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Cancer Prevention Research

Research Article

Lack of ABCG2 Shortens Latency of BRCA1-Deficient Mammary Tumors and This Is Not Affected by Genistein or Resveratrol

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Abstract

In addition to their role in drug resistance, the ATP-binding cassette (ABC) transporters ABCG2 and ABCB1 have been suggested to protect cells from a broad range of substances that may foster tumorigenesis. Phytoestrogens or their metabolites are substrates of these transporters and the influence of these compounds on breast cancer development is controversial. Estrogen-like properties might accelerate tumorigenesis on the one hand, whereas their proposed health-protective properties might antagonize tumorigenesis on the other. To address this issue, we used a newer generation mouse model of *BRCA1*-mutated breast cancer and examined tumor latency in $K14cre;Brca1^{F/F};p53^{F/F}$, $Abcb1a/b^{-/-};K14cre;Brca1^{F/F};p53^{F/F}$, or $Abcg2^{-/-};K14cre;Brca1^{F/F};p53^{F/F}$ animals, fed with genistein- or resveratrol-supplemented diets. Ovariectomized $K14cre;Brca1^{F/F};p53^{F/F}$ animals were included to evaluate whether any estrogen-mimicking effects can restore mammary tumor development in the absence of endogenous estrogens. Compared with the ABC transporter proficient model, ABCG2-deficient animals showed a reduced median tumor latency of 17.5 days ($P < 0.001$), whereas no significant difference was observed for ABCB1-deficient animals. Neither genistein nor resveratrol altered this latency reduction in $Abcg2^{-/-};K14cre;Brca1^{F/F};p53^{F/F}$ animals. Ovariectomy resulted in nearly complete loss of mammary tumor development, which was not restored by genistein or resveratrol. Our results show that ABCG2 contributes to the protection of genetically instable epithelial cells against carcinogenesis. Diets containing high levels of genistein or resveratrol had no effect on mammary tumorigenesis, whether mice were lacking ABCG2 or not. Because genistein and resveratrol only delayed skin tumor development of ovariectomized animals, we conclude that these phytoestrogens are no effective modulators of mammary tumor development in our mouse model. *Cancer Prev Res; 5(8); 1053–60. ©2012 AACR.*

Introduction

Several ATP-binding cassette (ABC) transporters act as cellular efflux pumps of drugs and cause multidrug resistance (1). In addition to this classical role, ABC transporters might have other functions in tumor biology (2). For instance, loss of ABC transporters has been claimed to decrease, as well as increase carcinogenesis. Mochida and colleagues (3) reported in heterozygous $Apc^{Min/+}$ mice, prone to develop intestinal malignancies, that loss of P-glycoprotein (P-gp/MDR1/ABCB1) decreased tumor formation. They disrupted the *Abcb1a* gene and found decreased intestinal polyp and tumor incidence compared with *Abcb1a* wild-type mice, suggesting that P-gp-mediated protection from xenotoxins allows epithelial cells with strong driver mutations to survive and progress into malignant tumors. This protective effect of P-gp may be specific for mice, as tumor-initiating cells of human colorectal tumors do not appear to be protected by ABCB1 (4). In contrast, Gupta and colleagues (5) found loss of ABCG2 protein and mRNA expression in human colorectal and cervical cancers and they hypothesized that increased exposure to carcinogenic genotoxins in premalignant cells might enhance tumor evolution through stimulated mutagenesis. Fletcher and colleagues (2) also discuss active transport-independent functions of ABC transporters in apoptosis and tumor cell proliferation, but no mechanisms for these functions have been established. Taken together, these studies illustrate that we still do not fully understand what roles ABCB1 and ABCG2 play in protecting normal cells from carcinogenic xenobiotics.

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Some members of the phytoestrogen family are ABCG2 substrates, and it has been suggested that these compounds competitively inhibit ABC transporter-mediated drug transport (6–8). In addition, phytoestrogens have received attention because epidemiological studies attributed the lower incidence of breast cancer in Asian countries to their traditional diets, consisting of phytoestrogen-rich soy food (9). A major phytoestrogen present in soy is the isoflavone genistein. Another phytoestrogen with putative health-promoting effects is the stilbenoid resveratrol, present in red grapes (10). Numerous studies impart preventive effects of resveratrol on cancer development, including breast cancer. To explain the benefit of phytoestrogen consumption various mechanisms have been suggested. These include inhibition of oncogenic signaling pathways, topoisomerases, cyclooxygenases, angiogenesis, proliferation, or endogenous estradiol production; SIRT1 activation; scavenging of oxygen radicals; and antiapoptotic effects (10–12). Consequently, phytoestrogens have become popular as dietary supplements. Given their claimed chemopreventive features, phytoestrogens may, in particular, appear attractive to women with an increased risk of developing breast cancer, such as carriers of *BRCA1* or *BRCA2* mutations.

The polyphenolic phytoestrogens genistein and resveratrol share structural characteristics with 17β-estradiol and, therefore, interact with the mammalian estrogen receptors (ER), although with lower binding affinity than estradiol itself (13, 14). Such estrogenic effects of phytoestrogens could alleviate discomforts of menopausal women and supplementing the diet of these women with phytoestrogens might appear an attractive alternative to estrogen-replacement therapy, which promotes breast tumorigenesis (15). Like estrogen-replacement therapy, however, substantial estrogen-mimicking effects of phytoestrogens might also increase the risk of carcinogenesis, especially in women who have a breast cancer predisposition. Despite several years of research, the disagreement on the positive versus negative effects of phytoestrogen-rich diets on breast cancer development remains (16, 17). This question has been addressed using rodent models of chemically induced or xenografted tumors, but the data reported are ambiguous and did not resolve the controversy (reviewed in refs. 12, 18).

To tackle these issues from a different angle, we investigated the effect of genistein or resveratrol in a newer generation mouse model. *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mice lose the tumor suppressor function of BRCA1 and p53 stochastically in mammary (and skin) epithelial cells early during development. BRCA1 dysfunction results in genomic instability and drives additional mutagenic events that result in spontaneous tumors when the mice are about 7 months old (19). The mammary tumors highly resemble BRCA1-associated breast cancer in humans and, like their human counterparts, these mouse tumors are "triple-negative," lacking expression of estrogen, progesterone or HER2 growth factor receptors. Despite this absence of hormone receptor expression, tumorigenesis is nevertheless estrogen-dependent: when young *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mice are ovariectomized, mammary tumor development is abolished, but can be restored when estradiol is reintroduced (van de Ven and colleagues, submitted for publication). This conditional mouse model, therefore, represents a suitable system to investigate whether any estrogenic effects of genistein- or resveratrol-rich diets affect mammary tumor formation. In particular, we studied the ability of phytoestrogens to restore mammary tumorigenesis in ovariectomized animals. The exposure of premalignant mammary epithelial cells to genistein or resveratrol might also be influenced by the ABC transporters ABCB1 or ABCG2. It has been shown that ABCG2-deficient animals have increased resveratrol or genistein plasma levels compared with wild-type mice (20–22). Hence, to increase the exposure of mammary epithelial cells to genistein or resveratrol, we also investigated mammary tumor formation in *Abcb1a/b*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ and *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females.

Materials and Methods

Mice and special diets

To study the effect of ABC transporters on phytoestrogen disposition, the *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mouse model (19) was crossed with *Abcg2*$^{-/-}$ (23) and *Abcb1a/b*$^{-/-}$ (24) mice on the same FVB/N genetic background to generate animals that develop spontaneous mammary tumors lacking functional ABCG2 or ABCB1A and ABCB1B. The *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ model was generated by backcrossing *FVB.129P2-Abcg2*^{tm1Ahs} *N7*, *FVB-Tg(KRT14-cre)8Brn*, *FVB.129P2-Trp53*^{tm1Brn}, or *FVB.129P2-Brca1*^{tm1Brn} mice on FVB/N animals for at least 8 generations (the first 5 generations using marker-assisted breeding) and eventually crossing these animals to generate the *FVB.Cg-Abcg2*^{tm1Ahs} *Trp53*^{tm1Brn} *Brca1*^{tm1Brn} *Tg(KRT14-cre)8Brn/A* compound mice. The *Abcg2*$^{\Delta 3\text{-}6/\Delta 3\text{-}6}$ genotype was confirmed by PCR with specific primers, as described previously (25). The *Abcb1a/b*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ model was generated in a similar fashion and described previously (26). Weened pups were genotyped by tail DNA PCR and littermates were fed either phytoestrogen-free, genistein-, or resveratrol-supplemented AIN-93G diets (27) from 6 weeks age onward. Subsequently, animals were monitored 3 times per week and sacrificed once a mammary tumor of 10 mm in diameter or a skin tumor of 5 mm in diameter was identified. Other reasons for sacrifice included discomfort because of weight loss (>20%), dyspnoe, or apathy. In addition to registration of age at sacrifice and tumor types, tumor and other tissue samples were collected during necropsy as previously described (25). To study tumor development in the absence of endogenous estrogens, 6-week-old *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females were ovariectomized and fed 1 of the 3 special diets until sacrifice. All experimental procedures were approved by the Animal Ethics Committee of the Netherlands Cancer Institute.

The genistein- and resveratrol-supplemented AIN-93G diets were formulated by mixing concentrated phytoestrogen stocks (Wuxi Gorunjie Technology Co., Ltd) to a final concentration of 300 mg/kg pelleted food (SDS Special Diet

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Services), packed into 5-kg bags and stored at −20°C until fed to the animals. To ensure that nutritional requirements of the mice were met continuously, each food batch was used for maximally 6 months after thawing.

Results

The latency in mammary tumor development is reduced in *Abcg2*$^{-/-}$ animals, but not in *Abcb1a/b*$^{-/-}$ animals

For experiments on topotecan resistance (25), we introduced *Abcg2* null alleles (23) into the *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ mouse model (19) on a mixed 129/Ola and FVB/N genetic background. These ABCG2-deficient females (Fig. 1A, red line) developed spontaneous mammary tumors with a 33-day shorter median latency (180 days, $P < 0.001$) than ABCG2-proficient ones (Fig. 1A, black line, 213 days). To eliminate artifacts caused by the mixed genetic background, we backcrossed the *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ and *Abcg2*$^{-/-}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ models to the FVB/N background. In addition, we generated *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ animals deficient for the *Abcb1a* and *Abcb1b* genes, which encode the mouse P-gp homologues (24). On the FVB/N background, we found that the median tumor latency of the *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females shortened from 213 to 201 days. The median mammary tumor latency was still 17.5 days shorter ($P < 0.001$) in ABCG2-deficient *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ FVB/N animals (Fig. 1B, red line, 183 days) than in transporter wild-type littermates (Fig. 1B, black line, 200.5 days). This was not the case in the ABCB1-deficient *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ FVB/N animals (Fig. 1B, green line, 194 days), however ($P = 0.770$). In addition to mammary tumors, *K14* promoter-driven Cre transgene expression also results in skin tumors, mainly squamous cell carcinomas and some hair follicle tumors (19). Compared with the base-line tumor model (Fig. 1C, top panel), no significantly altered tumor-type distributions were observed in the transporter deficient animals (Fig. 1C, middle and bottom). The FVB/N background or the introduction of *Abcb1a/b* or *Abcg2* null alleles did not alter the distribution of morphologic phenotypes that were described previously (19).

Because ABCG2 transports estrogen sulfates (28), ABCG2 ablation may stimulate tumorigenesis indirectly through elevating endogenous estrogen levels in the mammary epithelium. A more detailed analysis of overall survival per tumor type revealed, however, that both mammary and skin tumor latency were reduced in the *Abcg2*$^{-/-}$ animals, indicating that the transporter effect was not mammary gland specific (Fig. 1D).

Supplementing the diet with genistein or resveratrol does not restore mammary tumor development in ovariectomized *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females

To rigorously put any cancer-preventing effects of genistein or resveratrol to the test, we determined whether the shortened mammary tumor latency, observed in our ABCG2-deficient *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females, was reversed by supplementing these compounds to the diet. To exclude the possibility that the estrogen-like structure of these phytoestrogens promotes mammary tumorigenesis, we first fed ovariectomized *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females either a phytoestrogen-free diet (AIN-93G) or a diet containing 300-mg genistein or resveratrol per kg food (22, 29; Fig. 2A and B), starting at the age of 6 weeks. It has previously been shown that this dosage level yields plasma concentrations in mice which are also attainable in humans, not toxic when fed continuously, and which resulted in measurable effects on health and survival (30). Van de Ven and colleagues (submitted for publication) showed that ovariectomy does not result in mammary gland regression of *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ mice. Instead, the frequency of preneoplastic lesions, such as mammary duct dilatation, hyperplasia, or *carcinoma in situ*, is clearly reduced. Eventually, ovariectomy results in near complete loss of mammary tumor development and a shift to skin tumor development, which we also observed in this study (compare Figs. 1C and 2B). The relevance of estrogen on the tumor spectrum was shown by the full restoration of mammary tumor formation when 5- or 15-week-old ovariectomized females received subcutaneous pellets that released 17β-estradiol at physiological plasma levels (van de Ven and colleagues, submitted for publication). In contrast to the results with 17β-estradiol, we did not observe restoration of mammary tumor development by genistein or resveratrol, or a reduction in overall survival (Fig. 2A and B). Compared with the nonovariectomized *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females (Figs. 1B and 2A, black lines), there was a significant ($P = 0.001$, log-rank test) reduction in overall survival of the ovariectomized *K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females on phytoestrogen-free diet (Fig. 2A, blue line). Both genistein and resveratrol reversed this ovariectomy effect ($P = 0.004$ and 0.012, respectively, log-rank test). However, ovariectomized animals mainly developed skin tumors and if only the rate of these tumors was analyzed (Supplementary Fig. S1), the resveratrol effect was no longer significant, whereas the genistein effect still was ($P = 0.115$ and 0.037, respectively, log-rank test). This suggested that there may be a modest effect of genistein on preventing skin tumor formation in our model. In contrast, resveratrol may reduce the incidence of other pathological findings. Regarding tumor-type distributions (Fig. 2B), there was no effect of the phytoestrogens on mammary tumor incidence. However, there was a modest decrease in the incidence of degenerative disease or infection (genistein and resveratrol) and in the incidence of tumors, which did not originate from epithelial skin or mammary gland cells (resveratrol), such as thymoma or salivary gland adenocarcinoma (other tumors). When the relative incidences of these 2 death causes were compared between diet groups, the differences appeared to be appreciable, but they did not reach statistical significance ($P = 0.10$ and $= 0.11$, respectively, Fisher's exact test), presumably because of the low numbers.

Resveratrol and genistein do not change the tumor latency reduction in *Abcg2*$^{-/-}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females

Because we found a mild benefit of resveratrol on the overall survival of ovariectomized animals, we tested

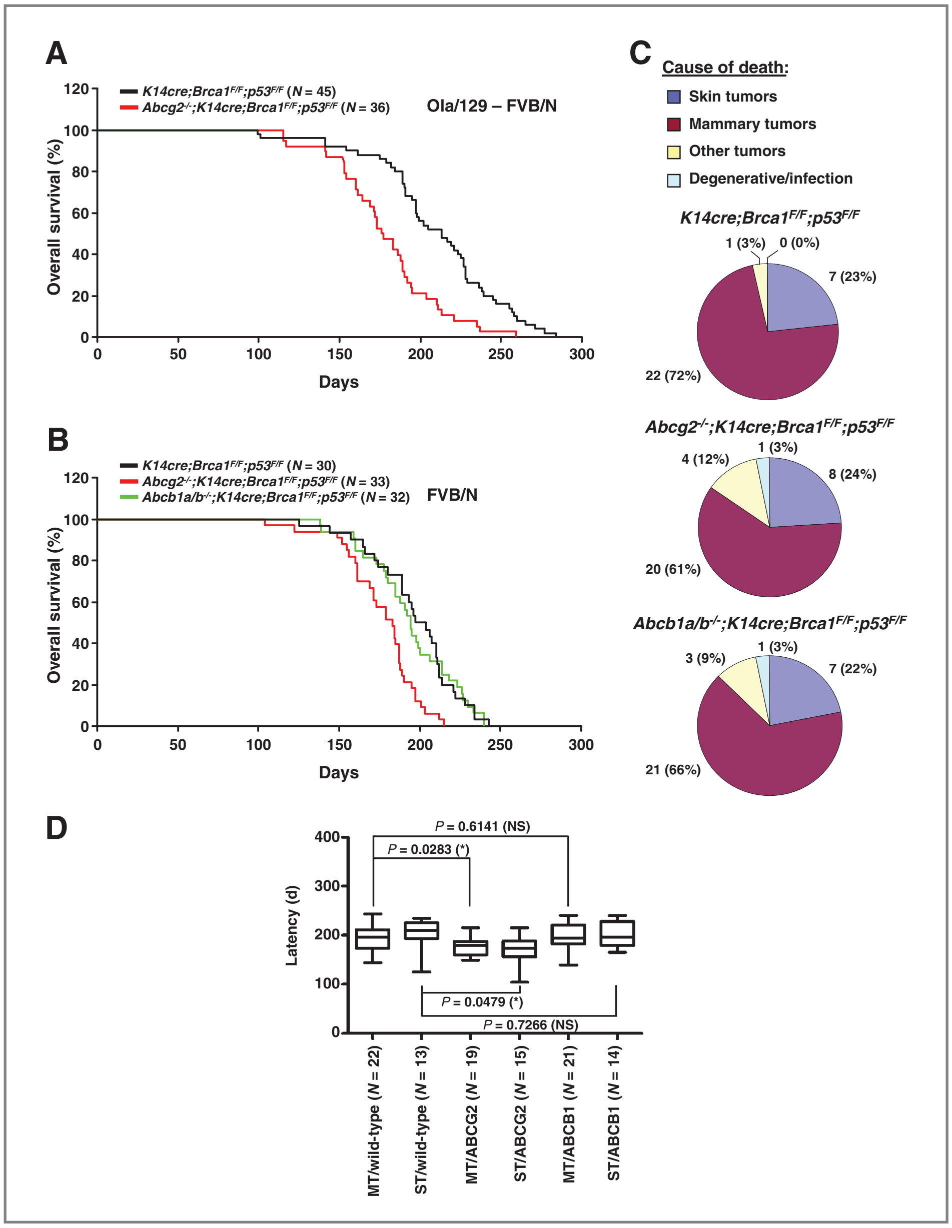

Figure 1. Overall survival, cause of death and tumor latency of ATP-binding cassette (ABC) transporter-proficient or -deficient *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females, fed with a phytoestrogen-free control diet. A, Kaplan–Meier (K–M) curves indicating overall survival (%) of *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ and *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females on a mixed Ola/129–FVB/N background. Compared with the base-line model (black line, $N = 45$), median mammary tumor latency was significantly shorter ($P < 0.001$) in the ABCG2-deficient animals (red line, $N = 36$). B, K–M curves indicating overall survival (%) of *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$, *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$, and *Abcb1a/b*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females on a pure FVB/N background. Compared with the base-line model (black line, $N = 30$), median mammary tumor latency was significantly shorter ($P < 0.001$) in the ABCG2-deficient animals (red line, $N = 33$), but not in the ABCB1-deficient animals (green line, $N = 32$, $P = 0.770$). C, Pie charts indicating tumor-type distributions of *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$, *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$, and *Abcb1a/b*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females on a pure FVB/N background. For each mouse strain the number (percentage) of animals killed because of skin tumors (dark blue), mammary tumors (brown), other tumors (yellow), or alternative pathology (light blue) is indicated. D, Boxplots of median mammary (MT) and skin tumor (ST) latencies in *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females (wild-type), *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ (ABCG2), or *Abcb1a/b*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ (ABCB1) animals. *P* values in A and B were calculated using the log-rank test, whereas *P* values in D were calculated using the Wilcoxon signed rank test.

whether the shortened latency of mammary tumors in the *Abcg2*$^{-/-}$*;K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ model (FVB/N) could be reversed by genistein or resveratrol. We have previously shown that lack of ABCG2 results in increased plasma levels of resveratrol and its metabolites in FVB/N mice (22). Hence, any antagonizing effects of this phytoestrogen on mammary tumor development should even be more pronounced in these ABCG2-deficient *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$

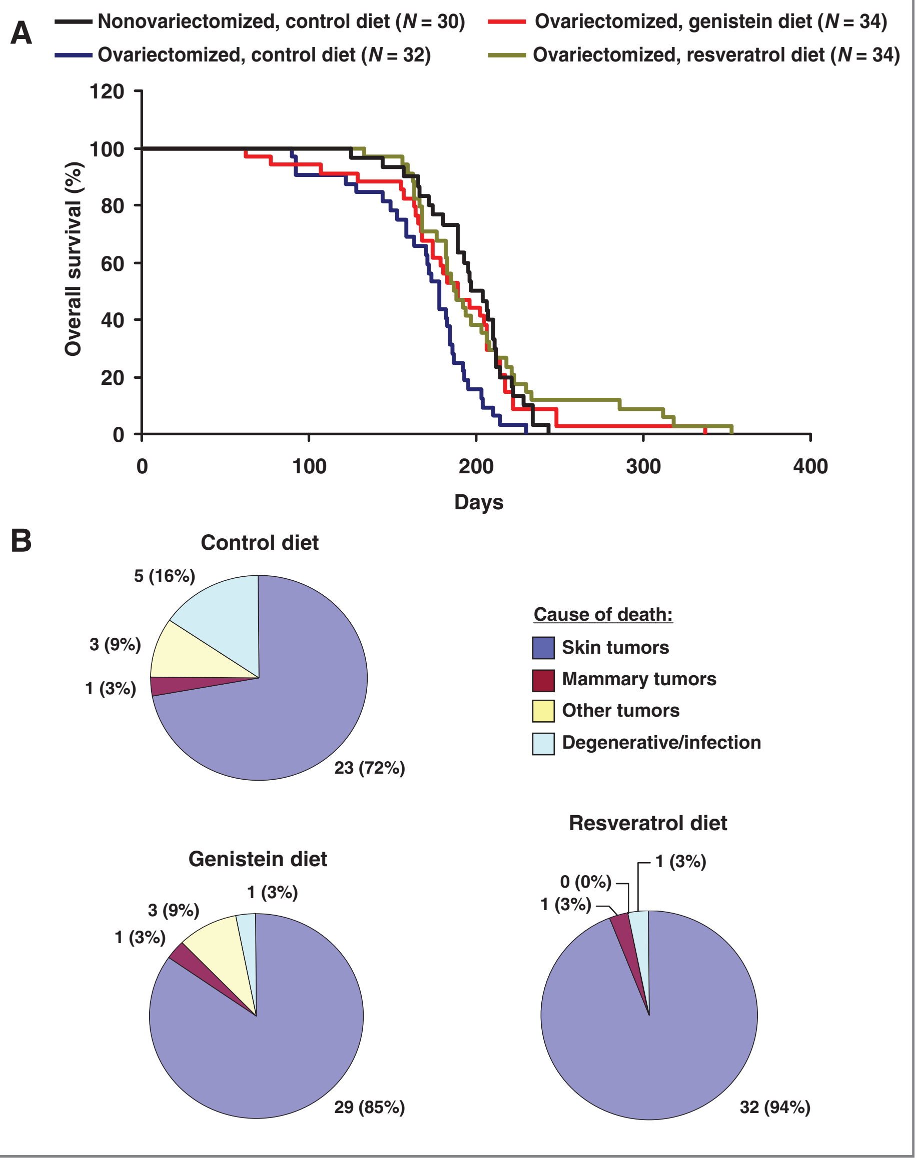

Figure 2. Overall survival and cause of death of ovariectomized *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females, fed with 3 special diets. A, Kaplan–Meier (K–M) curves indicating overall survival (%). As a reference the K–M curve of nonovariectomized *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ females (black line, $N = 30$) of Fig. 1B was added, which was significantly ($P = 0.001$) different from the K–M curve of the ovariectomized animals on phytoestrogen-free control diet (blue line, $N = 32$). Compared with these latter animals, median tumor latency was delayed in animals on genistein- (red line, $N = 34$, $P = 0.012$) and resveratrol- (green line, $N = 34$, $P = 0.004$) supplemented (300 mg/kg) test diets. B, Pie charts indicating tumor-type distributions. For each diet, the number (percentage) of animals killed because of skin tumors (dark blue), mammary tumors (brown), other tumors (yellow), or alternative pathology (light blue) is indicated. When death causes were compared between diet groups, no statistical significant differences could be detected. Even the categories degenerative/infection or other tumors, for which there seemed to be an appreciable effect in the pie charts, no significance was reached ($P = 0.10$ and $P = 0.11$, respectively, Fisher's exact test). The K–M curve *P* values were calculated using the log-rank test.

mice. However, neither the supplementation with genistein (Fig. 3A, red line) nor with resveratrol (Fig. 3A, green line) increased the overall survival in ABCG2-deficient animals compared with their control diet litter mates (Fig. 3A, blue line). The tumor-type distributions were not significantly influenced by the diet either (data not shown). This lack of dietary effects was also observed in the ABCG2-proficient animals (Fig. 3B), showing that diets with high concentrations of genistein or resveratrol do not retard mammary tumorigenesis in our mouse model.

Discussion

We show here that ablation of the ABC transporter ABCG2, but not ABCB1 (P-gp/MDR1), shortens mammary tumor latency in the *K14cre;Brca1*$^{F/F}$*;p53*$^{F/F}$ mouse model for hereditary breast cancer. This effect was not influenced by genistein or resveratrol, supplemented at the high dose of 300 mg/kg in the diet.

Following Cre-mediated deletion of floxed *Brca1* and *p53* alleles, mammary epithelial cells become genomically unstable in our conditional mouse model and accumulate additional (epi)genetic alterations. This stochastic process requires a long latency period for cells to form clonal outgrowths that become full-blown mammary tumors (19). Although tumors from different individual animals show identical histomorphology, tumor-specific signatures are found at the molecular level (19, 25, 31). What role xenobiotics play in this tumor formation process is not clear. *Abcg2*$^{-/-}$ mice are not tumor prone. However, lack of ABCG2 reduces the time required for mammary tumor development caused by loss of BRCA1 and p53. ABCG2 actively eliminates endogenous metabolites or dietary

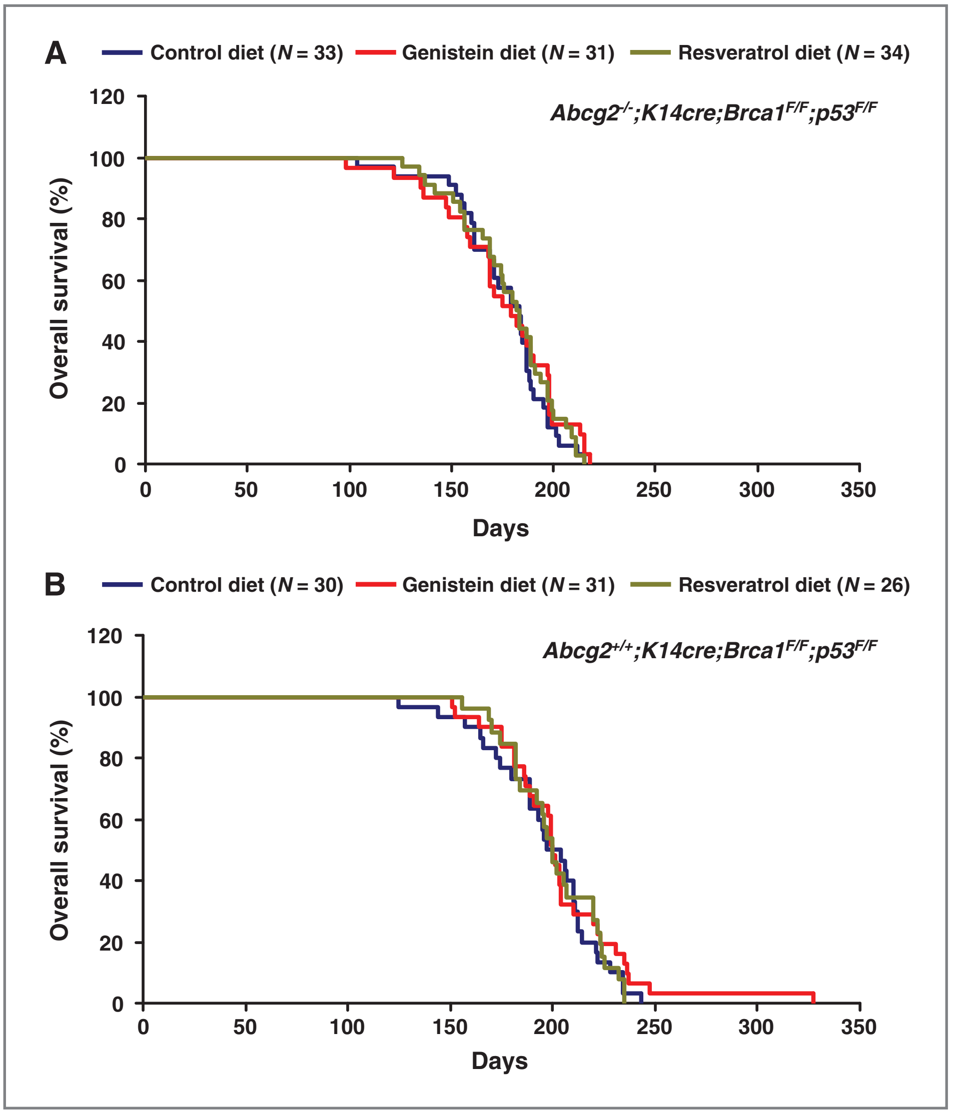

Figure 3. Overall survival of nonovariectomized *Abcg2*$^{-/-}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ and *Abcg2*$^{+/+}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females, fed with 3 special diets. A, Kaplan–Meier (K–M) curves indicating overall survival (%) of *Abcg2*$^{-/-}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females on phytoestrogen-free control diet (blue line, $N = 33$) or genistein- (red line, $N = 31$) and resveratrol- (green line, $N = 34$) supplemented (300 mg/kg) test diets. B, K–M curves indicating overall survival (%) of *Abcg2*$^{+/+}$;*K14cre;Brca1*$^{F/F}$;*p53*$^{F/F}$ females on phytoestrogen-free control diet (blue line, $N = 30$) or genistein- (red line, $N = 31$) and resveratrol- (green line, $N = 26$) supplemented (300 mg/kg) test diets.

xenobiotics from the body, and increased systemic exposure to a specific compound in *Abcg2*$^{-/-}$ animals may enhance the mutagenesis of BRCA1;p53-deficient epithelial cells. This compound might be an ABCG2-specific substrate, as ablation of ABCB1 did not alter latency. We do not know the compound(s) and mechanism that cause the observed effects. An unbiased, mass spectrometry-based approach (32) might be able to detect the relevant metabolite differences between the plasma or tumor tissues derived from *Abcg2*$^{-/-}$ versus *Abcg2*$^{+/+}$ animals. Moreover, it may be interesting to investigate in future experiments whether a shorter tumor latency is also found in response to ABCG2-specific inhibitors.

Our data suggest that functional ABCG2 polymorphisms might influence tumorigenesis in women with a familiar breast cancer predisposition. Of the ABCG2 single nucleotide polymorphisms (SNP) identified, only a few influence drug transport efficiency and, therefore, pharmacokinetics-related side effects in patients (33). The common SNP rs2231142 (421*C*>*A*), which encodes a Q141K loss of function mutation, causes at least 10% of all gout cases in the Caucasian American population of the United States (34). To our knowledge, no reports have yet been published linking ABCG2 loss-of-function SNPs to accelerated tumor progression in human BRCA1 or BRCA2 mutation carriers. Our mouse data suggest that ABCG2 dysfunction might increase the already high tumor incidence in the human population even further. Whether this effect is mammary gland-specific or also relevant to other cancer predispositions warrants further investigation.

We did not find an effect of genistein or resveratrol on mammary tumor development in our model. In other models, the exposure of female Sprague-Dawley rats to 500-ppm genistein for 2 years significantly increased the risk of mammary adenocarcinoma development, but decreased the number of benign mammary fibroadenomas (35). Overall, the evidence of carcinogenic activity of genistein in female rats was determined as being "equivocal." It

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was also found in rats that perinatal exposure to genistein renders animals more resistant to 7,12-dimethylbenz(*a*) anthracene–induced mammary tumorigenesis (36, 37), whereas no effect was seen when genistein was given to adult animals (38). These differential effects appear to be caused by genistein-induced alterations of mammary gland development (39). In nude mice, physiologically attainable concentrations of genistein stimulate the growth of ER-positive mammary tumor xenotransplants, such as MCF-7 cells (40). Ju and colleagues (41) also reported that genistein stimulates tumor outgrowth of mice that have low estradiol levels because of ovariectomy, and they conclude that "products containing genistein may not be safe for postmenopausal women with estrogen-dependent breast cancer." It is possible that this effect of genistein is specific for already existing ER-positive tumor cells. The BRCA1;p53-deficient tumors derived from our model are ER-negative, like their human counterparts (19). Nevertheless, their development is clearly hormone-dependent. As shown in Fig. 2B, ovariectomy results in nearly complete absence of mammary tumor development, which can be reversed by 17β-estradiol but not progesterone application (van de Ven and colleagues, submitted for publication). Hence, mammary tumor formation in our model is ER-dependent. In BRCA1 mutation carriers, ovariectomy is also an effective prophylactic strategy to prevent tumorigenesis (42, 43). Our model may be useful to investigate whether aromatase inhibitors or selective ER modulators have a similar effect as ovariectomy. The precise mechanisms responsible for this preventive effect are still unclear. It has been suggested that BRCA1-associated cancers arise from luminal progenitor cells that are still ER-positive (44). Another hypothesis is that paracrine RANK ligand signaling through ER-positive luminal mammary cells drives mammary stem cell expansion, which gives rise to tumors if additional genetic alterations during progression are acquired (45). Similarly, paracrine signaling through stromal cells has been shown to be critical during normal mammary gland development (46) and proposed as a mechanism for tissue specificity of tumor development in BRCA1 patients (47). While BRCA1-deficient cells usually undergo apoptosis, survival in the mammary gland may be promoted through supportive signals from an estrogen-responsive stroma (48). Our data show that even high concentrations of genistein and resveratrol in the diet do not have the same effect as 17β-estradiol. Most likely the binding affinity of these compounds to the ER is not sufficient to trigger any mammary tumor development (14, 49, 50).

In addition to a shift from mammary to skin tumor development, we observed that ovariectomy of *K14cre;* $Brca1^{F/F}$*;*$p53^{F/F}$ animals resulted in a significant (P = 0.001) reduction of overall survival (Fig. 2A). We do not know why. There were some ovariectomized animals on the phytoestrogen-free diet that died because of degenerative or infectious causes, whereas no such cases were present in the nonovariectomized animals. It is possible that the lack of endogenous estrogens increased the background pathology in our mouse model. Whether the animals on the phytoestrogen-supplemented diets experienced a health benefit in addition to the modest delay in skin tumor development warrants further investigation.

In summary, we found that in a genetically engineered mouse model for BRCA1-associated breast cancer ABCG2, but not ABCB1, ablation significantly reduced mammary tumor latency. This reduction was not affected by dietary supplementation with genistein or resveratrol.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.A.L. Zander, S. Rottenberg
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Cancer Prevention Research

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