[CANCER RESEARCH 63, 6607–6612, October 15, 2003]

Phytoestrogen-Rich Diets Modulate Expression of *Brca1* and *Brca2* Tumor Suppressor Genes in Mammary Glands of Female Wistar Rats[1]

Cécile Vissac-Sabatier, Véronique Coxam, Pierre Déchelotte, Christel Picherit, Marie-Nöelle Horcajada, Marie-Jeanne Davicco, Patrice Lebecque, Yves-Jean Bignon,[2] and Dominique Bernard-Gallon

Laboratoire d'Oncologie Moléculaire UMR 484 Institut National de la Santé et de la Recherche Médicale UdA, Centre Jean Perrin, Clermont-Ferrand, Cedex 01 [C. V-S., Y-J. B., D. B-G.]; Groupe Ostéoporose, Unité des Maladies Métaboliques et Micronutriments (U3M), I.N.R.A. de Clermont-Ferrand/Theix, 63122 Saint Genès Champanelle [V. C., C. P., M-N. H., M-J. D., P. L.]; and Service d'Anatomie Pathologique, Hôtel-Dieu, 63005 Clermont-Ferrand [P. D.], France

ABSTRACT

Phytoestrogens are natural compounds with anticancer, proliferation, differentiation, and chemopreventive effects, for which several mechanisms have been proposed. In the present study, modulation of Brca1 and Brca2 expression by different phytoestrogen-rich diets has been investigated in ovariectomized Wistar rats. Two hundred mammary glands were harvested in three independent experiments. *Brca1* and *Brca2* mRNAs were quantified by real-time quantitative reverse transcription-PCR, and their proteins by immunohistochemistry. The first experiment compared the influence of different phytoestrogens [flax-seed, isoflavones (IFs), or rutin]. A 10% increase in *Brca1* mRNA expression was shown after flax-seed consumption, whereas no variation was noted for *Brca2* mRNA, nor for Brca1 and Brca2 proteins. In the second experiment, two soy IFs sources (Novasoy or Soylife) were given at different concentrations to the animals. Only *Brca2* mRNA was increased and only at high doses. Finally, the effect of IFs was compared with that of estradiol. An increase in mRNA for both genes was noted after estradiol treatment and with the highest dose of IFs.

In conclusion, our results show that IFs, given in the diet at different doses, are able to increase *Brca1* and *Brca2* mRNA in ovariectomized female Wistar rat. However, no variation in Brca1 or Brca2 protein expression was demonstrated, whatever the experimental conditions were.

INTRODUCTION

There is a large amount of epidemiological and *in vitro* data suggesting that phytoestrogens may prevent carcinogenesis and inhibit tumor growth in breast cancer, as well as prevent osteoporosis (1–3) and cardiovascular disease (4).

Phytoestrogens belong to a broad group of biologically active compounds that have attracted attention recently. Those molecules occur widely in edible plants, and share with estradiol a phenolic ring and a pair of hydroxyl groups. The major classes of current interest from a nutritional and health perspective are the IFs[3] and the lignanes, although other polyphenols such as quercetin may exhibit light estrogenic activities.

Two major tumor suppressor genes of human breast cancer have been described, *BRCA1* and *BRCA2* (5, 6). In sporadic human breast cancers, although somatic mutations have not been detected (7), alterations in *BRCA1* and *BRCA2* mRNA level have been observed (8).

The rat *Brca1* gene has been map precisely to chromosome 10 (9), and its sequence is highly homologous to that of mice (88%) and humans (81%). *Brca1* mRNA is expressed in most tissues, with highest levels in testis, consistent with human and mouse data (5, 10). *Brca1* mRNA levels are also high in ovary, spleen, lung, and liver, and lower in mammary gland, kidney, heart, and brain, and undetectable in skeletal muscle (9).

Rat *Brca2* was cloned and mapped to chromosome 12 by Yamada *et al.* (11) in 1997 and shares a large homology with human *BRCA2* (12). Expression studies demonstrate an 11–12 kb transcript with tissue-specific patterns of expression consistent with human *BRCA2*.

Studies of BRCA1 and BRCA2 function strongly suggested that these genes play critical roles in the regulation of mammary epithelial cell growth (13). Variations in Brca1 and Brca2 expression were also observed in adult mouse tissues during mammary gland development associated with pregnancy (14, 15).

Previous *in vitro* studies demonstrated modulation of *BRCA1* and *BRCA2* expression[4] by genistein but not daidzein.[4] This is why the present study aimed to investigate the effect of other phytoestrogens in an OVX rat model.

MATERIALS AND METHODS

Animals and Diets. These studies were conducted in accordance with current legislation on animal experiments in France. Female Wistar rats (n = 190; 195 days old) were purchased from I.N.R.A. (Clermont-Ferrand Theix, France). All of the rats were housed individually at 21°C with a 12-h light/dark cycle in metal cages. Throughout the experimental period the rats had free access to water and were fed daily a humidified (1 ml water/g food) soy protein-free powdered semipurified diet (I.N.R.A., Jouy-en-Josas, France; Table 1).

First Experiment: Comparison of Different Phytoestrogen-Rich Diets (Fig. 1*A*). Ninety-day-old Wistar female rats were either SH (n = 10) or OVX (n = 30). On the day after surgery (day 0), OVX animals were randomly allocated to three groups of 10 rats each and given the standard diet supplemented with either 0.5% soy IFs, which corresponded to a dose of 16 μg/g body weight per day (Soylife, Giessen, the Netherlands), 0.25% rutin (a quercetine, glycoside; Sigma, France), or 10% flax-seeds (providing ~0.2% lignans; Biofar, France) for 90 days. Then, the animals were sacrificed by cardiac punction.

Second Experiment: Comparison of Two Soy IF Sources (Fig. 1*B*). In the same way, Wistar female rats were either SH (n = 10) or OVX (n = 60). At day 0, the OVX animals were randomly allocated to six groups of 10 rats each and given either Novasoy [A, Novasoy IF compound 152–400; Archer Daniels Midland Co., Decatur, IL; containing 348 mg/g as total IFs (genistein 159 mg, daidzein 156 mg, glycitein 33 mg, *i.e.* a genistein-daidzein:total IF ratio of 90%)] or Soylife [S, Soylife 100, batch n° 32K/154/H, containing 35 mg/g as total IFs (5.55 mg genistein, 15.36 mg daidzein, and 14.09 mg glycytein, *i.e.* a genistein-daidzein/total IFs ratio of 60%); Soylife] at three concentrations for each products: 20 (A1), 40 (A2), and 80 (A3), or 4 (S1), 8 (S2), and 16 (S3) μg/g body weight/day for 90 days. The six diets were prepared by mixing powdered soybean-IF concentrate with the semipurified diet.

Third Experiment: Comparison of IFs and Estradiol (Fig. 1*C*). Wistar female rats were either SH (n = 10) or OVX (n = 50). Ninety days after

Received 2/12/03; revised 7/16/03; accepted 7/24/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by the association de Recherche contre le Cancer (ARC). C. V-S. was a grant recipient of the M.E.N.R.T (Ministère de l'Education National de la Recherche et de la Technologie).

[2] To whom requests for reprints should be addressed, at Laboratoire d'Oncologie Moléculaire, Centre Jean Perrin, 58 rue Montalembert, BP 392, 63011 Clermont-Ferrand, cedex 01, France. Phone: 33-4-73-27-80-50; Fax: 33-4-73-27-80-42; E-mail: Yves-Jean.Bignon@cjp.fr.

[3] The abbreviations used are: IF, isoflavone; SH, sham-operated; OVX, ovariectomized; C_T, threshold cycle.

[4] Unpublished observations.

Table 1 *Composition of the soybean-protein-free powdered semipurified diet*

Ingredients	Content (g/kg)
Casein	180
Sucrose	210
Maize starch	430
Cellulose	100
Peanut oil	25
Rapeseed oil	25
Vitamin mixture (with cholecalciferol 32.25 μg/kg)	10
Mineral mixture [with (g/kg) Ca 2.3, P 1.6, Mg 0.42]	18.5
DL-methioninie	1.5

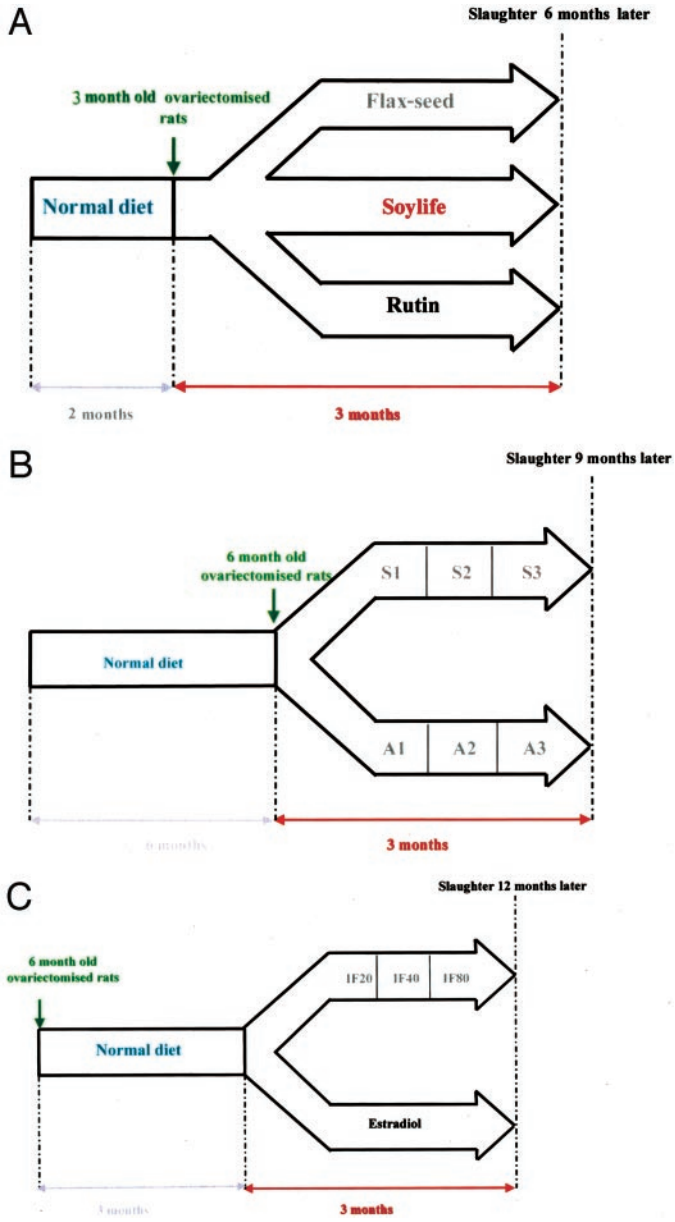

Fig. 1. *A*, schematic representation of the three experimental protocol for animals and diets. *B*, S and A corresponded respectively to the Soylife and the Novasoy. The rats were fed with Novasoy at three different doses (A1 = 20, A2 = 40, or A3 = 80 μg/g body weight) and with Soylife at three different doses (S1 = 4, S2 = 8, or S3 = 16 μg/g body weight). *C*, IF corresponds to the IFs (Novasoy). The rats received three different doses (IF20 = 20, IF40 = 40, or IF80 = 80 μg/g body weight).

surgery, the animals were randomly allocated to six groups of 10 rats each and given IF (Novasoy; ADM) at 0 (OVX), 20 (IF20), 40 (IF40), and 80 (IF80) μg/g body weight/day, or α -estradiol (30 μg/kg body weight) for 90 days, whereas the SH rats consumed the standard diet.

Mammary glands were harvested, then snap-frozen in liquid nitrogen at the time of collection.

mRNA Extraction. Mammary glands were weighed quickly, then ground thoroughly with a French press, and the power disrupted in a potter with the appropriate amount of RTL buffer containing β-mercaptoethanol, according to the weight of the gland. The solution was homogenized by passing lysate at least 10 times through a 30-gauge needle fitted to an RNase-free syringe. Total mRNA isolation was performed using RNA easy mini kit (Qiagen) according to the manufacture's protocol.

Real-Time Quantitative PCR. Total mRNA was reverse transcribed using a Pharmacia kit according to the manufacturer's conditions. Expression of *Brca1* and *Brca2* mRNAs was assessed by quantitative real-time PCR (system Taqman) by the comparative C_T method (16, 17).

Probes and primers used for the quantification were chosen with the help of Primer Express and are summarized in Table 2.

Single-stage PCR was carried out in a final volume of 25 μl containing 5 μl of the Reverse Transcription (RT) reaction mix (diluted to 25 ng) and 12.5 μl of Taqman Universal PCR Master mix, which containing 200 nM of each primer pair and probe (*Brca1* or *Brca2*), 200 μM DNTP, 400 μM dUTP, 5 mM $MgCl_2$, 1.25 units of Ampli Taq Gold, 0.5 units of amperase uracil-*N* glycosylase, and 50 nM of 18S rRNA primers and probe.

The PCR procedure consisted of 2 min at 50°C, 94°C for 10 min for the uracil-*N* glycosylase activation, 50 cycles with a initial denaturation of 15 s at 95°C, and finally an extension step of 1 min at 60°C, using a ABI prism 7700 sequence detection system (Applied Biosystems, ZA Courtabœuf, France).

The relative quantification of *Brca1* and *Brca2* expression was performed using the comparative C_T method (18), which consists of the normalization of the C_T of the target gene (*Brca1* or *Brca2*) to the C_T of an endogenous reference gene (*i.e.* 18S rRNA; $\Delta C_T = C_{T\ \text{target gene}} - C_{T\ 18S\ rRNA}$). C_Ts for *Brca1* or *Brca2* of each SH rat mammary gland rat were quantified, and the C_T average was determined with the other mammary gland tested ($\Delta\Delta C_T = \Delta C_{T\ \text{sham-opered}} - \Delta C_{T\ \text{target gene}}$).

Acetone Fixation of Frozen Mammary Glands and Immunohistochemistry. Cryostat sections (5 μm) were cut at −40°C. After 1 h stabilization, they were air-dried for 30 min then fixed in acetone at room temperature for 10 min and air-dried 10 min. Sections were washed in PBS and incubated for 45 min with antibodies against Brca1 or Brca2 (Table 3). After washing in PBS, the slides were incubated for 30 min with biotinylated goat antirabbit or rabbit antigoat immunoglobulins from Dako LSAB 2 system Peroxidase (Dako

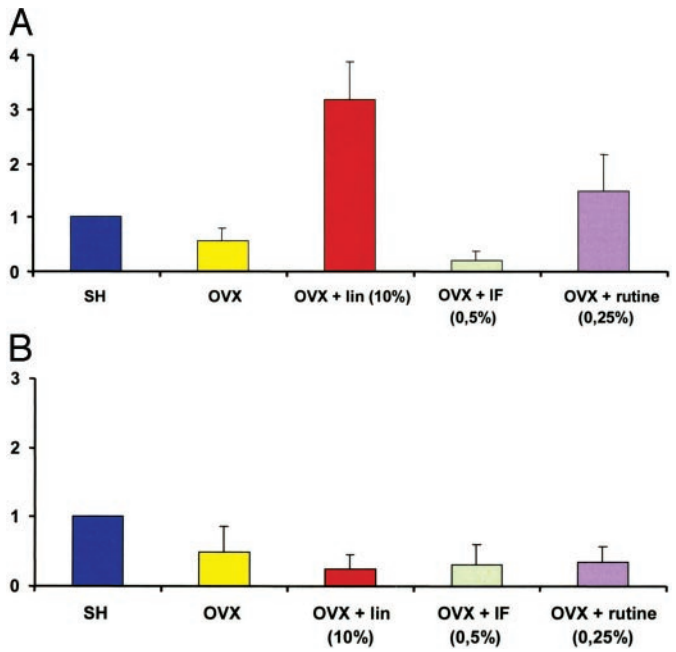

Fig. 2. Quantification of (A) *Brca1* and (B) *Brca2* mRNA by real time quantitative RT-PCR of mammary glands from SH or OVX rats after treatment for three months with Soylife (IF (0.5%)), flax-seed (lin; 10%) or rutin (0.25%). (A) *Brca1* mRNA. (B) *Brca2* mRNA.

Table 2 *Sequence of probes and primers for rat mRNA quantification*

Names	Forward primer	TaqMan Probe	Reverse primer
Brca1	5′-^{396}CAGATTGAGTCTGGAAGTGCCA^{417}[a]-3′	5′-^{428}CAGTCTCAGTGTCCAGTTGTCTAACCTTGGG^{448}-3′	5′-^{481}TGTTTTGAGGTTGTGTCTGCCTAT^{504}-3′
Brca2	5′-^{9745}TTGAGGACCCCAAGACCTGT^{9764}-3′	5′-^{9798}CTGCCCTTACCGCCACCGCTC^{9818}-3′	5′-^{9828}CCGGAGAGACAAAGGTGCA^{9846}-3′
18 S rRNA	5′-CGGCTACCACATCCAAGGAA-3′	5′-TGCTGGCACCAGACTTGCCCTC-3′	5′-GCTGGAATTACCGCGGCT-3′

[a] The numbers indicate the position of the first nucleotide in cDNA sequence of *Brca1* or *Brca2* (GenBank accession no. AF036760 for *Brca1*, and no. U89653 for *Brca2*).

Table 3 *Anti-Brca1 and anti-Brca2 antibodies*

Antibody names	Antigenic specificities	Types	Sources	Dilutions
Anti-Brca1				
M20	(aa 1793–1812) COOH-terminal	Goat PoAb	Santa Cruz Biotechnologies	1/15
D16	(aa 2–18) NH_2-terminal	Goat PoAb	Santa Cruz Biotechnologies	1/5
H100	(aa 1–100) NH_2-terminal	Rabbit PoAb	Santa Cruz Biotechnologies	1/5
Anti-Brca2				
H300	(aa 2520–2819) internal region	Rabbit PoAb	Santa Cruz Biotechnologies	1/5
N19	(aa 20–38) NH_2)-terminal	Goat PoAb	Santa Cruz Biotechnologies	1/5

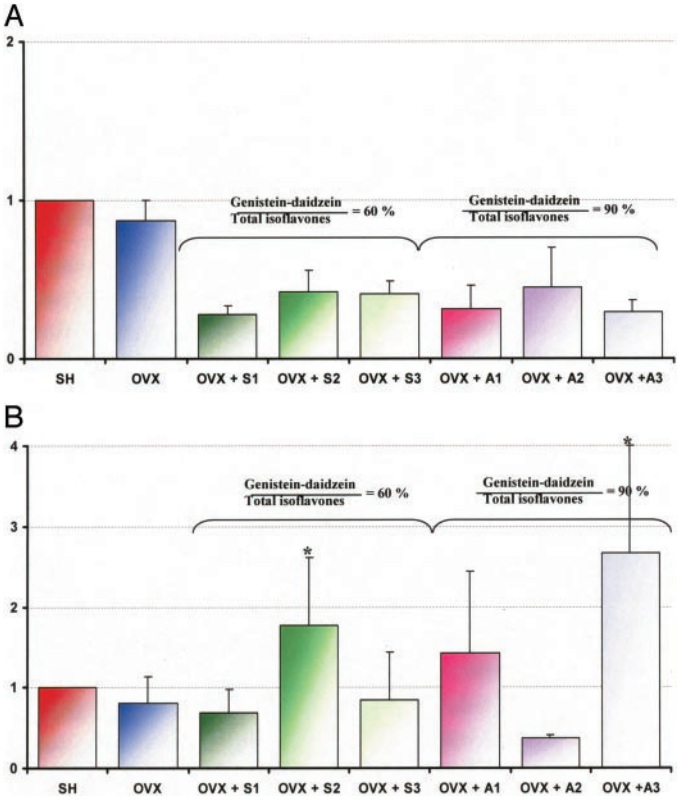

Fig. 3. Effects of Novasoy at three different doses (A1 = 20, A2 = 40, or A3 = 80 μg/g) or Soylife at three different doses (S1 = 4, S2 = 8, or S3 = 16 μg/g) on (*A*) *Brca1* or (*B*) *Brca2* mRNAs by comparison with untreated rats (*SH*). The quantification was performed with real time quantitative RT-PCR. *A, Brca1* mRNA. *B, Brca2* mRNA.

Corporation, Carpinteria, CA). After a wash in PBS, the slides were incubated for 20 min with streptavidin conjugated to horseradish peroxidase in PBS, before washing again with PBS for 10 min. The slides were then placed in 3-amino-9-ethylcarbazole with acetate buffer for 10 min, in the dark, washed in PBS, counterstained with Mayer's hematoxylin, and mounted in glycerin. All of the incubations and stainings were performed at room temperature. Negative control without primary antibody was performed on all of the cases (Fig. 5).

RESULTS

Quantification of *Brca1* and *Brca2* mRNA After Treatment with Phytoestrogens. In the first experiment (Fig. 2), the *Brca1* mRNA level in SH rats is defined as 1. As observed by Rajan *et al.* (19), *Brca1* mRNA decreased in OVX rats. The flax-seed-rich diet induced a 3-fold increase of *Brca1* mRNA, whereas the other diets did not elicit any difference compared with OVX values. Therefore, estrogen deficiency-induced changes in expression were not corrected by IFs or rutine. In contrast, none of the diets restored the basal levels of *Brca2* mRNA observed in SH rats.

The second experiment (Fig. 3) investigated the influence of the quality and dose of soy in relation to IF content on Brca1 and Brca2 expression in OVX rats. Neither Soylife nor Novasoy prevented the reduction in *Brca1* mRNA expression, compared with SH rats. Both diets provided the same amount of total IFs, whereas the ratio genistein:daidzein was different. In contrast, ovariectomy + S2 *i.e.* 8 μg IF or + A3 *i.e.* 80 μg IF increased the level of *Brca2* mRNA by comparison to SH rats.

In the last experiment (Fig. 4), which studied the potential improvement of ovariectomy-induced conditions by phytoestrogens in comparison with estradiol, the two highest doses of IFs (IF40 and IF80)

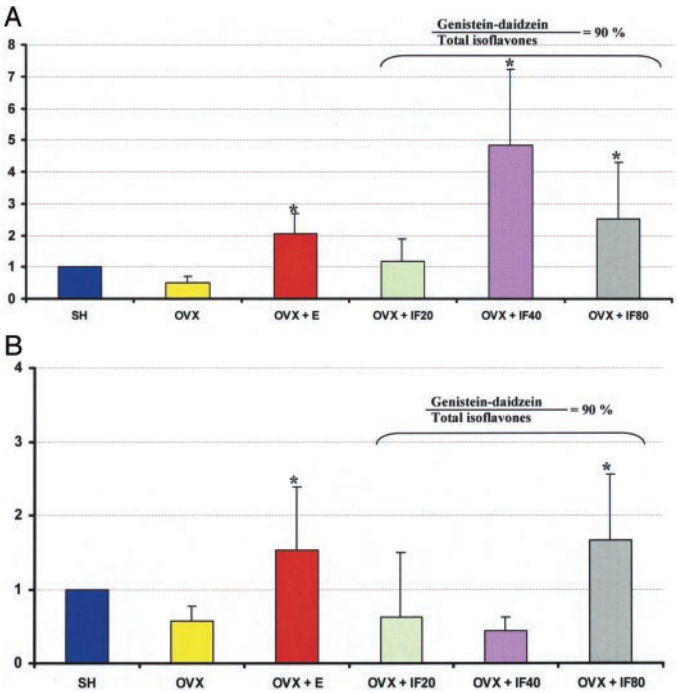

Fig. 4. *Brca1* (*A*) and *Brca2* (*B*) mRNA quantification after treatment with 20, 40, or 80 μg of IFs or 80 mg α-estradiol, 3 months after ovariectomy. *A, Brca1* mRNA. *B, Brca2* mRNA.

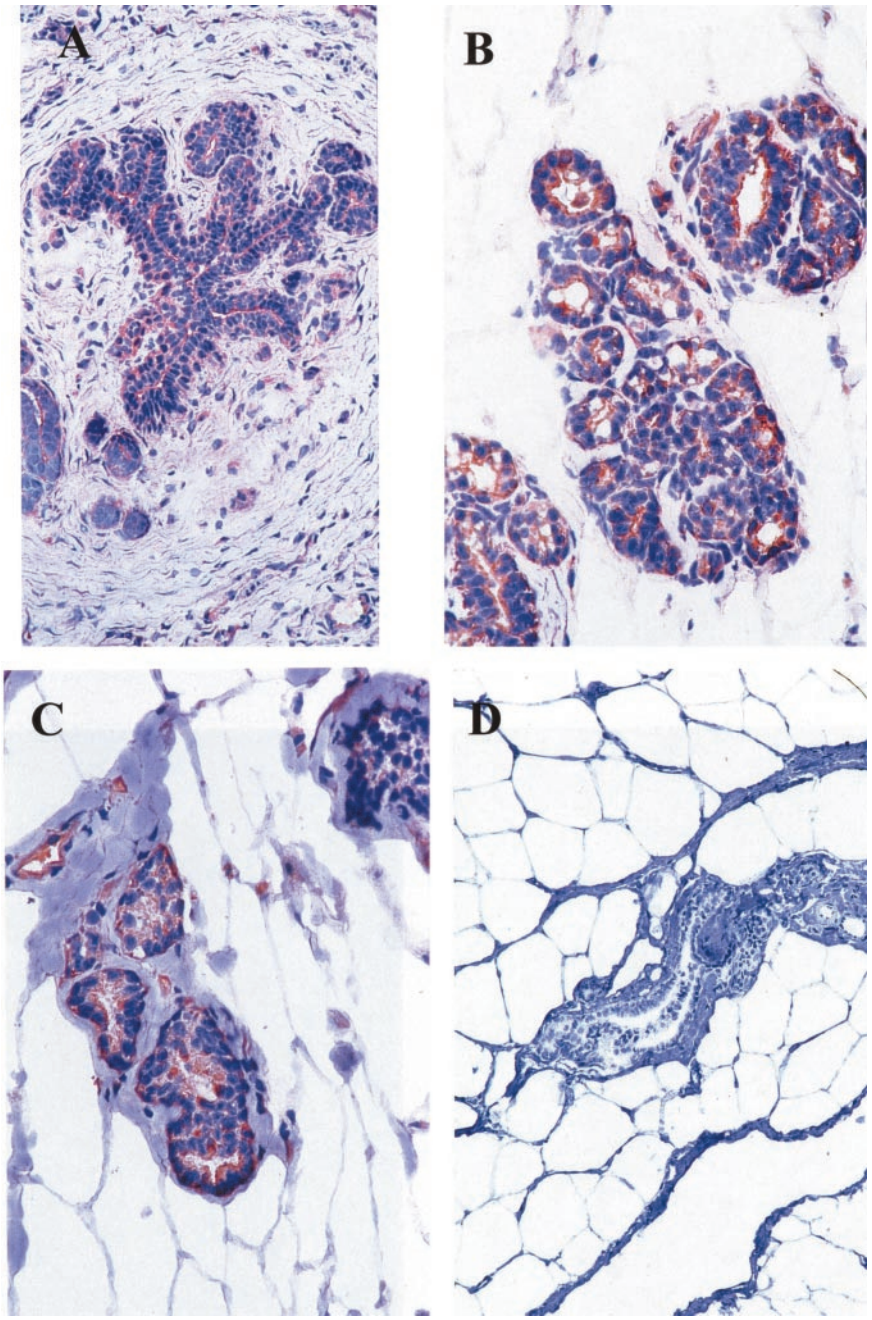

Fig. 5. Representative photographs of mammary glands showing the range of staining patterns observed with antibodies H100 raised against Brca1 protein in SH rats (*A*; ×160), OVX rats (*B*; ×320), and OVX + A2 rats (*C*; ×320). As control, an irrelevant primary antibody was used (*D*; ×160).

significantly increased *Brca1* mRNA, as did estradiol, to higher levels than in SH rats. The same trend was seen with the lowest dose (IF20), without reaching significance value. Only IF80 exhibited increased *Brca2* expression.

Distribution of Brca1 and Brca2 Proteins. We performed immunoperoxidase studies with different antibodies raised against Brca1 or Brca2 to ascertain whether different diets changed the intensity of staining or localization of these proteins.

With the three antibodies raised against Brca1 (H100, M2, and D16), staining was localized to galactophoric epithelial ducts and in the secretion. The same pattern was observed for antibodies against Brca2 (H300 and N19).

In each experiment, no variation in the range of staining or localization of Brca1 or Brca2 was elicited by any treatment (castration or diet; Fig. 5).

DISCUSSION

The purpose of this study was to determine whether phytoestrogen-rich diets might modulate *Brca1* and *Brca2* expression *in vivo*.

We demonstrated previously *in vitro* an increase of *BRCA2* mRNA in MDA-MB-231, an estrogen receptor-negative tumor cell line, and in MCF10a, a normal breast cell line after treatment with genistein. An increase of *BRCA1* mRNA was also observed in MCF7, an estrogen receptor-positive breast tumor cell line, after treatment with daidzein, and in MDA-MB-231 after genistein treatment. No variation in protein expression was noted for BRCA2.[4]

However, studies of cultured cells allow molecular dissection of pathways operative in a single cell, whereas research in animal models integrates the complexity of an organ and its different cell types with the dynamic hormonal and physiological status of the animal.

The ideal candidate animal should be relatively easy to characterize genetically and should allow both selective and rapid breeding. The rat is an extremely valuable model for studies of breast cancer susceptibility genes because the characteristics of rat and human breast cancer are so similar.

The rat model was chosen over the mouse model because: (*a*) the rat mammary tumor resembles that of the human in its spectrum of hormonal responsiveness; (*b*) mammary cancers in the rat, like the human but unlike the mouse, lack a major viral etiology; and (*c*) tumors in the rat, unlike the mouse, are rapidly induced by chemicals, radiation, or hormone treatment. The disadvantage of the rat model is that its genetic map is much less characterized than that of the mouse.

Phytoestrogens may be important antioxidants (20, 21). They also disrupt the action of DNA topoisomerase II and ribosomal S6 kinase, which could explain their observed effects on cell cycle, differentiation, proliferation, and apoptosis. In addition, genistein is a potent inhibitor of protein tyrosine kinase (22, 23). Rutin is the common flavonoid glycoside in the diet and is hydrolyzed by intestinal flora to produce biologically active aglycone (a sugar-free flavonoid; 24), and this quercetine compound has powerful antioxidant properties. Flaxseed is the precursor of enterolactone and enterodiol, which exhibit estrogenic properties. Urinary excretion of the mammalian lignans enterodiol and enterolactone has been shown to be linked to reduced risk of breast cancer (25, 26). However, Sathyamoorthy *et al.* (27) have demonstrated that phenolic compounds such as daidzein, genistein, and enterolactone were able to elicit an estrogen-like response, whereas enterodiol was not.

The risk of breast cancer is increased by exposure to high levels of endogenous and exogenous estrogens (28, 29). Soy foods are rich in precursors of the IFs daidzein and genistein, which are heterocyclic phenols similar in structure to estrogens, and it has been hypothesized that a high dietary intake of soy foods might reduce breast cancer risk by interfering with the action of endogenous estradiol (30). Furthermore, estrogens are able to activate BRCA1 and BRCA2 (31), so we investigated the effects of different phytoestrogens on the *Brca1* and *Brca2* expression.

These results show that phytoestrogen-rich diets containing various concentration of genistein and daidzein can increase *Brca1* and *Brca2* mRNA in a dose-dependent manner, whereas having no action at the protein levels. Indeed, a dose of 40 or 80 μg was sufficient to increase *Brca1*, whereas the highest level of consumption was necessary for *Brca2*. The time of exposure seems to be very important, as the results were different if the IFs-rich diet was administrated 3 days later or 3 months later after the ovariectomy. This raises the possibility that pathways activated by phytoestrogens may have differential effects on the regulation of *Brca1* and *Brca2* expression. Furthermore, Rajan *et al.* (19) have demonstrated that the magnitude of the up-regulation of *Brca1* mRNA that occurs either during early pregnancy or in OVX animals treated with estradiol and progesterone was significantly greater than that observed for *Brca2*.

We believe the environment of low endogenous estrogen created by ovariectomizing the rat affords genistein and daidzein the opportunity to be a significant source of estrogen and, hence, allows for the compounds to stimulate *Brca1* and *Brca2* mRNA. This hypothesis is corroborated by the control group, which received α-estradiol. These diets prevented the decrease in expression and even increased *Brca1* and *Brca2* mRNA to a 2-fold higher level than that of SH.

Human BRCA1 and BRCA2 proteins, like rat Brca1 and Brca2, are regulated during mammary proliferation and gland development (13–15, 32). Nevertheless, in the present study no variation was observed, whatever the dietary phytoestrogen.

In conclusion, the present study demonstrated that dietary IFs cannot compensate both immediately after surgery for reduced *Brca1* expression because of the ovariectomy but can restore after a delay for *Brca1* expression. Although IFs when given in high doses induced *Brca2* expression, so they can both be compensated and restore an ovariectomy status.

Furthermore, we will also study by microarray cDNA method a panel of genes that acts with *Brca1* and *Brca2* after treatment with a phytoestrogen-rich diet to determine the mechanism pathway of IFs.

ACKNOWLEDGMENTS

We thank Guy Ragonnaud for the pictures, and Jacqueline Avinain, and Christel Picard for the immunochemistry assistance. We also thank Dr. Nancy Uhrhammer for correcting the English.

REFERENCES

1. Picherit, C., Coxam, V., Bennetau-Pelissero, C., Kati-Coulibaly, S., Davicco, M. J., Lebecque, P., and Barlet, J. P. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. J. Nutr., *130:* 1675–1681, 2000.
2. Picherit, C., Bennetau-Pelissero, C., Chanteranne, B., Lebecque, P., Davicco, M. J., Barlet, J. P., and Coxam, V. Soybean isoflavones dose-dependently reduce bone turnover but do not reverse established osteopenia in adult ovariectomized rats. J. Nutr., *131:* 723–728, 2001.
3. Picherit, C., Chanteranne, B., Bennetau-Pelissero, C., Davicco, M. J., Lebecque, P., Barlet, J. P., and Coxam, V. Dose-dependent bone-sparing effects of dietary isoflavones in the ovariectomised rat. Br. J. Nutr., *85:* 307–316, 2001.
4. Barnes, S. Phytoestrogens and breast cancer. Baillieres Clin. Endocrinol. Metab., *12:* 559–579, 1998.
5. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., and *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science (Wash. DC), *266:* 66–71, 1994.
6. Wooster, R., Neuhausen, S. L., Mangion, J., Quirk, Y., Ford, D., Collins, N., Nguyen, K., Seal, S., Tran, T., Averill, D., and *et al.* Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12–13. Science (Wash. DC), *265:* 2088–2090, 1994.
7. Futreal, P. A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L. M., Haugen-Strano, A., Swensen, J., Miki, Y., and *et al.* BRCA1 mutations in primary breast and ovarian carcinomas. Science (Wash. DC), *266:* 120–122, 1994.
8. Thompson, M. E., Jensen, R. A., Obermiller, P. S., Page, D. L., and Holt, J. T. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. Nat. Genet., *9:* 444–450, 1995.
9. Chen, K. S., Shepel, L. A., Haag, J. D., Heil, G. M., and Gould, M. N. Cloning, genetic mapping and expression studies of the rat Brca1 gene. Carcinogenesis (Lond.), *17:* 1561–1566, 1996.
10. Bennett, L. M., Haugen-Strano, A., Cochran, C., Brownlee, H. A., Fiedorek, F. T., Jr., and Wiseman, R. W. Isolation of the mouse homologue of BRCA1 and genetic mapping to mouse chromosome 11. Genomics, *29:* 576–581, 1995.
11. Yamada, S., Nakagama, H., Toyota, M., Ushijima, T., Okada, K., Sato, K., Sugimura, T., and Nagao, M. Cloning of rat Brca2 and linkage mapping to chromosome 12. Mamm. Genome, *8:* 850–851, 1997.
12. McAllister, K. A., Haugen-Strano, A., Hagevik, S., Brownlee, H. A., Collins, N. K., Futreal, P. A., Bennett, L. M., and Wiseman, R. W. Characterization of the rat and mouse homologues of the BRCA2 breast cancer susceptibility gene. Cancer Res., *57:* 3121–3125, 1997.
13. Rajan, J. V., Wang, M., Marquis, S. T., and Chodosh, L. A. Brca2 is coordinately regulated with Brca1 during proliferation and differentiation in mammary epithelial cells. Proc. Natl. Acad. Sci. USA, *93:* 13078–13083, 1996.
14. Blackshear, P. E., Goldsworthy, S. M., Foley, J. F., McAllister, K. A., Bennett, L. M., Collins, N. K., Bunch, D. O., Brown, P., Wiseman, R. W., and Davis, B. J. Brca1 and Brca2 expression patterns in mitotic and meiotic cells of mice. Oncogene, *16:* 61–68, 1998.
15. Lane, T. F., Deng, C., Elson, A., Lyu, M. S., Kozak, C. A., and Leder, P. Expression of Brca1 is associated with terminal differentiation of ectodermally and mesodermally derived tissues in mice. Genes Dev., *9:* 2712–2722, 1995.
16. Favy, D. A., Lafarge, S., Rio, P., Vissac, C., Bignon, Y. J., and Bernard-Gallon, D. Real-time PCR quantification of full-length and exon 11 spliced BRCA1 transcripts in human breast cancer cell lines. Biochem. Biophys. Res. Commun., *274:* 73–78, 2000.
17. Vissac, C., Lemery, D., Le Corre, L., Fustier, P., Dechelotte, P., Maurizis, J. C., Bignon, Y. J., and Bernard-Gallon, D. J. Presence of BRCA1 and BRCA2 proteins in human milk fat globules after delivery. Biochim. Biophys. Acta, *1586:* 50–56, 2002.
18. Fink, L., Seeger, W., Ermert, L., Hanze, J., Stahl, U., Grimminger, F., Kummer, W., and Bohle, R. M. Real-time quantitative RT-PCR after laser-assisted cell picking. Nat. Med., *4:* 1329–1333, 1998.
19. Rajan, J. V., Marquis, S. T., Gardner, H. P., and Chodosh, L. A. Developmental expression of Brca2 colocalizes with Brca1 and is associated with proliferation and differentiation in multiple tissues. Dev. Biol., *184:* 385–401, 1997.
20. Ruiz-Larrea, M. B., Mohan, A. R., Paganga, G., Miller, N. J., Bolwell, G. P., and Rice-Evans, C. A. Antioxidant activity of phytoestrogenic isoflavones. Free Radic. Res., *26:* 63–70, 1997.

21. Wei, H., Bowen, R., Cai, Q., Barnes, S., and Wang, Y. Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proc. Soc. Exp. Biol. Med.., *208:* 124–130, 1995.
22. Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukami, Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem., *262:* 5592–5595, 1987.
23. Kurzer, M. S., and Xu, X. Dietary phytoestrogens. Annu. Rev. Nutr., *17:* 353–381, 1997.
24. Kuhnau, J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev. Nutr. Diet., *24:* 117–191, 1976.
25. Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J. T., Woods, M., Goldin, B. R., and Gorbach, S. L. Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. Lancet, *2:* 1295–1299, 1982.
26. Ingram, D., Sanders, K., Kolybaba, M., and Lopez, D. Case-control study of phytooestrogens and breast cancer. Lancet, *350:* 990–994, 1997.
27. Sathyamoorthy, N., Wang, T. T., and Phang, J. M. Stimulation of pS2 expression by diet-derived compounds. Cancer Res., *54:* 957–961, 1994.
28. Pike, M. C., Spicer, D. V., Dahmoush, L., and Press, M. F. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol. Rev., *15:* 17–35, 1993.
29. Thomas, H. V., Reeves, G. K., and Key, T. J. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control, *8:* 922–928, 1997.
30. Setchell, K. D., Borriello, S. P., Hulme, P., Kirk, D. N., and Axelson, M. Nonsteroidal estrogens of dietary origin: possible roles in hormone- dependent disease. Am. J. Clin. Nutr., *40:* 569–578, 1984.
31. Spillman, M. A., and Bowcock, A. M. BRCA1 and BRCA2 mRNA levels are coordinately elevated in human breast cancer cells in response to estrogen. Oncogene, *13:* 1639–1645, 1996.
32. Chodosh, L. A. Expression of BRCA1 and BRCA2 in normal and neoplastic cells. J. Mammary Gland Biol. Neoplasia, *3:* 389–402, 1998.

Phytoestrogen-Rich Diets Modulate Expression of *Brca1* and *Brca2* Tumor Suppressor Genes in Mammary Glands of Female Wistar Rats

Cécile Vissac-Sabatier, Véronique Coxam, Pierre Déchelotte, et al.

Cancer Res 2003;63:6607-6612.