth of *neu*-induced tumors versus human cancer cells could be due to differences between the species, it is more likely that other effects are responsible for the lack of growth inhibition in the mice. For example, *in vitro* growth conditions would not mimic the complex interactions of the live animal. Additionally, the doses of BCE with significant growth inhibition in the cell lines are usually high, >50 $\mu\text{g/mL}$ of culture

medium (27, 28), and may not be equivalent to the local or systemic dose in tumors or in other tissues in the mouse that influence tumor growth, such as stromal, immune, or endocrine tissues. Also, the growth response may be due to the specific type of tumor, because the breast cancer cell lines tested showing growth inhibition with BCE do not overexpress HER2/*neu* (25–28) unlike the mammary tumors in the mice. One study reported that individual components (actein) and fractions of BCE inhibited the growth of cultured breast cancer cells that overexpress HER2/*neu* (32). Their contrasting results to the tumors in the MMTV-*neu* mice may be due to potential differences between *in vitro* versus *in vivo* effects and to the separated components having different activities than that observed with the whole extract. Therefore, it cannot be predicted whether subfractions of BCE would elicit the same inhibitory effect in mice or women. Currently, only the complete extract is available as a supplemental therapy for women. Another reason that growth inhibition was not observed in the mice may be due to testing BCE in human cell lines obtained from existing breast tumors, unlike the MMTV-*neu* mice that were exposed to BCE before tumors developed. In the mice, tumors developed in the presence of BCE, which may have led to tumors that would be unresponsive to any potential BCE inhibition, which could also occur in women taking BCE before breast cancer development.

Due to the known benefits of estrogen for relieving menopausal symptoms, several reports have investigated whether BCE has estrogenic properties. Early reports identified estrogen agonist activity in BCE, which was thought to be due to formononetin (33). However, recent investigations do not detect formononetin in multiple BCE products, including the product tested in this mouse study (2, 34, 35), suggesting that this phytoestrogen is not present in the herbal extract. Other reports have associated BCE as a selective estrogen receptor modulator with estrogenic actions in the bone, fat, and hypothalamus/pituitary axis (36, 37). In this study, the comparable uterine weight and estrous cycle end points

Figure 5. Reproductive end points were not altered by black cohosh treatment. A, estrous cycling was evaluated for 30 continuous days starting at 2 mo of age. The number of days in estrus within the 30-d period is shown for control ($n = 25$) and black cohosh-treated mice ($n = 25$). B, average length of each complete estrus cycle within the 30-d period is shown for the treatment groups ($n = 25$). C, uterine weight/body weight (mg/g) for control ($n = 22$) and black cohosh-treated mice ($n = 22$) were determined from uterine wet weights and body weights obtained at necropsy for 3-mo-old females in estrus (designated as pretumor). Mice analyzed for tumor outcomes in diestrus are designated as tumor (average age 12.1 mo for control and 12.6 mo for black cohosh groups). Uterine weight/body weight (mg/g) for control ($n = 87$; 76% of mice in diestrus) and in black cohosh-treated mice ($n = 72$; 69% of females in diestrus). D, pretumor body weights (g) for 3-mo-old control ($n = 22$) and black cohosh-treated animals ($n = 22$) and tumor body weights (g) for control ($n = 115$) and black cohosh-treated mice ($n = 105$). Columns, mean; bars, SE; analyzed by the t test; no significant differences were detected for any of the end points shown ($P > 0.05$). All differences were also confirmed to be nonsignificant using the Mann-Whitney test.



(Fig. 5) suggest that BCE, at the tested dose, does not exert estrogenic effects on the reproductive tract. These findings correlate with results from previous BCE studies in women (8, 9, 38) and in mice and rats (10–12, 37). Furthermore, because the herbal therapy did not result in elevated estradiol levels (Fig. 4) or promote the formation of mammary tumors (Fig. 1), there is no evidence for increased estrogen action in the mammary gland. Similarly, in women, estrogen-responsive markers in the breast (nipple aspirate cytology and pS2 levels) and serum estradiol levels (7), mammographic breast density, and mammary cell proliferation (8) were unaffected by BCE treatment. Collectively, these data suggest that estrogenic mechanisms are unlikely to have a role in the BCE-induced increase in metastatic disease in the MMTV-*neu* mice.

BCE has been reported to have other receptor-mediated activities, including as serotonin, dopamine, and μ opioid receptor partial agonists (15–17), which may contribute to the resulting increased metastatic incidence observed in the MMTV-*neu* mice. Both dopamine and opioids that bind to the μ opioid receptor stimulate migration of breast cancer cells, an integral property of tumor invasion and metastasis (39, 40). In addition, some μ opioid agonists possess immunosuppressive properties involved in anti-tumor surveillance, including inhibition of natural killer cell activity, which is associated with increased metastatic progression (41, 42). Therefore, these reported neurotransmitter-like actions of BCE components may enhance the migration and survival of the mammary tumor cells leading to their colonization in the mouse lungs.

Based on the results in the MMTV-*neu* mice, the lack of effect on primary mammary tumor development suggests that risk of HER2⁺ breast cancer would not be increased or decreased in women taking the recommended dose (40 mg/d) of this black cohosh preparation. Due to the variability in BCE preparations and that the component(s) of BCE influencing metastatic progression are unknown, it is not possible to predict how these results would extrapolate to other black cohosh products. One retrospective case-control study examining multiple alternative therapies reported that BCE reduced breast cancer risk; however, only a small number of women reported taking BCE (4% of the study participants) and BCE use (assessed based on patient memory) included various black cohosh products, could be limited to 1 month, and could include coadministration with other alternative therapies (43). Furthermore, as the data included BCE use until the day of diagnosis, their results would reflect effects on existing tumors versus tumor development. Accordingly, additional studies will be needed to determine if these results conflict with the primary tumor outcomes in the MMTV-*neu* mice.

High doses of BCE were reported to reduce motility of human breast cancer cells (MDA-MB-231) *in vitro* (27); however, this MMTV-*neu* study is the first to examine the influence of BCE on mammary tumor metastasis *in vivo* at a comparable dose to what is recommended in women. Only one epidemiologic study has addressed the potential influence of black cohosh on tumor progression in breast cancer patients; however, the data include all stages of breast cancer, which included existing metastatic cancer and recurrence of primary as well as metastatic breast cancer (44). In addition, although their study reported a reduction in breast cancer recurrence in the BCE group, this group was defined as breast cancer patients receiving prescriptions for BCE instead of its use. As the control group had access to and likely also used BCE,

because it is an over-the-counter medication in Germany (where the study was performed), their findings may reflect other benefits derived from being treated by doctors that prescribe BCE. In addition, most breast cancer patients were also receiving chemotherapy and BCE has been reported to have differential effects on the cytotoxicity of some chemotherapies (45). Accordingly, any differences in recurrence may be related to coadministration of BCE with specific chemotherapies. However, conclusions from this human study should be considered with caution due to the lack of a verifiable control group. Therefore, randomized, controlled clinical studies that assess BCE use in relation to metastatic breast cancer progression would be needed to evaluate the safety of this herbal therapy in women with and without detectable breast tumors.

In contrast to primary mammary cancer, the increased metastatic incidence in the MMTV-*neu* mice suggests that the recommended dose of BCE may also influence the risk of developing metastatic breast cancer in women. As this study did not determine metastatic incidence at multiple time points, the increased number of mice with lung metastases may reflect an accelerated onset versus an increased incidence of metastatic cancer. However, either result would be undesirable for women taking this therapy as both could decrease survival. There is also the potential that this risk extends to other types of cancer with metastatic potential. One preclinical study reported that BCE did not modify the growth or metastasis of endometrial adenocarcinoma xenografts; however, animal numbers were very small, with only 6 rats per group, and treatment began after tumor formation (46). Therefore, additional studies will be required to determine if BCE influences other cancers arising in reproductive and nonreproductive tissues. In contrast, because the MMTV-*neu* mouse model mimics HER2⁺ breast cancer, the BCE-induced increase in metastatic progression may be restricted to HER2-overexpressing cancers.

Although few studies investigate metastatic cancer when assessing the safety of alternative therapies, the BCE-induced increase in this study stresses the importance of investigating metastatic progression, especially due to its direct link to patient survival. However, BCE treatment was continuous throughout most of the adult life of the mice, unlike the 6 months of use recommended for women. Further study will be necessary to determine whether this adverse effect of BCE requires treatment to be initiated before tumor development or may occur within a restricted timeframe after tumor onset, as short-term treatments may not carry the same level of risk. However, these results suggest caution for BCE use in women with and without breast cancer and indicate the need for examining its influence on metastatic breast cancer in clinical as well as additional preclinical studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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