[Cell Cycle 3:11, 1451-1456; November 2004]; ©2004 Landes Bioscience

Report

Brca1-Deficient Murine Mammary Epithelial Cells Have Increased Sensitivity to CDDP and MMS

Magdalene K. Sgagias[1,*]
Kay-Uwe Wagner[1]
Brad Hamik[1]
Scott Stoeger[1]
Rebecca Spieker[1]
L. Julie Huber[2]
Lewis A. Chodosh[2]
Kenneth H. Cowan[1]

[1]Eppley Institute for Research in Cancer and Allied Diseases; University of Nebraska Medical Center; Omaha, Nebraska USA

[2]Departments of Cancer Biology and Medicine; and Abramson Family Cancer Research Institute; University of Pennsylvania School of Medicine; Philadelphia, Pennsylvania USA

Correspondence to: Magdalene K. Sgagias, Ph.D.; Eppley Institute for Research in Cancer and Allied Diseases; 986805 Nebraska Medical Center; Omaha, Nebraska 68198-6805 USA; Tel: 402.559.3036; Fax: 402.559.4651; Email: msgagias@unmc.edu

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=1211

KEY WORDS

Bcra1-deficiency, DNA-damage and repair, breast cancer, CDDP, MMS

ABBREVIATIONS

MMECs	murine mammary epithelial cells
DAPI	4-6-diamidine-2-phenylidone dihydrochloride
CDDP	cis-platinum (II) diamine dichloride
MTT	3-(4-5 dimethylthiozol-2-yl) 2-5diphenyl-tetrazolium bromide
PBS	phosphate-buffered saline
MMS	methylmethane sulfonate

ACKNOWLEDGEMENTS

We like to thank Kevin Olson for technical assistance and Joerg Rahnenfuehrer (UC Berkeley) for statistical analysis. We also like to thank Dr. Charles A. Kuszynski and Linda M. Wilkie for the flow cytometry analysis.

ABSTRACT

In this report we describe the isolation of an isogenic pair of Brca1^{++} and Brca1$^{-/-}$ murine mammary epithelial cells (MMECs). These cells were isolated from Brca1 conditional knock-out mice which contained loxP sites flanking exon 11 of the Brca1 gene (Brca1$^{fl/f1}$) and then immortalized by infection with HPV-16E6 retrovirus to degrade p53 protein. Brca1$^{-/-}$ MMECs were generated by deletion of exon 11 following transduction of Brca1$^{fl/f1}$ MMECs with a retroviral vector expressing Cre recombinase. Brca1-deficiency rendered MMECs sensitive to cis-platinum (II) diamine dichloride (CDDP) and methyl-methane sulfonate (MMS). The Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs is the only known pair of isogenic mammary epithelial cell lines. The understanding of the mechanisms of the CDDP sensitivity of the BRCA1-deficient mammary epithelial cells would be very important in understanding how BRCA1-deficiency plays a role in tissue specific breast cancer chemotherapy. These studies support the role of BRCA1 in the CDDP-induced and MMS-induced DNA damage and repair by p53-independent pathways.

INTRODUCTION

Breast cancer is a major challenge for women in the United States and throughout the world. While germline mutations in the BRCA1 gene are commonly observed in cases of hereditary breast and ovarian cancer, transcriptional downregulation of BRCA1 is also frequently observed in sporadic breast cancer.[1-3] In mice, Brca1-deficiency results in growth retardation,[4] apoptosis, defective DNA damage repair, centrosome amplification, loss of G_2/M cell cycle checkpoint,[5] genetic instability[6,7] and Brca1$^{-/-}$ mice die in early gestation.[8] However, loss of p53 function is able to partially rescue Brca1$^{-/-}$ mice suggesting that p53 expression may be involved in cell death in Brca1-deficient cells.[8] Although, BRCA1 is not highly conserved across species,[9-11] human BRCA1 can rescue the embryonic lethality of Brca1 mutant mice,[12,13] indicating functional similarities between the human and murine BRCA1 proteins.

Several studies have suggested that BRCA1 participates in regulating gene expression, cell cycle control,[14,15] ubiquitination[16,17] and apoptosis.[18-20] BRCA1 is also involved in signal transduction following DNA damage and BRCA1 is phosphorylated following irradiation[21] and subsequently results in activation of Chk2 kinase[22] and Chk1 kinase.[15] In addition, BRCA1 appears to have a major role in DNA damage repair. Early studies had shown an interaction between BRCA1 and DNA repair machinery. For example, BRCA1 associates with RAD51,[23] RAD50, MRE11, NBS1in complexes involved in DNA repair by homologous recombination (HR) as well as nonhomologous end joining (NHEJ) DNA repair.[24,25] Despite advances in understanding the role of BRCA1 in DNA damage and repair, major gaps exist in understanding the molecular details of BRCA1 function in a tissue specific manner.

In this report we describe the isolation of a Brca1$^{-/-}$ mammary epithelial cell line. Since breast cancer originates in epithelial cells, these cells represent a useful model for studying the role of BRCA1 in DNA damage and repair and drug resistance in mammary epithelial cells. Preliminary studies suggest that lack of Brca1 renders MMECs more sensitive to agents that generate substrates for both nucleotide excision repair (NER) and base excision repair (BER). A role of BRCA1 in NER was previously shown.[26] For example, Brca1-deficiency in murine embryonic stem (ES) cells induces hypersensitivity to cis-platinum (II) diamine dichloride (CDDP).[27] Furthermore, BRCA1-deficient HCC1937 human breast cancer cells, which contain a mutation in the BRCA1 gene (5823insC), are more sensitive to γ-radiation compared with unrelated BRCA1 wild type MCF-7 human breast cancer

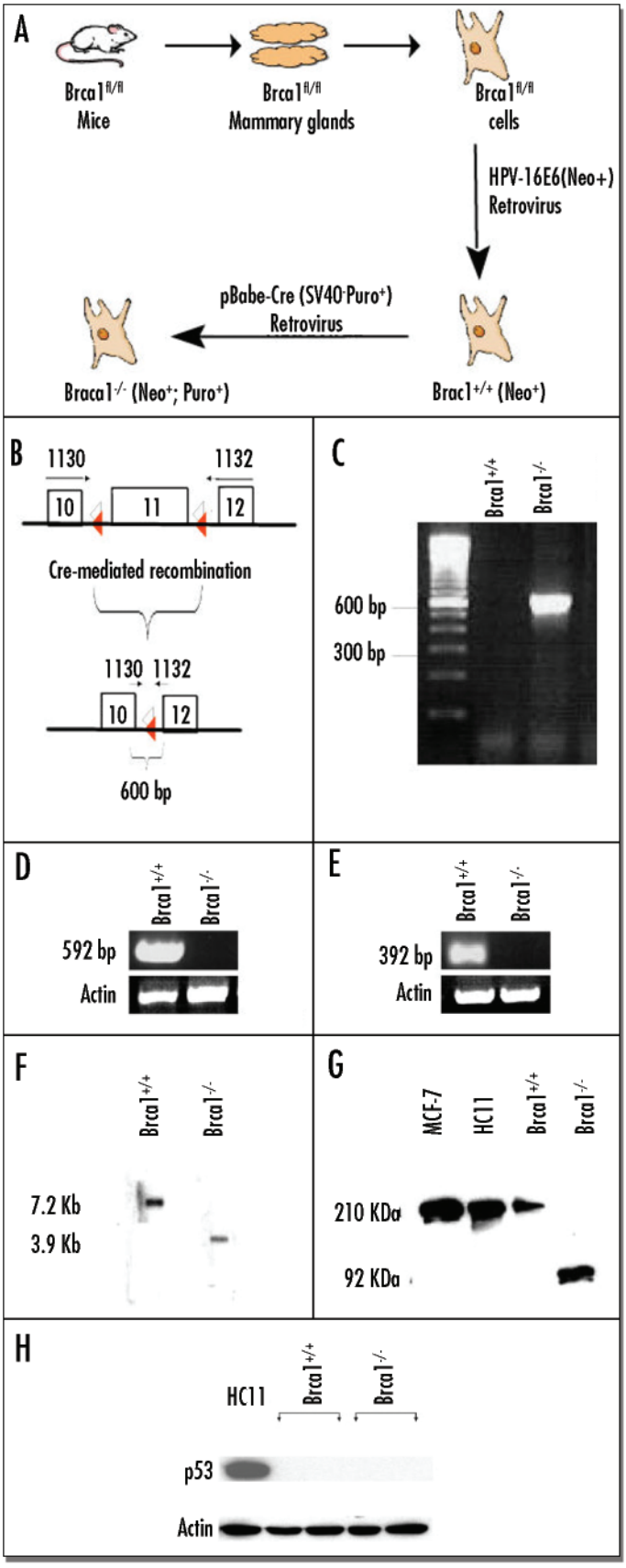

Figure 1. Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs. (A) Schema for the generation of Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs. Mammary glands were isolated from transgenic Brca1$^{fl/fl}$ mice and digested with collagenase and hyalouronidase to harvest cells. These cells were infected in vitro with HPV-16E6 retrovirus to generate Brca1$^{+/+}$ MMECs. Brca1$^{-/-}$ MMECs were generated from these cells by transduction with the pBabe-Cre retrovirus to delete exon 11 sequences from both alleles of Brca1 gene. (B) Schema for the detection of Cre-mediated recombination for the deletion of the Brca1 exon 11. (C) PCR amplification using primers (1130, 1132) that flank the loxP sites around exon 11 of Brca1 gene resulted in a 600 bp product in Brca1$^{-/-}$ MMECs but not in Brca1$^{+/+}$ MMECs. Deletions at the loxP sites generated a specific 600 bp PCR product. (D) PCR analysis using primers within exon 11 of Brca1 detects a 592 bp fragment in Brca1$^{+/+}$ MMECs but not in the Brca1$^{-/-}$ MMECs. (E) Total RNA was examined by RT-PCR. An exon 11-specific 392-bp product was formed in Brca1$^{+/+}$ MMECs but not in Brca1$^{-/-}$ MMECs. Actin gene expression was used as a control. (F) Northern analysis was performed on RNA from Brca1$^{-/-}$ and Brca1$^{+/+}$ MMECs. Brca1$^{+/+}$ MMECs contain the full length 7.2 kb transcript while the Brca1$^{-/-}$ MMECs contain a truncated 3.9 kb transcript. (G) Cell lysates from Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were examined by western blot with a specific anti-Brca1 antibody (mAb1) [32]. Brca1 $^{+/+}$ MMECs contain the wild type 210 kDa protein. In contrast, the Brca1$^{-/-}$ MMECs contain only a truncated 92 kDa immunoreactive protein. (H) Cell lysates were examined by western blot with a specific anti-p53 antibody (FL-393) (Santa Cruz). Actin was used as control.

cells and HBL-100 cells[28] or with MDA-MB-435S, T47D, U2OS and MDA-MB-231[29] and reconstitution with wild-type BRCA1 reverses the irradiation sensitivity of HCC1937 cells.[29]

Other studies suggest that BRCA1-deficient HCC1937 cells are more sensitive to MMS compared to MCF-7 breast cancer cells and T24 bladder cancer cells, suggesting a possible role of BRCA1 in base excision repair (BER).[24] In this report we describe the isolation of murine mammary epithelial cells deficient in Brca1 and examine the sensitivity of these cells to DNA damaging agents CDDP and MMS.

MATERIALS AND METHODS

Generation of Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs. Mammary epithelial cells were isolated from Brca1$^{fl/fl}$ mice, which contain loxP sites flanking exon 11 of Brca1 gene.[30] Mammary glands from five Brca1$^{fl/fl}$ mice, which develop normally, were removed and digested with a mixture of collagenase and hyalouronidase, leaving organoids intact. The organoids were grown in matrigel for three weeks, digested with collagenase and cultured in serum-free DMEM-F12 medium. Fibroblasts surrounding the organoids were eliminated by partial trypsinization. Epithelial cells from the organoids were isolated and cultured in fresh medium on plastic as monolayers.

Brca1$^{fl/fl}$ MMECs were infected with an HPV-16E6 (Neo^{+}) retrovirus (provided by Dr. D. Galloway, Seattle, WA), to inhibit p53 function and immortalize the cells. MMECs expressing the HPV-16E6 construct were selected with G418 (0.4 mg/ml) and were designated Brca1$^{+/+}$ MMECs. Brca1$^{-/-}$ MMECs were generated by deleting exon 11 of Brca1 following transfection with pBabe-Cre (Puro+) retrovirus, which expresses bacterial Cre recombinase.[31] The resulting Brca1$^{-/-}$ ^{M}MECs were selected in medium containing puromycin (7 μg/ml) and G418 (0.4 mg/ml).

Cell Culture and Drug Treatment. MMECs were grown in DMEM-F12 medium (GibcoBRL) containing 2% heat inactivated fetal bovine serum, 10 μg/ml insulin (Sigma), 5 ng/ml epidermal growth factor (Sigma), 1 mg/ml bovine serum albumin (Sigma), 5 μg/ml linoleic acid complex (Linoleic acid-BSA), 50 μg/ml gentamycin and 20 U/ml nystatin.

To determine growth kinetics, cells (2000 cells/well) were plated in 6-well plates and after incubation at 37°C for 3, 6, 9 or 12 days, cells stained with trypan blue and live cells were identified by trypan blue exclusion under the microscope and the results were expressed as the number of live cells at the indicated time points.

To determine drug cytotoxicity, cells were seeded at 50–60% confluency into 96-well microtitre plates (1000–2000 cells /well) and treated 24 hours later with increasing concentrations of cis-platinum (II) diamine dichloride

Table 1 **IC_{50} VALUES OF CDDP AND MMS IN Brca1$^{+/+}$ AND Brca1$^{-/-}$ MMECs**

	IC_{50}		
	CDDP	MMS	
Cell lines	(μM)	(μM)	
Brca$^{+/+}$	0.9	2600	$p = 0.0285$
Brca1$^{-/-}$	0.0398	500	$p = 0.0285$

The p-value of four experiments of the one-sided Wilcox rank sum test is $p = 0.0285$. The hypothesis is that if GR1 is smaller than GR2 it can be rejected at the level 0.05.

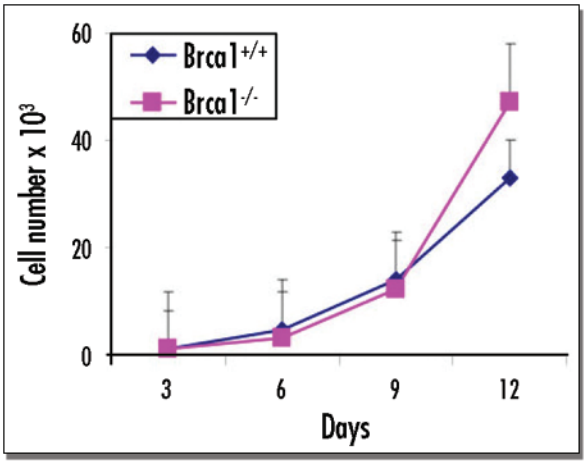

Figure 2 (Above). Growth Kinetics of Brca1$^{+/+}$ and Brca$^{-/-}$ MMECs. Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were incubated at 37°C for 3, 6, 9 or 12 days. Cells were stained with trypan blue and the number of surviving cells was counted microscopically by trypan blue exclusion. The results are shown as the mean ± S.D. of three independent experiments each done in triplicate samples.

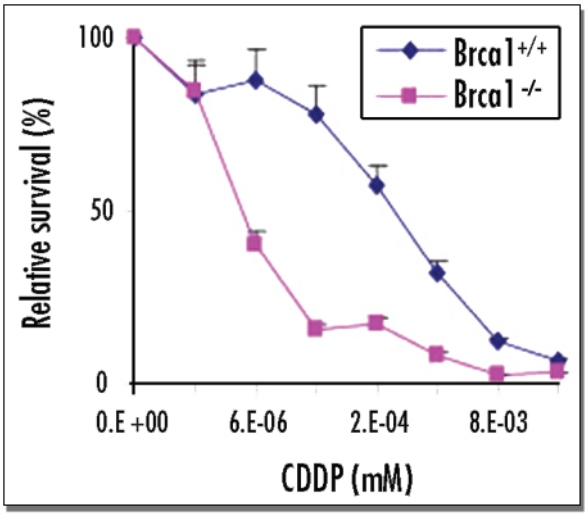

Figure 3 (Above). Sensitivity of Brca$^{-/-}$ MMECs to CDDP. Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were exposed to increasing concentrations of CDDP as described in materials and methods. After incubation at 37°C for 7 days, MTT assay was performed. Cell survival was expressed as the fraction of CDDP-treated surviving cells to the untreated cells. The results are shown as the mean ± S.D. of seven independent experiments each done in quadruplicate samples.

(CDDP) (Sigma) or methylmethane sulfonate (MMS) (ICN Biomedicals) in quadruplicate samples. After incubation at 37°C for 5–7 days, cell viability was measured by the MTT assay. Briefly, after incubation, 50 μl of 3-(4-5 dimethylthiozol-2-yl) 2-5diphenyl-tetrazolium bromide (MTT, Sigma) (2 mg/ml) was added to each well. The yellowish MTT was reduced to dark color formazan by viable cells only. After incubating the cells with MTT for four hours, the formazan crystals formed solubilized in dimethyl sulfoxide (DMSO) (150 μl/well). The color developed was quantitated with an ELISA microplate reader (ELx 808 Ultra Microplate Reader, BIO-TEK Instruments, Inc.) (measuring wave length: 570 nm, reference wavelength: 630 nm). The results were expressed as the ratio of the results from drug-treated cells to that of the untreated control cells.

Genetic Analysis of MMECs. Cre-mediated recombination of the Brca1 gene was detected by PCR amplification with primers that flank exon 11 (Forward: 5'-GGGTAGTTTGTAAGCATGC, Reverse: 5'-CTGCGAGC-AGTCTTCAGAAAG). A 600 bp product indicates excision of exon 11. The deletion was confirmed by a second PCR experiment with primers within exon 11 (Forward: 5'-ATCAGTAGTAGAAATCCAAGCCCACC, Reverse: 5'-TGCCACTCCCAGCATTGTTAG). Wild-type exon 11 generated a 592-bp PCR product. PCR analysis for the actin gene was used as a control.

Brca1 RNA was assayed in total RNA obtained from MMECs using reverse transcription with oligo dT, followed by PCR amplification of Brca1 exon 11 sequences (Forward primer: 5'-TTCCCTGCTTCCAACACTT-CATG, Reverse primer: 5'-TCCTCATTCCCACACTGGTGACTC). The transcribed exon 11 of Brca1 generated a 322-bp product.

Brca1 transcripts were also examined by northern blot analyses using a probe encompassing nucleotides 4827 to 5354 that recognizes both full length and exon 11-deleted Brca1 transcripts.

Western Blot Analysis. Brca1 and p53 proteins were examined in cell lysates by western blot analyses with an anti-murine Brca1 antibody (mAb1)[32] and a rabbit polyclonal anti-p53 antibody (FL-393) (Santa Cruz).

Cell Cycle Analysis. The cells were harvested and fixed in 70% ethanol. Fluorescent-activated cell sorting (FACS) analysis for DNA content was performed using FACSCalibur instrument (BD PharMingen) by measuring the intensity of the fluorescence emitted by the propidium iodide.

DAPI Nuclear Fluorescence Staining. Cells were seeded on plastic chamber slides and treated as indicated. Cells were then fixed with 4% paraformaldehyde for 30 min at room temperature and washed with PBS. The fixed cells were incubated with 0.8 mg/ml DAPI (4'-6-diamidine-2'-phenylidone dihydrochloride) for 30 min and washed with PBS. DAPI-staining was visualized by fluorescence microscopy. Apoptotic cells were identified by nuclear condensation and fragmentation. Results were expressed as the proportion of apoptotic to total cells x 100 in a field of 400 cells.

RESULTS

In order to study the tissue-specific effects of Brca1-deficiency, we isolated MMECs from conditional Brca1$^{fl/fl}$ mice (carrying loxP sites flanking exon 11 of the Brca1 gene, which shows wild-type activity). We used these latter mice to isolate Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs as described in the materials and methods. Since MMECs generally grow for only a short time in culture, MMECs isolated from Brca1$^{fl/f1}$ mice were immortalized by transfection with a retrovirus containing HPV-16E6, to inactivate p53. Immortalized Brca1$^{fl/f1}$ MMECs were subsequently transduced with a retroviral vector expressing Cre recombinase (pBabe-Cre SV40-Puro^{+}) which resulted in the deletion of exon 11 of the Brca1 gene by Cre-mediated recombination resulting in the generation of Brca1$^{-/-}$ MMECs.

The schema for generating of Brca1-deficient MMECs is shown in Figure 1A. Briefly, MMECs were harvested from five conditional Brca1$^{fl/f1}$ mice carrying loxP sites flanking exon 11 of the Brca1 gene.[30] These MMECs were infected with a retrovirus expressing HPV-16E6 to generate immortalized Brca1$^{fl/f1}$ MMECs. Since Brca1$^{fl/f1}$ mice develop normally and express full-length Brca1 protein, Brca1 protein expressed in these animals is thought to be normal. MMECs derived from these animals are referred to

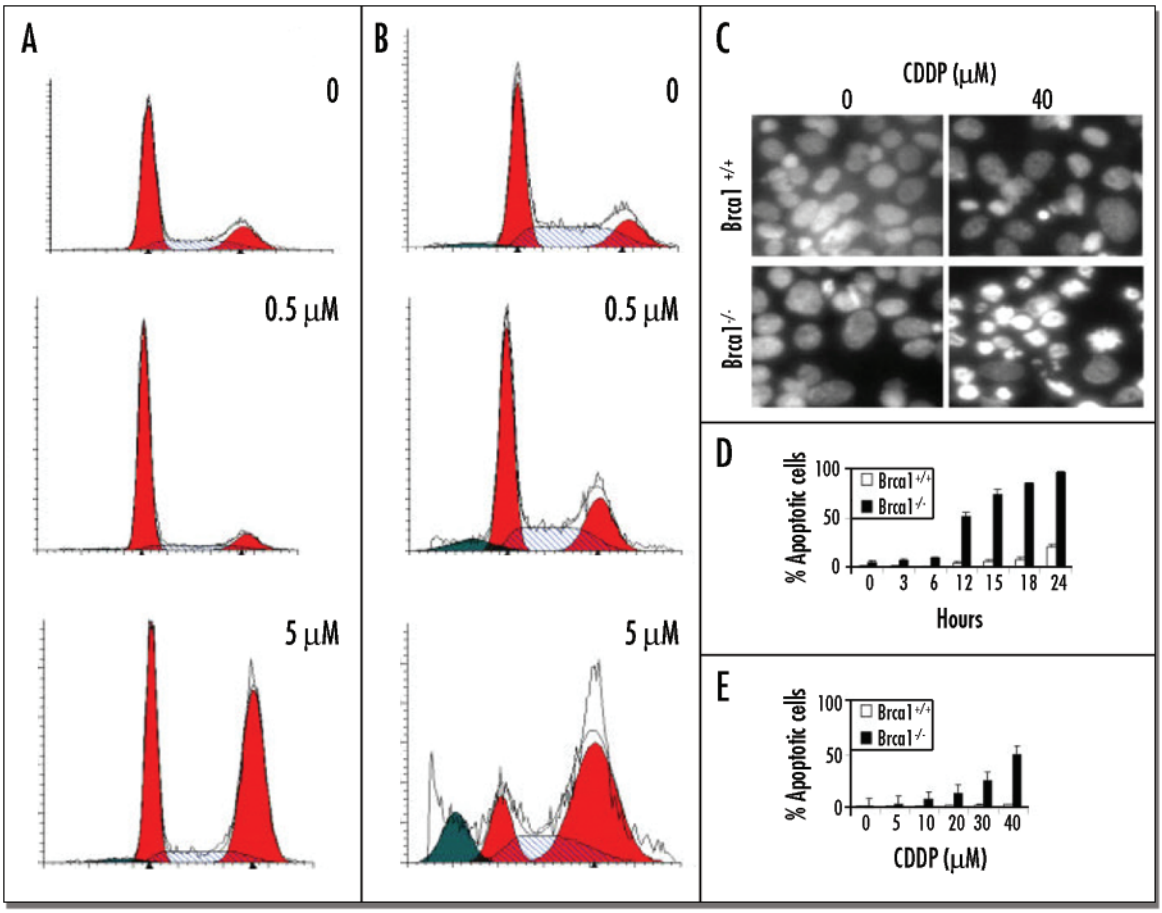

Figure 4. CDDP treatment induces apoptosis in Brca1-deficient MMECs. (A) Brca1$^{+/+}$ MMECs and (B) Brca1$^{-/-}$ MMECs were either untreated or treated with increasing doses of CDDP for 72 hours. Seventy two hours after CDDP treatment, FACS analysis was performed as described in Materials and Methods. (C) Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were either untreated or treated with 40 μM of CDDP for 48 hours. Cells were identified for apoptosis by DAPI staining and fluorescence microscopy as described in materials and methods. (D) Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were either untreated or treated with 40 μM of CDDP for 0–24 hours or, (E) 0–40 μM of CDDP for 12 hours. Cells were stained with DAPI and the percentage of DAPI positive cells is shown as the mean ± S.D. of quadruplicate samples.

as Brca1$^{+/+}$ MMECs. Following transduction of Brca1$^{+/+}$ MMECs with a retrovirus expressing Cre recombinase,[31] Brca1$^{-/-}$ MMECs were isolated by selection in puromycin.

To confirm deletion of exon 11 in Brca1$^{-/-}$ MMECs, the segment flanking Brca1 exon 11 was amplified by PCR and the results shown in Figure 1C reveal the presence of a 600 bp Brca1 product formed as a result of Cre-mediated recombination and subsequent deletion of exon 11 in Brca1$^{-/-}$ MMECs. As shown in Figure 1C, this 600 bp product is detected in Brca1$^{-/-}$ MMECs only and not detected in Brca1$^{+/+}$ MMECs. The deletion of exon 11 was confirmed by a second PCR assay using primers that amplify a region of exon 11 (Fig. 1D). In this experiment, a 592 bp PCR product corresponding to exon 11 is observed in Brca1$^{+/+}$ but not in Brca1$^{-/-}$ MMECs (Fig. 1D). This indicates homozygous deletion of exon 11 in Brca1$^{-/-}$ MMECs. The presence or absence of exon 11 sequences in Brca1 transcripts in Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs was also confirmed by RT-PCR (Fig. 1E). In this analysis, only the Brca1$^{+/+}$ cells show a 392 bp-long exon 11-specific RT-PCR product.

To examine whether the Brca1$^{-/-}$ MMECs generated the predicted truncated transcript, northern blot analysis was performed using a probe encompassing nucleotides 4827 to 5354 that recognizes both full length and exon 11-deleted Brca1 transcripts. As shown in Figure 1F, a 3.9 kb truncated Brca1 transcript was detected in the Brca1$^{-/-}$ MMECs whereas only a full-length 7.2 transcript was detected in the Brca1$^{+/+}$ MMECs (Fig. 1F).

Brca1 protein expression by western blot analysis is shown in (Fig. 1G). In these experiments, Brca1$^{+/+}$ MMECs were found to express the full-length

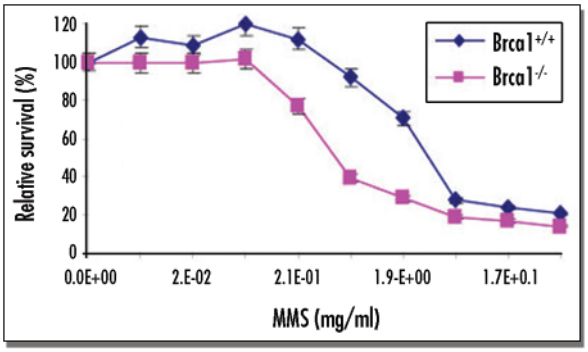

Figure 5. Sensitivity of Brca$^{-/-}$ MMECs to MMS. Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were exposed to increasing concentrations of MMS as described in Materials and Methods. After incubation at 37°C for 7 days, MTT assay was performed. Cell survival was expressed as the fraction of MMS-treated surviving cells to the untreated cells. The results are shown as the mean ± S.D. of four independent experiments each done in quadruplicate samples.

Brca1 protein (210 kDa), similar to that found in mouse (HC11) epithelial cells and human MCF-7 breast cancer cells. In contrast, Brca1$^{-/-}$ MMECs expressed a truncated Brca1 immunoreactive protein (92 kDa).

We also examined the levels of p53 protein expression in the immortalized Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs (Fig. 1H). Western blot analysis with anti-p53

antibody showed a p53-specific band in control murine mammary epithelial cells (HC11) that are known to express high levels of p53 protein, but not in Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs.

Growth and Sensitivity of Brca1$^{-/-}$ MMECs to CDDP and MMS. As shown in Figure 2, the loss of Brca1 function in Brca1$^{-/-}$ MMECs did not affect the growth rate relative to Brca1$^{+/+}$ MMECs (Fig. 2). Therefore, these paired cell lines represent useful models to examine the effect of Brca1 function on the sensitivity of mammary epithelial cells to various agents.

We first examined the biological consequence of Brca1-deficiency in MMECs on the sensitivity of cells to CDDP (Fig. 3). CDDP is the most effective drug for treatment of ovarian cancer[33,34] and has also been suggested for the treatment of breast cancer.[35] As shown in Figure 3 Brca1-deficient MMECs are more sensitive to CDDP than MMECs expressing wild-type Brca1. There is a dose-dependent decrease in cellular survival in both Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs following exposure to increasing doses of CDDP (Fig. 3). However, deletion of Brca1 exon 11 was associated with a 22.6-fold increase in sensitivity to CDDP relative to Brca1$^{+/+}$ MMECs. Thus, the IC50 for CDDP is 0.0398 μM in Brca1$^{-/-}$ MMECs and 0.9 μM in Brca1$^{+/+}$ MMECs (Table 1). These results in MMECs as well as these of previous studies in human breast cancer cells[36,37] suggest that BRCA1 is involved in the repair of CDDP-induced DNA damage in mammary epithelial cells.

In order to determine whether increased CDDP sensitivity in Brca1$^{-/-}$ MMECs is associated with enhanced CDDP-induced apoptosis, both Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were exposed to CDDP at the indicated doses. After 72 hours of incubation at 37ºC, MMECs nuclei were stained with propidium iodide and analyzed by FACS for apoptosis, as described in materials and methods. As shown in Figure 4A, CDDP treatment in Brca1$^{+/+}$ MMECs resulted in a G_2/M phase arrest in a dose dependent manner (0.5-5 μM). In contrast, CDDP treatment in Brca1$^{-/-}$ MMECs resulted in induction of apoptosis as indicated by the presence of a sub-G_1 peak, as is shown in Figure 4B.

In order to confirm these results and quantitate the induction of apoptosis, both Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs were treated with 40 μM CDDP for 48 h and stained with DAPI as described in materials and methods. Brca1$^{-/-}$ MMECs treated with 40 μM CDDP resulted in accumulation of cells with condensed and fragmented nuclei, indicative of apoptosis compared to Brca1$^{+/+}$ MMECs (Fig. 4C). Furthermore, as shown in Figure 4D and E, the increased apoptosis in Brca1$^{-/-}$ MMECs is observed in a time-dependent (3–24 hours) and dose-dependent (5–40 μM) manner following exposure to CDDP for 12 hours compared to Brca1$^{+/+}$ MMECs.

Previous studies have also indicated that BRCA1-deficient human breast cancer (HCC1937) cells were found to be more sensitive to the alkylating agent MMS in DNA, relative to the MCF-7 human breast cancer cells that express BRCA1.[24] Since MMS is a potent inducer of BER,[38,39] we compared the sensitivity of Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs to increasing exposure to MMS. As shown in Figure 5 and Table 1, loss of Brca1 expression in Brca1$^{-/-}$ MMECs was associated with a 5.2-fold increased sensitivity to MMS compared to Brca1$^{+/+}$ MMECs (IC50 of 2600 μM and 500 μM respectively). These studies indicate that Brca1 also plays a role in the repair of MMS-induced DNA damage.

DISCUSSION

Although BRCA1 is broadly expressed in various tissues, germline mutations in the BRCA1 are predominantly associated with increased breast and ovarian cancers. The reason for this tissue selectivity is not known. In order to study the role of the BRCA1 gene in the development of mammary cancer and the sensitivity of mammary epithelial cells to DNA damaging agents, we have isolated a pair of murine mammary epithelial cell lines (MMECs) that differ only in Brca1 expression (Brca1$^{+/+}$ and Brca1$^{-/-}$ MMECs). Studies presented in this report indicate that Brca1-deficiency renders MMECs sensitive to agents (MMS, CDDP) that induce DNA damage.

Brca1$^{-/-}$ MMECs are more sensitive to CDDP- and MMS exposure compared to Brca1$^{+/+}$ MMECs, suggesting that loss of Brca1 causes a deficiency in repair of CDDP- and MMS-induced DNA damage. Since CDDP-induced DNA adducts are removed from DNA by NER[40,41] and MMS-induced DNA damage is repaired by BER,[39] these finding simply that loss of Brca1 is involved in both NER and BER pathways.

CDDP is an effective drug in the treatment of ovarian cancer,[33,34] and recent studies suggest that it is useful in the treatment of breast cancer as well.[35] Studies in Brca1$^{-/-}$ MMECs are consistent with clinical studies suggesting improved survival in BRCA1-deficient ovarian cancer patients following treatment with chemotherapy regimens which include CDDP.[42,43,39]

Previous studies have also suggested a role for BRCA1 in the repair of MMS-induced DNA damage. For example, HCC1937 human breast cancer cells, which are deficient in BRCA1 and p53, were found to be more sensitive to MMS compared with MCF-7 human breast cancer cells, which express wild-type BRCA1 and wild-type p53.[24] MMS alkylates DNA to form adducts that are repaired by BER. Since BRCA1 interacts with p53, which is known to be involved in BER, a role of BRCA1 in p53-mediated BER has previously been postulated.[44,45] However, results in Brca1$^{+/+}$ vs Brca1$^{-/-}$ MMECs which lack p53 expression suggest that Brca1 also functions in BER through a p53-independent pathway(s).

A role for BRCA1 in BER could have important implications for breast cancer risk. Previous studies have suggested that DNA damage that induces substrates of BER (oxidized bases and apurinic sites) may be associated with an increased risk of breast cancer. Thus, intracellular metabolism of estrogen to catechol quinones can generate reactive oxygen species that can react with DNA which are subsequently removed and form apurinic sites in DNA[46] which has been shown to be mutagenic.[47] A possible role for estrogen quinone in the development of breast cancer has been suggested by studies that demonstrate higher levels of 4-catechol estrogen quinones in normal breast tissue obtained from women with breast cancer compared to women without breast cancer.[47] Studies in this report indicating a role for BRCA1 in BER in mammary epithelial cells may provide some insights into the role of Brca1 in breast cancer and for the development of specific strategies to treat BRCA1-deficient breast and ovarian cancers.

References

1. Antoniou AC, Pharoah PD, McMullan G, Day NE, Stratton MR, Peto J, Ponder BJ, Easton DF. A comprehensive model for familial breast cancer incorporating *BRCA1*, *BRCA2* and other genes. Br J Cancer 2002; 86:76-83.
2. Rahman N, Stratton MR. The genetics of breast cancer susceptibility. Annu Rev Genet 1998; 32:95-121.
3. Thull DL, Vogel VG. Recognition and management of hereditary breast cancer syndromes. Oncologist 2004; 9:13-24.
4. Cressman VL, Backlund DC, Avrutskaya AV, Leadon SA, Godfrey V, Koller BH. Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice. Mol Cell Biol 1999; 19:7061-75.
5. Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang XW, Harris CC, Ried T, Deng CX. Centrosome amplification and a defective G_2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol Cell 1999; 3:389-95.
6. Deng CX, Brodie SG. Roles of BRCA1 and its interacting proteins. Bioessays 2000; 22:728-37.
7. Deng CX, Scott F. Role of the tumor suppressor gene Brca1 in genetic stability and mammary gland tumor formation. Oncogene 2000; 19:1059-64.
8. Hakem R, de la Pompa JL, Mak TW. Developmental studies of Brca1 and Brca2 knock-out mice. J Mammary Gland Biol Neoplasia 1998; 3:431-45.
9. Abel KJ, Xu J, Yin GY, Lyons RH, Meisler MH, Weber BL. Mouse Brca1: Localization sequence analysis and identification of evolutionarily conserved domains. Hum Mol Genet 1995; 4:2265-73.
10. Sharan SK, Wims M, Bradley A. Murine Brca1: Sequence and significance for human missense mutations. Hum Mol Genet 1995; 4:2275-8.

11. Bennett LM, Brownlee HA, Hagavik S, Wiseman RW. Sequence analysis of the rat Brca1 homolog and its promoter region. Mamm Genome 1999; 10:19-25.
12. Chandler J, Hohenstein P, Swing DA, Tessarollo L, Sharan SK. Human *BRCA1* gene rescues the embryonic lethality of Brca1 mutant mice. Genesis 2001; 29:72-7.
13. Lane TF, Lin C, Brown MA, Solomon E, Leder P. Gene replacement with the human BRCA1 locus: Tissue specific expression and rescue of embryonic lethality in mice. Oncogene 2000; 19:4085-90.
14. MacLachlan TK, Takimoto R, El-Deiry WS. BRCA1 directs a selective p53-dependent transcriptional response towards growth arrest and DNA repair targets. Mol Cell Biol 2002; 22:4280-92.
15. Yarden RI, Pardo-Reoyo S, Sgagias M, Cowan KH, Brody LC. BRCA1 regulates the G_2/M checkpoint by activating Chk1 kinase upon DNA damage. Nat Genet 2002; 30:285-9.
16. Choudhury AD, Xu H, Baer R. Ubiquitination and proteasomal degradation of the BRCA1 tumor suppressor is regulated during cell cycle progression. J Biol Chem 2004; 279:33909-18.
17. Starita LM, Parvin JD. The multiple nuclear functions of BRCA1: Transcription, ubiquitination and DNA repair. Curr Opin Cell Biol 2003; 15:345-50.
18. Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, Englert C, Christians FC, Ellisen LW, Maheswaran S, Oliner JD, Haber DA. Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. Cell 1999; 97:575-86.
19. Thangaraju M, Kaufmann SH, Couch FJ. BRCA1 facilitates stress-induced apoptosis in breast and ovarian cancer cell lines. J Biol Chem 2000; 275:33487-96.
20. Yan Y, Haas JP, Kim M, Sgagias MK, Cowan KH. BRCA1-induced apoptosis involves inactivation of ERK1/2 activities. J Biol Chem 2002; 277:33422-30.
21. Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science 1999; 286:1162-6.
22. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. Nature 2000; 404:201-4.
23. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston DM. Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 1997; 88:265-75.
24. Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH. Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science 1999; 285:747-50.
25. Moynahan ME, Cui TY, Jasin M. Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. Cancer Res 2001; 61:4842-50.
26. Hartman AR, Ford JM. BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair. Nat Genet 2002; 32:180-4.
27. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene *BRCA1* is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 2000; 275:23899-903.
28. Abbott DW, Thompson ME, Robinson-Benion C, Tomlinson G, Jensen RA, Holt JT. BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair. J Biol Chem 1999; 274:18808-12.
29. Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M, Livingston DM. Genetic analysis of BRCA1 function in a defined tumor cell line. Mol Cell 1999; 4:1093-9.
30. Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX. Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. Nat Genet 1999; 22:37-43.
31. Wagner KU, Krempler A, Qi Y, Park K, Henry MD, Triplett AA, Riedlinger G, Rucker III EB, Hennighausen L. Tsg101 is essential for cell growth, proliferation, and cell survival of embryonic and adult tissues. Mol Cell Biol 2003; 23:150-62.
32. Huber LJ, Yang TW, Sarkisian CJ, Master SR, Deng CX, Chodosh LA. Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. Mol Cell Biol 2001; 21:4005-15.
33. McGuire WP, Ozols RF. Chemotherapy of advanced ovarian cancer. Semin Oncol 1998; 25(3):340-8.
34. McGuire WP. Current aspects of adjuvant therapy of early stage ovarian cancer. Zentralbl Gynakol 1998; 120:93-7.
35. Van Poznak C, Tan L, Panageas KS, Arroyo CD, Hudis C, Norton L, Seidman AD. Assessment of molecular markers of clinical sensitivity to single-agent taxane therapy for metastatic breast cancer. J Clin Oncol 2002; 20:2319-26.
36. Husain A, He G, Venkatraman ES, Spriggs DR. BRCA1 up-regulation is associated with repair-mediated resistance to cis-diamminedichloroplatinum(II). Cancer Res 1998; 58:1120-3.
37. Quinn JE, Kennedy RD, Mullan PB, Gilmore PM, Carty M, Johnston PG, Harkin DP. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res 2003; 63:6221-8.
38. Hanna M, Chow BL, Morey NJ, Jinks-Robertson S, Doetsch PW, Xiao W. Involvement of two endonuclease III homologs in the base excision repair pathway for the processing of DNA alkylation damage in Saccharomyces cerevisiae. DNA Repair (Amst) 2004; 3:51-9.
39. Chen KH, Srivastava DK, Wilson SH. Relationship between base excision repair capacity and DNA alkylating agent sensitivity in mouse monocytes. Mutat Res 2001; 487:121-6.
40. Kartalou M, Essigmann JM. Recognition of cisplatin adducts by cellular proteins. Mutat Res 2001; 478:1-21.
41. Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. Mutat Res 2001; 478:23-43.
42. Sloots K, Ausems MG, de Haan HH. Ovarian cancer in BRCA-positive women: Vigilance is mandatory despite screening programs. Eur J Obstet Gynecol Reprod Biol 2002; 101:196-8.
43. Aida H, Takakuwa K, Nagata H, Tsuneki I, Takano M, Tsuji S, Takahashi T, Sonoda T, Hatae M, Takahashi K, Hasegawa K, Mizunuma H, Toyoda N, Kamata H, Torii Y, Saito N, Tanaka K, Yakushiji M, Araki T, Tanaka K. Clinical features of ovarian cancer in Japanese women with germ-line mutations of BRCA1. Clin Cancer Res 1998; 4:235-40.
44. Offer H, Erez N, Zurer I, Tang X, Milyavsky M, Goldfinger N, Rotter V. The onset of p53-dependent DNA repair or apoptosis is determined by the level of accumulated damaged DNA. Carcinogenesis 2002; 23:1025-32.
45. Seo YR, Fishel ML, Amundson S, Kelley MR, Smith ML. Implication of p53 in base excision DNA repair: In vivo evidence. Oncogene 2002; 21:731-7.
46. Cavalieri EL, Rogan EG, Chakravarti D. Initiation of cancer and other diseases by catechol ortho-quinones: A unifying mechanism. Cell Mol Life Sci 2002; 59:665-81.
47. Rogan EG, Badawi AF, Devanesan PD, Meza JL, Edney JA, West WW, Higginbotham SM, Cavalieri EL. Relative imbalances in estrogen metabolism and conjugation in breast tissue of women with carcinoma: Potential biomarkers of susceptibility to cancer. Carcinogenesis 2003; 24:697-702.