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BRCA1 Induces Antioxidant Gene Expression and Resistance to Oxidative Stress

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ABSTRACT

Mutations of the breast cancer susceptibility gene 1 (BRCA1), a tumor suppressor, confer an increased risk for breast, ovarian, and prostate cancers. To investigate the function of the BRCA1 gene, we performed DNA microarray and confirmatory reverse transcription-PCR analyses to identify BRCA1-regulated gene expression changes. We found that BRCA1 up-regulates the expression of multiple genes involved in the cytoprotective antioxidant response, including glutathione *S*-transferases, oxidoreductases, and other antioxidant genes. Consistent with these findings, BRCA1 overexpression conferred resistance while BRCA1 deficiency conferred sensitivity to several different oxidizing agents (hydrogen peroxide and paraquat). In addition, in the setting of oxidative stress (due to hydrogen peroxide), BRCA1 shifted the cellular redox balance to a higher ratio of reduced to oxidized glutathione. Finally, BRCA1 stimulated antioxidant response element-driven transcriptional activity and enhanced the activity of the antioxidant response transcription factor nuclear factor erythroid-derived 2 like 2 [also called NRF2 (NFE2L2)]. The ability of BRCA1 to stimulate antioxidant response element-dependent transcription and to protect cells against oxidative stress was attenuated by inhibition of nuclear factor erythroid-derived 2 like 2. These findings suggest a novel function for BRCA1, *i.e.,* to protect cells against oxidative stress. This function would be consistent with the postulated role of BRCA1 as a caretaker gene in preserving genomic integrity.

INTRODUCTION

Inherited mutations of the breast cancer susceptibility gene breast cancer susceptibility gene 1 (BRCA1) confer an increased risk for breast, ovarian, and prostate cancers (1, 2). In addition, BRCA1 expression is often decreased or absent in sporadic breast and ovarian cancers due, in part, to promoter methylation or other causes, suggesting a role for BRCA1 in nonhereditary tumors (3, 4). The specific functions of the BRCA1 gene that contribute to tumor suppression are unclear. However, established functional roles for BRCA1 include the regulation of cell cycle progression, DNA damage signaling and repair, maintenance of genomic integrity, and the regulation of various transcriptional pathways [reviewed by Rosen *et al.* (5)].

A role for BRCA1 in transcriptional regulation was first suggested by the finding that BRCA1 has a conserved acidic COOH-terminal transcriptional activation domain (6). Although BRCA1 is not known to bind to specific DNA sequences, it may regulate transcription through protein:protein interactions with components of the basal transcription factor (*e.g.,* RNA helicase A and RNA pol II), transcriptional coactivators and corepressors [*e.g.,* p300 and its functional homologue CBP (the cAMP-responsive element binding protein-binding protein), retinoblastoma 1, retinoblastoma 1-associated proteins (RbAp46/48), and several histone deaceylases (HDAC-1/2)], and/or sequence-specific DNA-binding transcription factors (*e.g.,* p53, c-Myc, estrogen receptor, and other proteins; refs. 7–12).

Some of the functions of BRCA1 cited above may be due, in part, to regulation of specific transcriptional pathways by BRCA1, but the linkage of these functions to BRCA1-regulated transcription is not well understood. We used cell culture models of BRCA1 overexpression, underexpression, and mutational inactivation to identify patterns of BRCA1-regulated gene expression. The identification of antioxidant genes as transcriptional targets of BRCA1 led to the findings that BRCA1 regulates the activity of the antioxidant response transcription factor nuclear factor erythroid-derived 2 like 2 [also called NRF2 (NFE2L2)] and protects cells against oxidative stress.

MATERIALS AND METHODS

Cell Lines and Culture

Human prostate (DU-145, LNCaP) and breast (MCF-7, T47D) cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA) and cultured as described before (12, 13). The stable wild-type BRCA1 (wtBRCA1) and control (Neo) DU-145 cell clones were isolated and characterized earlier (14, 15). A mouse embryonic fibroblast (MEF) cell line homozygous for a deletion of Brca1 exon 11 and the control wild-type ($Brca1^{+/+}$) MEFs (16) were provided by Dr. Chuxia Deng (National Institutes of Diabetes, Digestive and Kidney Diseases, Bethesda, MD). All of the above cell types were grown in DMEM supplemented with 5% (DU-145) or 10% (all other cell types) v/v fetal calf serum, L-glutamine (5 mmol/L), nonessential amino acids (5 mmol/L), penicillin (100 units/mL), and streptomycin (100 μg/mL; all obtained from BioWhittaker, Walkersville, MD).

EBV-immortalized peripheral blood lymphocyte cell lines R794 and R1041 were derived from a female BRCA1 (185delAG) and BRCA2 (6174delT) mutation carrier, respectively. These lymphoblastoid cell lines were provided by the Tissue Culture Shared Resource of the Lombardi Comprehensive Cancer Center. The genotypes of the cells were confirmed by the Familial Cancer Registry of the Lombardi Comprehensive Cancer Center.

BRCA1 Expression Vectors and Transfections

For transient expression experiments, cells were transfected with a wild-type BRCA1 expression vector (wtBRCA1) consisting of the full-length BRCA1 cDNA within the pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA) or within the pCMV-Tag2B vector (Stratagene, La Jolla, CA), which allows expression of the full-length protein containing a NH_2-terminal FLAG epitope tag. Both the untagged and the FLAG-tagged proteins are expressed well and exhibit identical biological activities (13). Methodologies used for transient transfections have been reported previously (13, 15) and are also briefly described below.

Small Interfering (si) RNAs

The BRCA1 and control (scrambled-sequence) siRNAs were described earlier (15). All siRNAs were chemically synthesized by Dharmacon, Inc. (Lafayette, CO). For siRNA treatments, subconfluent proliferating cells were treated with each siRNA (50 nmol/L), with siPORT Amine reagent (Ambion, Austin, TX). The cells were incubated with siRNA for 72 hours (to reduce BRCA1 protein levels to <25% of control) before the start of the experiment. The control siRNA has no effect on BRCA1 levels (15), and neither siRNA is toxic to the cells under these

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Note: Supplementary data for this article can be found at Cancer Research Online (http://cancerres.aacrjournals.org).

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Table 1 *PCR primers and expected product sizes for semi-quantitative reverse transcription-PCR analyses*

Gene name	Symbol	Primer sequences (5′→3′)	Genbank accession no.	Product size (bp)
Breast cancer susceptibility gene 1	BRCA1	F: ttgcgggaggaaaatgggtagtta B: tgtgccaagggtgaatgatgaaag	U14680	292
Annexin 1	ANX1	F: agtcatccaaggtggtcccgg B: ggccctggcatctgaatcagcc	NM_000700	539
Mesoderm specific transcript homologue	MEST	F: actatttcctaaaatcacaggacattaagg B: atcacttaataatcctgctttgactaagac	NM_002402	411
Microphthalmia-associated transcription factor	MITF	F: ctaacctgtacaacaactctcgatctc B: atcacttaataatcctgctttgactaagac	XM_010977	486
Microsomal GST1	MGSTI	F: aaaagaccacgagctcagga B: 5 aaagacctgacccaaaac′	AY368173	346
Cardiac ankyrin repeat protein	CARP	F: gtgttgtgaagatgtacctaatgaagtt B: taatcagccctctcttaaaaactcttac	XM_051726	297
Coch-5B2	COCH1	F: aaagcagatgtcctctgcccaggggg B: tttcctgtattggaattacccccctctg	XM_007471	300
Brain-derived neutrophic factor	BDNF	F: gattcataaggatagacacttcttgtg B: aaggaatgtaatgcagacttttttaagtt	XM_006027	287
G_0-S transition 2	G0S2	F: gctgtttttttatttggacttaacttcagag B: ccttttgatgttaaaatgctaaatctcacg	M72885	531
Tumor necrosis factor α	TNFA	F: tacagatgaatgtatttatttgggagac B: atcagcattgtttagacaacttaatcag	M10988	198
Prostacyclin-stimulating factor		F: gtatctcctctaagtaaggaagatgctg B: tgtgatctttattttgtatttctctgtg	S75725	299
Expressed sequence tags, weakly similar to TAL-6		F: ttcttctggtactggagaataataacaac B: aatcccataagcctagaatctgtaaggaaac	R16604	249
Ceruloplasmin	CP	F: tatagttctgatgtctttgacattttcc B: atgcttccagtcttcttttaatgtttat	XM_011006	293
Tumor necrosis factor-inducible protein	TSG6	F: atggatggctaagggcagagttgg B: gctcatctccacagtatcttccc	NM_007115	386
Dickkopf homologue 3	DKK3	F: tctaatgaagacggtgatgttgacactg B: ctttaaaccttaagaactctggatgaat	NM_015881	337
Caveolin 1	CAV1	F: gcaagtgtacgacgcgcacacc B: ctgatgcactgaatctcaat	NM_001753	318
NAD(P)H quinone oxidase 1	NQO1	F: ggtcagaagggaattgctca B: ctccagcctgggtaacagag	NM_000903	302
GST ζ 1	GSTZ1	F: gtggggtggagtagggagat B: aggcaagtggctgactgact	AH006398	334
β-Actin	ACTB	F: tagcggggttcacccacactgtgccccatcta B: ctagaagcatttgcggtggaccgatggaggg	XM_004814	661

experimental conditions, as determined by the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays.

Isolation of RNA

The total cellular RNA was extracted with TRIzol Reagent (Life Technologies, Inc., Rockville, MD), according to the manufacturer's instructions, additionally purified with chloroform and precipitated with 95% etomidate before cDNA synthesis. The quality of isolated RNA was verified by electrophoresis through 1.0% agarose-formaldehyde gels, and its quantity was determined from absorbance measurements at 260 and 280 nm.

DNA Microarray Analyses

cDNA Spotted Microarrays. cDNA-spotted slides corresponding to 9216 human genes (including expressed sequence tags) and 9568 mouse genes (including expressed sequence tags) were prepared at the Albert Einstein College of Medicine microarray facility (Bronx, NY). cDNA synthesis, hybridizations, scanning, gridding, and analysis have been described earlier (ref. 17; also see web site).[4] On the basis of our experience suggesting that cDNA spotted microarrays often underestimate differences in gene expression (17), ratios of gene expression were considered to be significant if they were $\geq$1.5 or $\leq$ 0.7 in at least two independent experiments.

Microarray Comparisons. For DU-145 cells, we compared gene expression in two different wtBRCA1 *versus* Neo clone pairs, with two independent experiments per clone pair, for a total of $n = 4$ independent experiments. For MCF-7 cells, subconfluent proliferating cells were transiently transfected with wtBRCA1 or empty pcDNA3 vector (15) and postincubated for 24 hours to allow gene expression. Three independent experiments comparing wtBRCA1- *versus* pcDNA3-transfected cells were made after confirming that the wtBRCA1 gene was expressed in each experiment. For MEFs, we performed three independent comparisons of Brca1-deficient (Δ exon 11) *versus* wild-type MEFs. In each case, the ratios of gene expression were considered to be significant if they were $\geq$1.5 or $\leq$0.7 in at least two independent experiments.

Affymetrix Oligonucleotide Microarrays. Affymetrix microarray analyses were performed at the North Shore-Long Island Jewish Research Institute core facility. RNA isolation, cRNA synthesis, gene chip hybridizations, and data analysis were performed as described earlier (18). We performed one experiment each comparing a DU-145 wtBRCA1 *versus* Neo clone pair and comparing Brca1-deficient MEFs *versus* wild-type MEFs. The gene chips used for these experiments were HG-U133A (which contains ~16,000 human probe sets) and MG_U74Av2 (which contains ~12,000 mouse genes plus expressed sequence tags). Differences in gene expression were considered to be significant if the log signal ratios were $\geq +1$ or ≤ -1 and the P values were significant according to the Affymetrix algorithm. These log signal ratio cutoffs correspond to ratios of $\geq$2.0 or $\leq$0.5, respectively.

Semiquantitative Reverse Transcription-PCR Analysis

Rigorously controlled semiquantitative reverse transcription-PCR assays were performed as described before (15, 17). The PCR primers, reaction conditions, and cycle numbers are shown in Tables 1 and 2. The PCR reactions were individually optimized so that each reaction fell within the linear range of product amplification. The first-strand cDNA template was generated from 1 μg of total RNA in a final volume of 20 μL, with SuperScript II reverse transcriptase (Life Technologies, Inc.) and oligo(dT) primers. One microliter (of 20 μL) of 1:2.5-diluted cDNA template was amplified in a total volume of 50 μL, containing 200 μmol/L each of all four deoxynucleoside triphosphates, 2 μmol/L each of specific primers, and 1 unit of Tag DNA polymerase (Perkin-Elmer, Norwalk, CT). β-Actin, whose expression is unaffected by BRCA1, was used as a control for loading. The PCR products were analyzed by electrophoresis through 1.0% agarose gels containing ethidium bromide (0.1 mg/mL) and photographed under UV illumination.

[4] Internet address: http://www.aecom.yu.edu/cancer/new/cores/microarray.

Table 2 *PCR reaction conditions*

Gene	PCR cycle parameters	No. of cycles
BRCA1	94°C (30 s); 56°C (30 s); 72°C (30 s)	23
ANX1	94°C (1 min); 55°C (1 min); 72°C (1 min)	30
MEST	94°C (30 s); 53°C (30 s); 72°C (30 s)	35
MITF	94°C (30 s); 53°C (30 s); 72°C (30 s)	35
MGST1	94°C (30 s); 50°C (30 s); 72°C (30 s)	30
CARP	94°C (30 s); 55°C (30 s); 72°C (30 s)	35
COCH1	94°C (30 s); 55°C (30 s); 72°C (30 s)	38
BDNF	94°C (30 s); 55°C (30 s); 72°C (30 s)	35
G0S2	94°C (60 s); 55°C (60 s); 72°C (60 s)	35
TNFA	94°C (30 s); 53°C (30 s); 72°C (30 s)	35
Prostacyclin-stimulating factor	94°C (30 s); 55°C (30 s); 72°C (30 s)	35
Expressed sequence tags, weakly similar to TAL-6	94°C (30 s); 50°C (30 s); 72°C (30 s)	35
CP	94°C (30 s); 55°C (30 s); 72°C (30 s)	35
TSG6	94°C (30 s); 53°C (30 s); 72°C (30 s)	35
DKK3	94°C (30 s); 55°C (30 s); 72°C (30 s)	38
CAV1	94°C (30 s); 55°C (30 s); 72°C (30 s)	32
NQO1	94°C (30 s); 55°C (30 s); 72°C (30 s)	35
GSTZ1	94°C (1 min); 53°C (1 min); 72°C (1 min)	42
ACTB	94°C (30 s); 56°C (30 s); 72°C (30 s)	23

Assays of Oxidant Sensitivity

MTT Dye Reduction. Subconfluent proliferating cells in 96-well dishes were treated with different doses of H_2O_2 or paraquat (Sigma Chemical Co., St. Louis, MO) for 24 hours (or for different time intervals) and then assayed for MTT dye reduction, a measure of mitochondrial viability (14, 19). Cell viability was normalized to 0 dose control cells. Cell viability values were calculated as means ± SE of $n = 10$ replicate wells or as means ± SE for three independent experiments, each of which used $n = 10$ replicate wells per cell type per assay condition.

Trypan Blue Dye Exclusion. This assay measures the ability of intact cell membranes of viable cells to exclude trypan blue dye. Subconfluent proliferating DU-145 cells in 100-mm plastic Petri dishes were transfected overnight using Lipofectamine with a FLAG-wtBRCA1 expression vector or the empty pCMV-Tag2B vector (15 μg of plasmid DNA per dish), washed, and allowed to recover and express the transgene for 24 hours. wtBRCA1-transfected, empty vector-transfected, and untransfected control cells were harvested, plated into 2-cm^2 wells (8×10^4 cells per well in quadruplicate wells), allowed to attach, and exposed to different doses of H_2O_2 for T = 24 hours at 37°C. The cells were then collected, suspended in a solution containing 0.4% trypan blue, and counted with a hemocytometer. For each experiment and dose of H_2O_2, at least 200 cells were counted per well. Three independent experiments were performed; and the cell viability values were expressed as means ± SE.

Assays of the Cellular Redox State

Subconfluent proliferating cells in 24-well dishes were transfected overnight with wtBRCA1 or empty pcDNA3 vector (0.25 μg per well) with Lipofectamine, washed, and postincubated in fresh culture medium for 24 hours to allow gene expression. The cells were then treated with different doses of H_2O_2 for T = 24 hours and assayed for reduced (GSH) or oxidized (GSSG) forms of glutathione using a kit from Oxis, Inc. (Portland, OR).

Transcriptional Assays

The wild-type NRF2 vector, dominant negative NRF2 vector (DN-NRF2), NQO1-ARE-Luc reporter, and mutant or truncated BRCA1 expression vectors have been described earlier (12, 20). The NQO1-ARE-Luc reporter contains the antioxidant response element (ARE) of NAD(P)H dehydrogenase quinone 1 (NQO1), driving a minimal promoter upstream of the luciferase gene. Transient transfection assays were performed to measure transcriptional activity, as described earlier (12, 15). Briefly, subconfluent proliferating cells in 24-well dishes were transfected overnight with the indicated expression vector(s) (0.25 μg per well) and luciferase reporter (0.25 μg per well), with Lipofectamine. The cells were washed and postincubated for 24 hours to allow luciferase expression. Luciferase values (minus background) were normalized to the control (reporter only) and expressed as means ± SE of quadruplicate wells. Transfection efficiency was monitored using the control plasmid pRSV-β-gal (15).

Western Blotting

Whole cell lysates were prepared and subjected to Western blotting, as described earlier (14, 15). Briefly, equal aliquots of total cellular protein (50 μg per lane) were electrophoresed on a 4 to 13% SDS-polyacrylamide gradient gel, transferred to nitrocellulose membranes (Millipore, Bedford, MA), and blotted with primary antibodies directed against human BRCA1 (C-20, rabbit polyclonal, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) and α-actin (I-19, goat polyclonal, , 1:500; Santa Cruz Biotechnology). After incubation with the appropriate horseradish peroxidase conjugated secondary antibody (Amersham Lifescience), immune complexes were visualized by using an enhanced chemiluminescence detection system (Amersham Lifescience, Buckinghamshire, UK), with colored markers (Bio-Rad, Hercules, CA) as molecular size standards.

Statistical Methods

Where appropriate, statistical comparisons were made using the two-tailed Student's *t* test.

RESULTS

Microarray Analysis of BRCA1-overexpressing Cell Lines. To determine the effect of BRCA1 overexpression on the transcriptosome, we compared the gene expression profiles of DU-145 human prostate cancer cell clones stably expressing a wild-type BRCA1 gene (wtBRCA1) with control (Neo) clones with cDNA spotted microarrays. These wtBRCA1 and Neo cell clones have been described and extensively characterized in previous studies (13–15). A partial list of genes up-regulated in the wtBRCA1 clones, categorized by function, is shown in Table 3 (see Supplemental Material for complete list). The wtBRCA1 cells showed up-regulation of various types of genes, including those involved in transcription, stress responses, DNA replication and repair, signal transduction, metabolism, differentiation, and RNA and protein processing. As a check on the methodology, a separate experiment revealed that a large number of genes identified by the cDNA-spotted arrays were concordantly up-regulated with an Affymetrix oligonucleotide microarray (Table 3).

Noticeably, the wtBRCA1 cell lines overexpressed many genes that protect against oxidative stress, including microsomal glutathione *S*-transferases (GSTs; MGST1 and MGST2), cytoplasmic GSTs (GSTT1 and GSTZ1), a glutathione peroxidase (GPX3), and various oxidoreductases [*e.g.,* NQO1, alcohol dehydrogenase 5 (ADH5), and malic enzyme (ME2)]. wtBRCA1 also up-regulated other potential antioxidant genes, including paraoxonase 2 (PON2), an enzyme that hydrolyzes toxic organophosphates (*e.g.,* pesticides) and oxidized lipids (*e.g.,* oxidized low density lipoprotein; ref. 21), the Klotho gene (KL), a deficiency of which causes oxidative brain damage and a shortened life span in mice (22), and ubiquitin carboxyl-terminal esterase L1 (UCHL1), an oxidation-sensitive ubiquitin recycling enzyme that has been implicated in Parkinson's disease (23). A number of these genes are involved in xenobiotic and drug metabolism: *e.g.,* GSTs, NQO1, PON2, and member of PAS protein 2 (MOP2, also called HIF2α), an aryl hydrocarbon receptor (AhR) family gene.

Somewhat fewer genes were down-regulated by wtBRCA1 than were up-regulated (see Table 4 for a partial list and see Supplemental Material for the complete list). Again, an Affymetrix microarray experiment identified many of the same genes found with cDNA spotted arrays (Table 4). Only one GST, GSTP1, was decreased in wtBRCA1 clones. Interestingly, the overexpression of this particular isoform of GST in cancer cell lines is associated with cellular chemoresistance (24). Various genes involved in cell cycle regulation and DNA repair were down-regulated, including the retinoblastoma susceptibility gene retinoblastoma 1, which is known to be down-regulated by wtBRCA1 (13).

Table 3 *Genes for which expression is increased in DU-145 wtBRCA1 [versus control (Neo)] cell clones*

Accession no.	Gene name	Symbol	Ratio: Mean ± SE (Range)	Ratio: Affymetrix oligonucleotide array ($N = 1$)
Transcription/Nuclear proteins				
AA969184	Cytokine inducible nuclear protein (= cardiac ankyrin repeat protein)	CARP	10.1 ± 4.6 (3)	
H45711	Zinc finger transcription factor hEZF	EZF	3.4 ± 1.2 (2)	
AA115076	Human msg1-related gene 1 (mrg1) mRNA	MRG1	2.9 ± 0.3 (2)	
T72202	Transcription factor IL-4 Stat (also known as Stat6)	STAT6	2.7 ± 0.2 (3)	
AA425238	Proto-oncogene AML1 {alternative products}	CBFA2	2.6 ± 0.3 (3)	6.1
AA911236	v-Myb avian myeloblastosis viral oncogene homologue-like 1 (= AMYB)	MYBL1	2.6 ± 0.2 (3)	13
N66177	Microphthalmia-associated transcription factor	MITF	2.6 ± 0.3 (2)	2.8
H96235	v-Ets avian erythroblastosis virus E26 oncogene homologue 2	ETS2	2.4 ± 0.1 (2)	12
Stress response: oxidative stress and xenobiotic detoxification				
AA495936	GST, microsomal 1	MGST1	10.2 ± 4.4 (3)	
AA670438	Ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	UCHL1	8.2 ± 4.0 (2)	18
AA455538	NAD(P)H:menadione oxidoreductase (also called NQO1)	NMOR1	3.8 ± 0.9 (3)	4.6
AA680300	Member of PAS protein 2 (MOP2, also called HIF2α)	MOP2	3.4 ± 0.5 (3)	
W73474	GST, microsomal 2	MGST2	3.3 ± 1.3 (2)	5.2
N93686	Aldehyde dehydrogenase 7	ALDH7	3.2 ± 0.4 (2)	
AA625806	Ninjurin1 (nerve injury-induced protein 1)	NINJ1	2.7 ± 0.3 (4)	
AA677655	Klotho protein	KL	2.6 ± 0.5 (2)	
H99813	GST θ1	GSTT1	2.5 ± 0.2 (3)	2.3
AA664180	Glutathione peroxidase 3 (plasma)	GPX3	2.4 ± 0.5 (2)	5.3
AA453859	Alcohol dehydrogenase 5 (χ subunit, class III)	ADH5	2.3 ± 0.4 (3)	
AA446301	Paraoxonase (PON2 protein)	PON2	2.3 ± 0.4 (3)	
AA428859	Glutathione transferase ζ 1 (GSTZ1)	GSTZ1	2.1 ± 0.1 (2)	
H99699	Aconitase 2, mitochondrial	ACO2	2.0 ± 0.1 (2)	
H21041	Activating transcription factor 3	ATF3	1.9 ± 0.3 (2)	
Replication/Cell cycle/DNA repair and metabolism				
R60343	5′; nucleotidase (CD73, placental purine ecto-5′-nucleotidase)	NT5E	6.2 ± 2.9 (3)	
AA429895	Human multidrug resistance-associated protein homologue (MRP3)	MRP3	2.6 ± 0.1 (2)	
AA931758	Putative lymphocyte G_0-G_1 switch gene (G0S2)	G0S2A	2.6 ± 0.4 (4)	
N72115	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	CDKN2C	2.6 ± 0.5 (2)	2.3
H85464	Deleted in split hand/split foot syndrome 1 (DSS1)	DSS1	2.5 ± 0.3 (2)	2.2
AA430382	Nucleoside phosphorylase	NP	1.9 ± 0.0 (2)	
Signal transduction				
AA598601	Insulin-like growth factor binding protein 3	IGFBP3	5.3 ± 1.3 (2)	2.1
T72877	Interleukin 1 receptor antagonist	IL1RN	4.0 ± 1.6 (3)	
AA460286	G protein γ-10 subunit (guanine nucleotide-binding protein GBGA)		2.4 ± 0.3 (3)	
H26426	Protein tyrosine phosphatase, receptor type, mu polypeptide	PTPRM	2.4 ± 0.4 (2)	2.9
AA460841	Insulin receptor substrate-1 [human, skeletal muscle, mRNA, 5828 nt]	IRS1	2.3 ± 0.3 (3)	6.1
AA443302	Rho-related GTP binding protein RhoE	RHOE	2.1 ± 0.2 (2)	4.9
AA411640	Ras-related GTP-binding protein A (ragA protein)	RAGA	2.1 ± 0.3 (2)	
T57556	Putative protein kinase C inhibitor PKCI-1 (protein kinase C-interacting 1)	PRKCNH1	1.9 ± 0.0 (2)	
AA910443	Nephroblastoma-overexpressed gene	NOV	1.7 ± 0.2 (2)	2.3
Biosynthesis and metabolism				
H87471	Human L-kynurenine hydrolase (kynureninase)	KYNU	4.0 ± 1.5 (2)	5.3
AA401111	Glucose phosphate isomerase	GPI	4.4 ± 0.6 (4)	
AA424937	Glucose-6-phosphate dehydrogenase	G6PD	3.1 ± 0.6 (2)	2.1
AA599158	Multifunctional aminoacyl-tRNA synthetase	SYHUQT	2.9 ± 0.3 (2)	
AA894557	Creatine kinase B	CKB	2.9 ± 0.9 (2)	3.4
H44956	Fumarylacetoacetate hydrolase	FAH	2.9 ± 0.2 (4)	
AA011215	Spermidine/spermine N1-acetyltransferase	SAT	2.8 ± 0.4 (3)	
T71782	Branched chain Acyl-CoA oxidase	BRCOX	2.7 ± 0.1 (2)	7.3
AA669689	Malate oxidoreductase (NADH-dependent malic enzyme)		2.5 ± 0.3 (4)	3.7
AA485653	Mannosyl (α-1,6) glycoprotein-β-1,2-*N*-acetylglucosaminyltransferase II	MGAT2	2.4 ± 0.0 (4)	2.7
W07099	*N*-acetylglucosaminidase, α- (Sanfilippo disease IIIB)	NAGLU	2.3 ± 0.1 (2)	
AA444009	Acid α-glucosidase	GAA	2.3 ± 0.5 (2)	
Growth factor/Cytokine and receptors				
H15718	XL receptor tyrosine kinase	AXL	5.1 ± 3.1 (2)	99
T55558	Colony-stimulating factor 1 (M-CSF)	CSF1	3.2 ± 0.6 (3)	3.4
AA262988	Brain-derived neurotrophic factor	BDNF	3.0 ± 0.1 (2)	23
R19956	Vascular endothelial growth factor	VEGF	2.6 ± 0.6 (3)	
N31467	Coxsackie virus and adenovirus receptor	CXADR	2.6 ± 0.3 (2)	2.3
AA504461	Low-density lipoprotein receptor precursor	LDLR	2.6 ± 0.0 (2)	2.3
T56316	Nerve growth factor β	NGFB	2.2 ± 0.2 (2)	17
AA053285	Interleukin 15 receptor α chain	IL15RA	2.2 ± 0.2 (3)	2.6
AA453831	Hepatoma-derived growth factor	HDGF	2.1 ± 0.0 (2)	
T47813	Macrophage-stimulating 1 (hepatocyte growth factor-like)	MST1	1.9 ± 0.2 (2)	
Cytoskeleton/Cell adhesion/Cell and organelle structure				
H15662	Cytoplasmic linker 2	CYLN2	4.2 ± 1.3 (2)	
H15662	Cytoplasmic linker 2 isoform 2 (Williams Beuren syndrome) [KIAA0291]		4.1 ± 1.1 (2)	
R78725	Vitamin A responsive; cytoskeleton related	JWA	3.3 ± 0.8 (2)	2.8
AA490684	Non-lens β γ-crystallin like [absent in melanoma 1 (AIM1)]	AIM1	3.1 ± 0.3 (2)	36
R06417	Junction plakoglobin	JUP	2.6 ± 0.6 (3)	
R13558	Activated leucocyte cell adhesion molecule	ALCAM	2.6 ± 0.5 (2)	31
H50993	Actinin, α 4	ACTN4	2.1 ± 0.2 (2)	2.1
Extracellular matrix				
T77595	Hexabrachion (tenascin C, cytotactin)	HXB	6.8 ± 4.4 (2)	10
AA677534	*H. sapiens* mRNA for laminin		4.8 ± 2.0 (3)	
AA001432	Laminin, α 3 [nicein (150 kDa), kalinin (165 kDa), BM600 (150 kDa), epilegrin]	LAMA3	2.7 ± 0.5 (3)	39
AA427561	Heparan sulfate proteoglycan (HSPG2)	HSPG2	2.6 ± 0.2 (3)	

Table 3 *Continued*

Accession no.	Gene name	Symbol	Ratio Mean ± SE (Range)	Ratio Affymetrix oligonucleotide array ($N = 1$)
Apoptosis/Cell death				
AA454646	Lymphotoxin-β receptor precursor	LTB	9.9 ± 4.3 (2)	
AA452556	TRAMP protein (translocating chain-associating membrane protein)	TRAMP	2.6 ± 0.2 (4)	
AA025275	DAP-kinase (death associated protein kinase)	HSDAPK	2.5 ± 0.1 (2)	
Inflammation/Immune and antiviral response/Histocompatibility				
H63077	Annexin I (lipocortin I)	ANX1	9.3 ± 2.7 (2)	145
AA458965	Natural killer cells protein 4 precursor		3.0 ± 1.0 (2)	
AA406020	IFN-induced 17 kDa protein (ubiquitin cross-reactive protein)	UCRP	2.4 ± 0.4 (4)	
AA488367	Host cell factor homologue	LCP	2.4 ± 0.3 (2)	
AA491191	IFN-γ induced protein IFI 16 (myeloid differ. transcriptional activator)	IFI16	1.7 ± 0.2 (2)	
Differentiation/Development/Tissue-specific expression or function				
AA598610	Mesoderm-specific transcript (mouse) homologue	MEST	4.9 ± 0.4 (3)	6.1
R43605	Homeobox protein Cux-2 (Cut-liKe 2) [KIAA0293]	CUTL2	4.7 ± 0.5 (2)	
R60995	*Homo sapiens* Coch-5B2 (Cochlin precursor)	COCH1	3.8 ± 1.1 (3)	
AA432066	Sarcoglycan, epsilon (dystrophin complex protein, muscular dystrophy)	SGCE	3.0 ± 0.7 (2)	7.1
H99813	Keratin 19	KRT19	2.5 ± 0.4 (4)	8.3
T97762	IFN-related developmental regulator 1	IFRD1	2.7 ± 0.3 (2)	3.7
AA700054	Adipophilin (adipose differentiation-related protein)	ADRP	2.4 ± 0.2 (2)	
R26960	Peripheral myelin protein 22	PMP22	2.0 ± 0.2 (2)	
AA884015	Tubby-related protein 2 (TULP2)	TULP2	2.0 ± 0.1 (2)	
AA676598	Nerve growth factor-inducible PC4 homologue	IFRD2	1.9 ± 0.2 (3)	2.9
Transmembrane/Membrane/Integrins				
AA488073	Mucin 1, transmembrane	MUC1	3.3 ± 1.2 (3)	
AA424695	Integrin α-3 subunit	ITGA3	2.2 ± 0.3 (4)	2.3
AA425451	Integrin, α E (CD103, mucosal lymphocyte antigen 1; α-polypeptide)	ITGAE	1.9 ± 0.0 (2)	2.1
AA464601	Tetraspanin Tspan-5 (TSPAN-5) gene	TM4SF9	1.9 ± 0.0 (2)	
Channels/Pore structure/Transport				
N62620	Two P-domain K+ channel, subfamily K, member 1	TWIK1	3.5 ± 1.2 (2)	8.8
AA292226	Creatine transporter mRNA	SLC6A8	2.7 ± 0.1 (3)	
AA598814	ATPase, Na+/K+ transporting, β 1 polypeptide	ATP1B1	2.0 ± 0.1 (2)	
RNA or protein processing/Protein modification and proteolysis				
H99816	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2	PLOD2	5.7 ± 2.0 (3)	2.5
AA628430	Sm-like protein CaSm (U6 SnRNA-associated LSm1 protein)		2.6 ± 0.1 (4)	
H59231	Human metalloprotease/disintegrin/cysteine-rich protein (MDC9)	MDC9	2.6 ± 0.3 (4)	
T70122	Ribonuclease L (2′,5′-oligoisoadenylate synthetase-dependent) inhibitor	RNASELI	2.5 ± 0.4 (3)	
AA047338	Proteasome ι chain (prosomal p27K protein, PROS-27)	PSA6	2.5 ± 0.1 (2)	
AA047039	eIF-1A, Y isoform (eukaryotic translation initiation factor 1A, Y isoform)	EIF1AY	2.4 ± 0.3 (4)	1260
AA934762	26S proteasome subunit p44.5 (26S proteasome regulatory subunit 9)	PSDB	2.3 ± 0.3 (4)	
Miscellaneous or function unknown				
T74192	Plasma protein S (α) (blood coagulation protein)	PROS1	3.3 ± 0.6 (3)	
AA670382	*H. sapiens* mRNA for 3′; UTR of unknown protein		3.6 ± 0.0 (2)	
AA875953	KIAA0404 protein (unknown function)		2.6 ± 0.2 (2)	

On the basis of previous experience with cDNA-spotted microarrays, we expected a low rate of false positivity with the selected filtering criteria (ref. 17; see Materials and Methods). To confirm this expectation, we tested $n = 15$ genes with rigorously controlled semiquantitated reverse transcription-PCR assays (14, 15, 17) of RNA samples from parental DU-145 cells and three clones each of Neo and wtBRCA1 cells. β-Actin, which is unaffected by BRCA1, was used as a control gene. The expression of BRCA1 in the wtBRCA1 relative to control cell lines is shown in Fig. 1*A*. For all 15 genes, the expected increases (Fig. 1*B*) or decreases (Fig. 1*C*) in gene expression were confirmed. In some cases, the fold changes (determined by densitometry and expressed relative to β-actin) were greater by reverse transcription-PCR than by microarray assays, consistent with our impression that cDNA spotted arrays often underestimate gene expression changes. We tested the effect of BRCA1 knockdown with a previously validated siRNA (15) on the expression of three antioxidant response genes that were up-regulated by wtBRCA1 (MGST1, NQO1, and GSTZ1). In each case, the mRNA levels were decreased by BRCA1-siRNA but not by control-siRNA (Fig. 1*D*). These findings suggest that BRCA1 regulates the expression of some antioxidant response genes over a very wide range of intracellular BRCA1 protein levels.

We also examined the effect of overexpression of wtBRCA1 on gene expression in MCF-7 human breast cancer cells. In these studies, gene expression was compared in MCF-7 cells transiently transfected with wtBRCA1 *versus* empty pcDNA3 vector. Gene expression was compared in wtBRCA1 *versus* control (pcDNA3)-transfected cells in the absence (−E2) or presence (+E2) of exogenous estrogen (17β-estradiol, 1 μmol/L × 24 hours). Although the cell types and duration of BRCA1 expression differed, we identified >40 genes concordantly regulated by wtBRCA1 in DU-145 *versus* MCF-7 cells (Table 5). These include MGST1, ANX1, ADH5, DSS1, MOP2, IGFBP3, GNG10, NOV, G6PD, KRT19, IFRD2, and others. In the absence and/or presence of E2, wtBRCA1 up-regulated expression of genes involved in the oxidative stress response or the detoxification of xenobiotics and drugs in MCF-7 cells, including MGST1, MOP2, ADH5, ALDH8, an epoxide hydrolase (EPHX2), several selenoproteins (SEPHS1 and SEPW1), and several other antioxidant proteins (PRDX4 and TSA).

Microarray Analysis of Brca1 Mutant Cell Lines. Next, we determined the effect of loss of the endogenous full-length Brca1 protein on gene expression. Thus, we compared gene expression in Brca1-deficient (exon 11-deleted) *versus* wild-type ($Brca1^{+/+}$) MEFs. The Brca1 Δ exon 11 MEFs express a M_r 92,000 Brca1 protein that is defective in DNA repair function (25). Examples of genes down-regulated in Brca1-deficient MEFs are listed in Table 6 (see Supplemental Material for the full list.) Categories of genes underexpressed in the Brca1-deficient cells included those involved in transcription, stress responses, cell cycle regulation, DNA replication and repair, signal transduction, and other processes. Stress response genes up-regulated in Brca1-deficient cells included a GST (Gsta2), a glutathione peroxidase (Gpx3), the antioxidant response transcription

Table 4 *Genes whose expression is decreased in DU-145 wtBRCA1 [versus control (Neo)] cell clones*

Accession no.	Gene name	Symbol	Ratio	
			Mean ± SE (range)	Affymetrix array ($N = 1$)
Transcription/Nuclear proteins				
AA857131	HIV Tat-SF1 (Tat-specific factor 1)	HTATSF1	0.29 ± 0.00 (2)	
W00975	p300/CBP-associated factor (P/CAF)	PCAF	0.33 ± 0.09 (2)	
H27379	Transcription elongation factor S-II (also called transcription elongation factor A)	TCEA2	0.39 ± 0.00 (2)	
AA504265	PINCH protein (LIM and senescent cell antigen like domains 1)	LIMS1	0.41 ± 0.05 (2)	
Stress response: oxidative stress and xenobiotic detoxification				
H86554	Ceruloplasmin (ferroxidase) [multicopper oxidase, Fe & Cu homeostasis]	CP	0.18 ± 0.09 (3)	0.030
R33755	GST π-1	GSTP1	0.39 ± 0.00 (2)	0.27
Replication/Cell cycle/DNA repair and metabolism				
AA042990	Semaphorin E (protein associated with drug resistance)	SEMA3C	0.26 ± 0.13 (3)	
R19031	Photolyase homologue [similar to Drosophila (6-4) photolyase]	PH	0.35 ± 0.10 (3)	
R10662	DNA mismatch repair protein MLH1	MLH1	0.37 ± 0.05 (2)	
N54344	KIAA0074 (also called condensin subunit 2, Barren homologue protein 1)	BRRN1	0.38 ± 0.09 (2)	
AA598887	KIAA0178 (SMC1 structural maintenance of chromosomes 1-like 1)	SMC1L1	0.42 ± 0.01 (2)	
AA683578	Adenosine deaminase	ADA	0.42 ± 0.06 (2)	0.28
H94617	Replication factor C (activator 1) 3 (38 kDa)	RFC3	0.47 ± 0.00 (2)	
AA045192	Retinoblastoma 1 (including osteosarcoma)	RB1	0.49 ± 0.01 (2)	
Signal transduction				
S75725	Prostacyclin-stimulating factor (also known as IGFBP7)	IGFBP7	0.21 ± 0.04 (2)	
AA055835	Caveolin, caveolae protein, 22 kDa	CAV1	0.31 ± 0.04 (3)	
AA284634	Janus kinase 1 (a protein tyrosine kinase)	JAK1	0.32 ± 0.09 (3)	0.26
AA425947	RIG (regulated in glioma) [putative tumor suppressor, Ras-related gene]	RIG	0.32 ± 0.08 (3)	0.29
NM_015881	Dickkopf homologue 3 (regulator of Wnt signaling)	DKK3	0.37 ± 0.12 (2)	
AA418999	Smad5 (a MAD-like protein, putative tumor suppressor)	SMAD5	0.40 ± 0.04 (2)	
AA101793	Lipid-activated, protein kinase PRK2	PRK2	0.43 ± 0.06 (3)	
Biosynthesis and metabolism				
AA599187	Phosphoglycerate kinase 1 (hypoxia-inducible glycolytic enzyme)	PGK1	0.32 ± 0.12 (2)	0.35
AA018372	Liver glutamate dehydrogenase	GLUD1	0.36 ± 0.12 (2)	
AA489611	Lactate dehydrogenase A	LDHA	0.40 ± 0.02 (2)	
H07926	Mitochondrial 3-oxoacyl-CoA thiolase (acetyl-Coenzyme A acyltransferase 2)	ACAA2	0.44 ± 0.02 (3)	
Growth factor/Cytokine and receptors				
AA425028	Human cytokine receptor EB13 (induced by EBV infection)	EBI3	0.35 ± 0.05 (2)	0.018
AA424629	Latent transforming growth factor β binding protein 2	LTBP2	0.38 ± 0.11 (2)	0.32
R72075	Human heregulin-β gene	HRGA	0.39 ± 0.01 (2)	
T64134	Monocyte chemoattractant protein MCP-4 (= small inducible cytokine A13)	CCL13	0.40 ± 0.05 (2)	
R39227	Cytokine IK (also called IK factor, RER protein, red protein)	IK	0.40 ± 0.06 (2)	
H98636	CD40L receptor precursor (CD40 antigen, member of TNF receptor family)	CD40	0.41 ± 0.03 (2)	
Cytoskeleton/Cell adhesion/Cell and organelle structure				
AA196000	Actinin α-3	ACTN3	0.31 ± 0.08 (3)	
AA669758	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	NPM1	0.38 ± 0.01 (2)	
AA452566	Peroxisomal membrane protein 3 (35 D, Zellweger syndrome)	PXMP3	0.46 ± 0.00 (2)	0.47
Extracellular matrix				
W93163	TNF-inducible protein TSG-6 precursor (a hyaluron binding protein)	TNFAIP6	0.24 ± 0.10 (3)	0.038
AA134871	Fibulin 1	FBLN1	0.32 ± 0.07 (2)	0.19
AA418811	Fibrillin 1 (Marfan syndrome)	FBN1	0.44 ± 0.04 (2)	0.092
Apoptosis/Cell death				
AA699697	Tumor necrosis factor	TNF	0.18 ± 0.01 (2)	0.043
N69204	Chromosome segregation gene homologue CAS (apoptosis susceptibility)	CSE1L	0.29 ± 0.03 (2)	
AA455413	Ceramide glucosyltransferase (glucosylceramide synthase, GCS)	UGCG	0.40 ± 0.03 (3)	
Inflammation/Immune and antiviral response/Histocompatibility				
AA625981	FK506-binding protein 1 (12 kDa) [an immunophilin]	FKBP1	0.40 ± 0.08 (4)	0.066
AA625981	FK506-binding protein 1A (12 kDa) [an immunophilin]	FKBP1A	0.41 ± 0.08 (2)	0.35
AA419108	Annexin IV (placental anticoagulant protein II)	ANX4	0.40 ± 0.02 (2)	0.36
Differentiation/Development/Tissue-specific expression or function				
AA400739	SRY (sex-determining region Y)-box 9 (campomelic dysplasia, sex-reversal)	SOX9	0.23 ± 0.03 (2)	0.090
H20758	ZyginI (synaptogamin-interacting protein) [axonal growth and fasciculation]		0.25 ± 0.06 (2)	
AA911661	Homeo box B2	HOXB2	0.29 ± 0.01 (2)	0.045
W58092	Tropomyosin α chain (skeletal muscle)	TPM1	0.31 ± 0.07 (3)	0.17
N79708	Fragile X mental retardation protein 1 homologue FXR1	FXR1	0.34 ± 0.12 (2)	0.49
AA598517	Keratin 8	KRT8	0.38 ± 0.07 (3)	
Transmembrane/Membrane/Integrins				
N46419	Synaptogyrin 3 (integral membrane protein, function unclear)	SYNGR3	0.48 ± 0.02 (3)	0.10
H78244	Intestinal and liver tetraspan membrane protein (il-TMP)	TM4SF4	0.40 ± 0.06 (2)	
RNA or protein processing/Protein modification and proteolysis				
AA476294	Nucleolin (nuclear phosphoprotein) [synthesis & maturation of ribosomes]	NCL	0.27 ± 0.09 (2)	
AA143201	Matrix metalloproteinase 1 (interstitial collagenase)	MMP1	0.35 ± 0.07 (2)	0.36
Function unknown				
R16604	ESTs, weakly similar to TAL-6		0.14 ± 0.09 (2)	
AA455476	*Homo sapiens* cDNA: FLJ22921 fis, clone KAT06711 (unnamed protein product)		0.38 ± 0.06 (2)	
AA431438	*H. sapiens* cDNA FLJ10500 fis, clone NT2RP2000369		0.38 ± 0.06 (2)	
R67042	KIAA0265 (Kelch motif containing protein of unknown function)		0.40 ± 0.00 (2)	0.49

factor Nfe2l2 (also called Nrf2), a Nrf2 binding partner [activating transcription factor 2 (Atf2)], a superoxide dismutase (Sod1), a selenoprotein (Sepp1), the aryl hydrocarbon receptor (Ahr), several etomidate-responsive genes, and several heat shock proteins. In contrast, very few genes were up-regulated, and the magnitude of the increases was small (see Supplemental Material). Examples of genes concordantly increased in BRCA1-overexpressing (DU-145 or MCF-7) cells and decreased in Brca1-deficient MEFs include PROS1, GPX3, BNIP3, and TGFB2 (see Table 7*A*).

We compared our findings with published microarray studies of

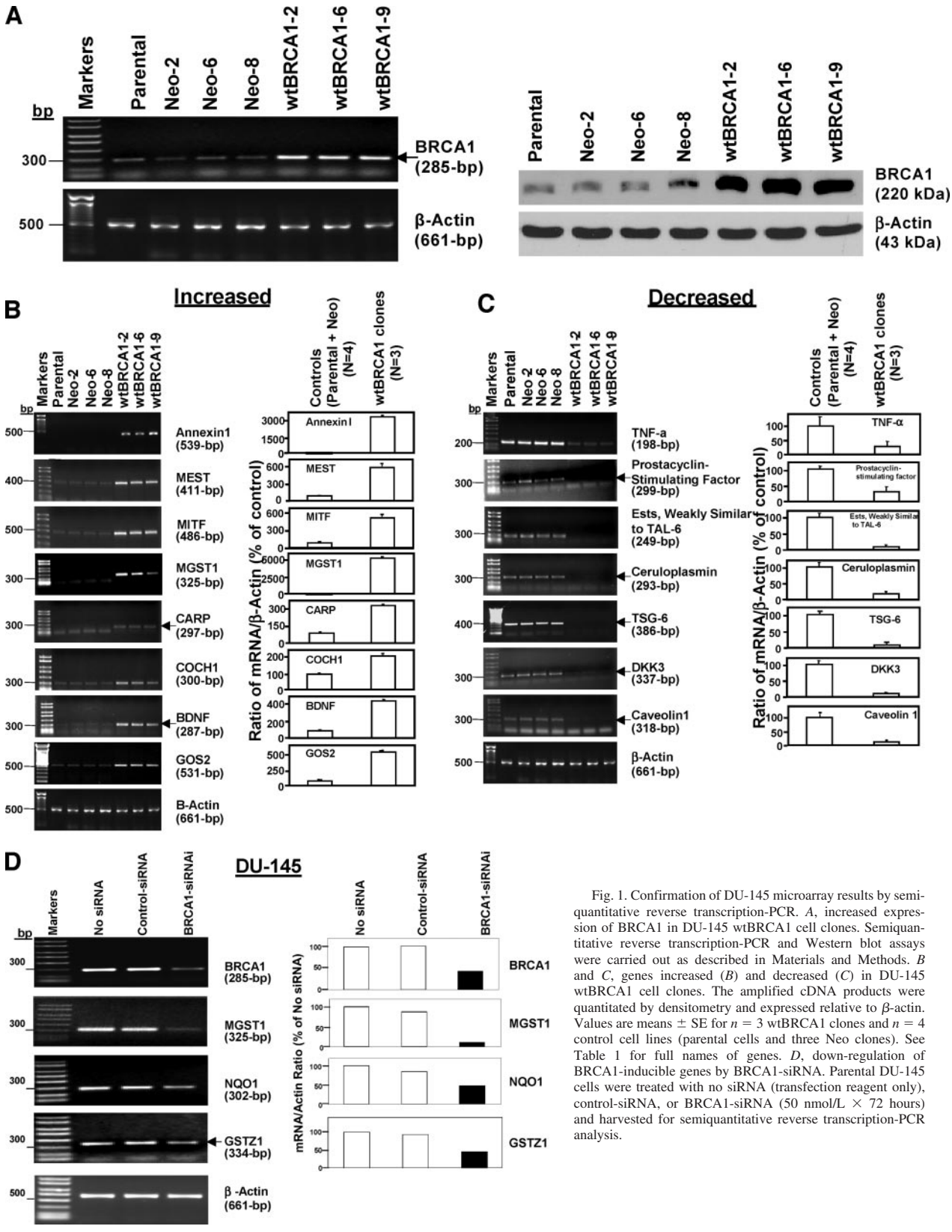

Fig. 1. Confirmation of DU-145 microarray results by semiquantitative reverse transcription-PCR. *A*, increased expression of BRCA1 in DU-145 wtBRCA1 cell clones. Semiquantitative reverse transcription-PCR and Western blot assays were carried out as described in Materials and Methods. *B* and *C*, genes increased (*B*) and decreased (*C*) in DU-145 wtBRCA1 cell clones. The amplified cDNA products were quantitated by densitometry and expressed relative to β-actin. Values are means $\pm$ SE for $n = 3$ wtBRCA1 clones and $n = 4$ control cell lines (parental cells and three Neo clones). See Table 1 for full names of genes. *D*, down-regulation of BRCA1-inducible genes by BRCA1-siRNA. Parental DU-145 cells were treated with no siRNA (transfection reagent only), control-siRNA, or BRCA1-siRNA (50 nmol/L $\times$ 72 hours) and harvested for semiquantitative reverse transcription-PCR analysis.

gene regulation by BRCA1 in 293T cells (26), colon cancer cells (27), and Brca1-deficient mouse embryonic stem cells (28). Examples of genes concordantly regulated in our study *versus* published studies are provided in Table 7*B*. These include (*a*) genes commonly induced by wtBRCA1 in 293T and in DU-145 and/or MCF-7 cells (MGAT2, CCNG2, FSTL1, LAMA3, and KCNK1), (*b*) genes induced by wtBRCA1 in 293T cells and decreased in Brca1-deficient MEFs (SEPP1, ZNF148, and ENPP2), (*c*) genes induced by wtBRCA1 in colon cancer cells and decreased in Brca1-deficient MEFs (TOP1 and SOD1), (*d*) genes decreased by wtBRCA1 in colon cancer and

Table 5 *Effect of exogenous wtBRCA1 on gene expression in MCF-7 breast cancer cells (selected)*

Accession no.	Gene name (symbol)	Ratio (wtBRCA1/control) DU-145	MCF-7 (−E2)	MCF-7 (+E2)
Increased by BRCA1				
Transcription factors/Nuclear proteins				
H27379	Transcription elongation factor (S-II)			2.1 ± 0.5 (2)
	Pre-B-cell–enhancing factor (PBEF)			2.0 ± 0.2 (2)
AA600217	Activating transcription factor-4 (ATF4)			1.9 ± 0.4 (2)
AA685085	High mobility group protein 1 (HMG1)	1.9 ± 0.0 (2)		1.8 ± 0.0 (2)
AA418670	JunD proto-oncogene (JUND)			1.8 ± 0.0 (2)
T99236	JunB proto-oncogene (JUNB)			1.7 ± 0.1 (2)
AA62599	Zf9 (cellular nucleic acid binding protein) [ST12, ZF9, KLF6]			1.7 ± 0.0 (2)
AA421977	Dr1-associated transcriptional corepressor (DRAP1)			1.7 ± 0.2 (2)
T55801	Transcription factor TFIIA, γ (GTFA2)	2.1 ± 0.3 (4)		1.6 ± 0.0 (2)
AA291513	B-cell lymphoma 7B (BCL7)	1.9 ± 0.3 (2)		1.6 ± 0.0 (2)
Stress response/Xenobiotic detoxification/Mitochondrial function				
AA629719	Cytochrome *c* oxidase VIIc subunit (COX7C)		2.6 ± 0.7	
T90999	UDP glucuronosyl transferase 1A, microsomal (UGT1A)			2.5 ± 0.7 (2)
AA453859	Alcohol dehydrogenase 5 (ADH5)	2.2 ± 0.4 (3)	1.5 (1)	2.3 (1)
AA680322	Ubiquinone oxidoreductase MLRQ subunit (NUOMS)			2.2 ± 0.6 (2)
W77812	*Cis*-platinum resistance-associated α protein		2.1 ± 0.1 (2)	
AA680300	Member of PAS protein 2 (MOP2)	3.4 ± 0.5 (3)	1.9 ± 0.1 (2)	1.6 (1)
AA495936	GST, microsomal (MGST1)	10.2 ± 4.4 (3)		1.8 ± 0.1 (3)
R73525	Epoxide hydrolase 2, cytoplasmic [EPHX2]			1.8 ± 0.1 (2)
AA488081	Selenium donor protein (selenoprotein synthetase) [SelD, SEPHS1]			1.7 ± 0.0 (3)
AA443630	Aldehyde dehydrogenase-8 (ALHD8)		1.9 ± 0.2 (2)	1.7 ± 0.1 (2)
AA459663	Anti-oxidant protein AOE37-2 (thioredoxin peroxidase) [PRDX4]			1.6 ± 0.1 (2)
H68845	Thiol-specific anti-oxidant (thioredoxin peroxidase 1) [TSA, PRP]			1.6 ± 0.0 (2)
AA283629	Selenoprotein W (Se1W, SEPW1)			1.6 ± 0.1 (2)
Replication/Cell cycle/DNA repair				
AA489752	Cyclin G2 (CCNG2)			4.0 ± 0.8 (2)
R19031	Brain mRNA for photolyase homologue		2.1 ± 0.5 (2)	
N70463	B-cell translocation gene 1, antiproliferative (BTG1)		2.1 ± 0.4 (2)	1.8 ± 0.2 (3)
H54417	Nucleoside diphosphate kinase		2.1 ± 0.4 (2)	1.8 ± 0.1 (2)
AA496013	Protein serine/threonine kinase 2 (STK2, also called NEK4)			1.9 ± 0.0 (2)
H85464	Deleted in split hand/split foot syndrome 1 (DSS1)	2.5 ± 0.3 (2)		1.8 ± 0.1 (2)
AA291715	DNA ligase I, ATP-dependent (LIG1)		1.7 ± 0.1 (2)	
AA885642	H2B/g (= histone H2B.1) [H2BFG]		1.7 ± 0.1 (2)	
AA156571	Alanyl tRNA synthase (AARS)		1.7 ± 0.1 (2)	
Signal transduction				
AA598601	Insulin-like growth factor binding protein 3	5.3 ± 1.3 (2)	3.8 ± 0.8 (2)	3.6 ± 0.5 (2)
H08899	Homologue of yeast IPP isomerase	2.6 ± 0.8 (3)		3.2 ± 0.2 (2)
AA460286	G protein γ-10 subunit	2.4 ± 0.3 (3)	1.8 ± 0.1 (2)	2.5 ± 0.3 (2)
R24543	Guanine nucleotide regulatory protein (NET1)			2.0 ± 0.2 (2)
W73473	Bone morphogenetic protein-7 (BMP7)		2.0 ± 0.4 (2)	
T72202	IL4-STAT (STAT6)	2.7 ± 0.2 (3)	1.8 ± 0.1 (2)	1.6 ± 0.1 (2)
AA521411	Calcium modulating ligand (CAMLG)			1.7 ± 0.0 (2)
AA482328	Myristolated ala-rich C-kinase substrate (MACS, MARCKS)			1.7 ± 0.1 (2)
T57556	Protein kinase C inhibitor PKCI-1 (PRKCNH1)	1.9 ± 0.0 (2)		1.7 ± 0.1 (3)
AA490300	PDGF receptor-associated protein	2.1 ± 0.2 (3)		1.7 ± 0.1 (3)
AA910443	Nephrobastoma overexpressed gene (NOV)	1.7 ± 0.2 (2)	1.6 ± 0.1 (2)	1.6 (1)
AI017398	Sodium channel 2 (amiloride-sensitive) [BNaC2, ACCN2]	2.1 ± 0.1 (2)		1.5 ± 0.0 (2)
Biosynthesis and metabolism				
AA424937	Glucose-6-phosphate dehydrogenase (G6PD)	3.1 ± 0.6 (2)	1.7 ± 0.1 (2)	2.6 (1)
H87471	Human L-kynurenine hydrolase (KYNU)	4.0 ± 1.5 (2)		2.5 ± 0.5 (2)
H51574	Arachidonate 5-lipoxygenase (ALOX5)			2.0 ± 0.2 (2)
AA444009	Acid-α glucosidase (GAA)	2.3 ± 0.5 (2)	1.9 ± 0.3 (2)	1.6 (1)
AA434024	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase) [LSS]			1.8 ± 0.1 (3)
AA633882	Retinol dehydrogenase 1 (11-cis) [RDH1]	2.7 ± 0.7 (2)		1.7 ± 0.0 (2)
AA157955	Methyl sterol oxidase (sterol-C4-methyl oxidase-like) [ERG25]		1.7 ± 0.1 (2)	
Ligands and receptors				
H22652	Glial maturation factor β (GMFB)			2.6 ± 0.7 (2)
AA452556	Translocation-associated membrane protein 1 (TRAMP)	2.6 ± 0.2 (4)		
R19956	Vascular endothelial growth factor (VEGF)	2.6 ± 0.6 (3)		2.2 ± 0.5 (2)
T77810	RYK receptor-like tyrosine kinase (RYK)			2.0 ± 0.4 (3)
AA679177	Follistatin-related protein precursor (follistatin-like 1) [FSTL1]			2.0 ± 0.2 (2)
N48698	Hybrid receptor gp250 precursor (SORLA, LRP9)	2.1 ± 0.0 (2)		1.7 ± 0.1 (2)
AA233809	Transforming growth factor β2 (TGFB2)		1.6 ± 0.1 (2)	
Cytoskeleton/Cell adhesion/Cell structure				
H72027	Gelsolin precursor, plasma (Finnish amyloidosis) [GSN]		2.4 ± 0.7 (2)	
N77754	Lysosome-associated membrane protein 2 (LAMP2)			2.0 ± 0.1 (2)
R06417	Junction plakoglobin (JUP)	2.6 ± 0.6 (3)	1.8 ± 0.1 (2)	
Extracellular matrix				
AA029997	Procollagen type IV, α 5 (Alport's Syndrome) [COL4A5]		2.0 ± 0.4 (2)	
Apoptosis/Cell death				
AA063521	E1B 19K/Bcl-2 binding protein Nip3 (BNIP3)		1.6 (1)	2.8 ± 0.1 (2)
AA598483	Tax1 binding protein-151 (TXBP151)			2.1 ± 0.1 (2)
Inflammation/Immune responses				
H63077	Annexin I (lipocortin) [ANX1]	9.3 ± 2.7 (2)		2.3 ± 0.6 (2)
AA630328	Galectin 3 (GALS3)	1.8 ± 0.2 (2)	2.1 ± 0.3 (2)	
AA634103	Thymosin β-4 (THYB4)	2.0 ± 0.1 (2)	1.6 ± 0.1 (2)	1.6 ± 0.1 (2)

Table 5 *Continued*

Accession no.	Gene name (symbol)	Ratio (wtBRCA1/control) DU-145	MCF-7 (−E2)	MCF-7 (+E2)
Differentiation/Development/Tissue-specific expression				
AA402207	Eyes absent homologue (EAB1, EYA2)		2.6 ± 0.6 (3)	3.0 ± 1.0 (2)
R26960	Peripheral myelin protein 22 (PMP22)	2.0 ± 0.2 (2)	2.1 (1)	2.8 ± 0.8 (2)
AA464250	Keratin 19 (KRT19)	2.5 ± 0.4 (4)		1.8 ± 0.2 (2)
AA676598	Nerve growth factor-inducible PC4 homologue (IFRD2)	1.9 ± 0.2 (3)		1.8 ± 0.2 (2)
Channels/Pore structure/Transport				
R39446	WHITE protein homologue		2.4 ± 0.8 (2)	
N62620	Two P-domain K+ channel (TWIK1)	3.5 ± 1.2 (2)	1.5 ± 0.0 (2)	
RNA or protein processing/Protein modification				
W73874	Cathepsin L (CTSL)			2.6 ± 0.3 (2)
AA490124	Ubiquitin conjugating enzyme E2 (Drosophila bandless) [UBE2N]			2.1 ± 0.3 (2)
AA011215	Spermine/spermidine N1-acetyltransferase (SAT)	2.8 ± 0.4 (3)	2.0 ± 0.1 (2)	
AA459039	Placental bikunin (serine protease inhibitor) [SPINT2, HAI2]			2.0 ± 0.2 (3)
AA628430	Sm-like protein CaSm (LSM1)	2.6 ± 0.1 (4)		1.7 ± 0.2 (2)
Miscellaneous or function unknown				
AA418918	Nuclear autoantigen GS2NA (striatin-3) [GS2NA]	3.4 ± 0.7 (3)		2.2 ± 0.2 (2)
Decreased by BRCA1				
Transcription factors/Nuclear proteins				
H32858	X2 box repressor (putative zinc finger transcriptional regulator)		0.43 ± 0.13 (2)	
N67262	Zinc finger protein 135 (clone PH8-17) [ZNF135]		0.51 ± 0.17	
N49526	Myb proto-oncogene (MYB)		0.52 ± 0.20	
AA704809	SIL [TAL1 (SCL) interrupting locus] (hypoxia-sensitive) [SIL]	0.40 ± 0.09 (2)	0.64 ± 0.08 (2)	
Stress response/Xenobiotic detoxification/Mitochondrial function				
AA099134	Oxygen-regulated protein, 150 kDa (ORP150)		0.40 ± 0.11 (2)	0.41 ± 0.22 (2)
AA133187	Iron-regulated protein (IRP2, IREB2)		0.42 ± 0.25 (2)	
N55459	Metallothionein hMT-1f (functional) [MT1F]		0.69 ± 0.05 (2)	0.50 ± 0.06 (2)
AA621218	Carnitine acetyltransferase (CRAT)			0.58 ± 0.05 (3)
N66957	Cytochrome P450 subfamily XXVII (CYP27, CYP27A1)		0.66 ± 0.06	
Signal transduction				
AA284634	Janus kinase 1 (JAK1)	0.32 ± 0.09 (3)	0.34 ± 0.13 (3)	0.50 ± 0.22 (2)
AA282196	*Homo sapiens* serine/threonine protein kinase		0.40 ± 0.23 (2)	0.56 ± 0.01 (2)
AA101793	Lipid-activated, protein kinase 2 (PRK2)	0.43 ± 0.06 (3)	0.43 ± 0.11 (2)	
AA418999	Smad5 (a MAD-like protein) [SMAD5]	0.40 ± 0.04 (2)	0.50 ± 0.18 (2)	
Biosynthesis and metabolism				
AA070357	Transketolase (Wernicke-Korsakoff syndrome) [TKT]		0.29 ± 0.07 (3)	
R84263	CAD protein (multifunctional enzyme) (CAD)		0.46 ± 0.23	
AA018372	Liver glutamate dehydrogenase (GLUD1)	0.36 ± 0.12 (2)	0.53 ± 0.17 (2)	
Growth factor/Cytokine and receptors				
AA425028	Human cytokine receptor EBI3 (EBI3)	0.35 ± 0.05 (2)		0.61 ± 0.05 (2)
AA019320	Adducin 2 (β) [ADD2, ADDB]		0.65 ± 0.08 (3)	0.41 ± 0.07 (2)
Cytoskeleton/Cell Adhesion/Cell structure				
AA598659	NuMA (nuclear matrix protein) [NUMA, NUMA1]		0.20 ± 0.12	
W31983	X104 (tight junction protein 2, zona occludens 2) [TJP2, ZO2]		0.32 ± 0.17 (2)	0.59 ± 0.09 (2)
W13290	Tight junction protein 1 (TJP1)		0.45 ± 0.14 (3)	
AA455062	Zyxin (YX)		0.48 ± 0.16 (3)	
AA669758	Nucleophosmin (B23, numatrin) [NPM1]	0.38 ± 0.01 (2)	0.58 ± 0.14 (2)	
AA452566	Peroxisomal membrane protein 3 (XMP3)	0.46 ± 0.00 (2)	0.69 ± 0.01 (2)	
Extracellular matrix				
AA464630	Thrombospondin 1 (HBS1)		0.50 ± 0.13 (3)	0.41 ± 0.16 (2)
H24650	Laminin γ 1 (AMC1)		0.49 ± 0.16 (3)	0.46 ± 0.18 (2)
W93163	TNF-inducible protein 6 (TSG-6) [TNFAIP6]	0.24 ± 0.10 (3)	0.70 ± 0.01 (2)	
Apoptosis/Cell death				
AA476272	Putative DNA-binding protein A20 (TNFα-induced) [TNFAIP3]		0.56 ± 0.05 (2)	
Differentiation/Development/Tissue specific				
AA455145	Semaphorin V (guidance of neuronal growth cones) [SEMA3B]			0.64 ± 0.09 (2)
Channels/Pore structure/Transport				
AA457050	Treacher-Collins syndrome susceptibility-1 (treacle) [TCOF1]		0.50 ± 0.07	
N46843	Aquaporin 4 (AQP4)		0.66 ± 0.01	
RNA or protein processing/Protein modification				
AA284634	Antithrombin III [AT3, SERPINC1]		0.25 ± 0.11 (2)	
Miscellaneous or function unknown				
AA056465	54 kDa protein (RNP repeat domain protein)	0.25 ± 0.06 (2)	0.44 ± 0.23 (2)	

DU-145 cells (CD59), (*e*) genes decreased in Brca1-deficient embryonic stem cells and MEFs (Rock2, Qk, and Nfl), and (*f*) genes decreased in Brca1-deficient embryonic stem cells and up-regulated in DU-145 wtBRCA1 cells (CKB, SYHUQT, and PSMC2).

BRCA1 Protects against Oxidative Stress and Restores Cellular Redox Balance. To determine whether the ability of BRCA1 to stimulate the expression of antioxidant response genes has functional consequences, we measured the effects of BRCA1 on the cellular sensitivity to two different oxidizing agents, hydrogen peroxide (H_2O_2), and paraquat. DU-145 wtBRCA1 or Neo clones were exposed to different doses of H_2O_2 for 24 hours, after which, the cell viability was determined by using MTT assays. The wtBRCA1 cells were significantly more resistant to H_2O_2 over a wide range of doses ($P < 0.001$, two tailed *t* tests; Fig. 2*A*). In concordance with these findings, pretreatment of parental DU-145 cells with a BRCA1-siRNA caused significant sensitization to H_2O_2 ($P < 0.001$; Fig. 2*B*). Please note that the experiments shown in Fig. 2, *A* and *B*, are representative of two or three independent experiments of each type that showed similar results. These results suggest that both exogenous and endogenous BRCA1 protects DU-145 cells against oxidative stress due to H_2O_2.

We also tested the ability of endogenous Brca1 to protect MEFs against H_2O_2 induced cytotoxicity. Consistent with the results obtained with DU-145 cells, Brca1-deficient MEFs were more sensitive

Table 6 *Selected genes whose expression is decreased in Brca1$^{\Delta ex11\Delta ex}$ (versus Brca1$^{+/+}$) mouse embryo fibroblasts*

Accession no.	Gene name	Symbol	Ratio	
			Mean ± SE (range)	Affymetrix Array (N = 1)
Transcription/Nuclear proteins				
AA208865	Nuclear receptor corepressor 1	Ncor1	0.47 ± 0.08 (2)	
AA276365	Myocyte enhancer factor 2c	Mef2c	0.47 ± 0.07 (2)	0.44
AA244944	CCAAT/enhancer binding protein α (C/EBP), related sequence 1	Cebpa-rs1	0.51 ± 0.02 (2)	
AA212695	*Trans*-acting transcription factor Sp1 (cell cycle-regulated)	Sp1	0.51 ± 0.09 (2)	
AA036347	Kruppel-like factor 9	Klf9	0.52 ± 0.11 (2)	
AA125037	Zinc finger protein 148	Zfp148	0.52 ± 0.01 (2)	
AA138529	Zinc finger protein 62	Zfp62	0.56 ± 0.15 (2)	
AA501045	Nuclear receptor subfamily 2, group H, member 2	Nr2c2	0.56 ± 0.11 (2)	0.061
AA155377	Zinc finger protein X linked	Zfx	0.57 ± 0.01 (2)	
W83524	Transcription factor 12	Tcf12	0.58 ± 0.06 (3)	
AA097341	Nuclear receptor coactivator 1	Ncoa1	0.59 ± 0.07 (3)	
Stress response: oxidative stress and xenobiotic detoxification				
AA200734	Ethanol decreased 2	Etohd2	0.47 ± 0.07 (2)	
AA445861	Activating transcription factor 2 (stress response transcription factor)	Atf2	0.50 ± 0.07 (2)	
AA139271	Glutathione peroxidase 3	Gpx3	0.51 ± 0.05 (3)	0.12
W54349	GST, α 1 (Ya)	Gsta1	0.51 (1)	
AA120574	Superoxide dismutase 1, soluble	Sod1	0.53 ± 0.07 (2)	
AA044475	Nuclear factor, erythroid-derived 2, like 2 (also called Nrf2)	Nfe2l2	0.53 ± 0.07 (2)	
AA033466	Myeloperoxidase	Mpo	0.53 ± 0.06 (2)	
AA060716	Early growth response 1 (stress-responsive transcription factor)	Egr1	0.54 ± 0.16 (2)	
AA276440	Selenoprotein P, plasma, 1	Sepp1	0.57 ± 0.02 (3)	
W82873	Aryl hydrocarbon receptor	Ahr	0.59 ± 0.15 (2)	
AA250120	GST, α 2 (Yc2)	Gsta2	0.61 ± 0.10 (2)	
Heat shock-related proteins				
AA212162	Chaperonin subunit 8 (θ)	Cct8	0.48 ± 0.12 (2)	
AA498713	Heat shock protein, 74 kDa	Hsp74	0.58 ± 0.13 (2)	
W62018	Heat shock cognate protein, 70	Hsc70	0.61 ± 0.03 (2)	
Replication/Cell cycle/DNA repair and metabolism				
AA17363	Ectonucleotide pyrophosphatase/phosphodiesterase 2	Enpp2	0.38 ± 0.22 (2)	
AA386895	Tetratricopeptide repeat domain 3 (chromosome segregation)	Ttc3	0.43 ± 0.07 (2)	
AA288567	Suppressor of Ty 4 homologue (*S. cerevisiae*)	Supt4 h	0.44 ± 0.04 (3)	
AA268478	Serine/threonine kinase 2 (homologous to mitotic egulator NIMA)	Stk2	0.45 ± 0.03 (2)	
AA273291	Chromodomain helicase DNA binding protein 1	Chd1	0.47 ± 0.04 (3)	
AA498480	Centrosomin A	Csma	0.49 ± 0.06 (2)	
AA542278	Clusterin	Clu	0.50 ± 0.04 (3)	0.46
AA122530	SNM1 protein (required for repair of DNA double-strand cross-links)	SNM1	0.55 ± 0.14 (2)	
AA285484	G_1 to phase transition 1	Gspt1	0.55 ± 0.05 (2)	
AA475568	5′-3′; exoribonuclease 2	Xrn2	0.57 ± 0.05 (2)	
AW544661	Serine/threonine kinase 10 (Polo-like kinase)	Stk10	0.57 ± 0.18 (2)	
AA170792	Topoisomerase (DNA) I	Top1	0.58 ± 0.08 (2)	
AA108933	CDC-like kinase (serine/threonine kinase, cell cycle regulatory)	Clk	0.58 ± 0.09 (3)	
AA020165	Uridine phosphorylase	Upp	0.59 ± 0.05 (3)	
Signal transduction				
AA254238	Rho-associated coiled-coil forming kinase 2	Rock2	0.44 ± 0.11 (2)	
AA286039	Ras-binding protein SUR-8 (also called suppressor of clear, Soc-2)	Sur8	0.50 ± 0.02 (2)	
AA200336	Tumor protein D52 (? Role in calcium-mediated signal transduction)	Tpd52	0.51 ± 0.09 (2)	
AA177814	MAD homologue (Drosophila) [also called Smad1]	Madh1	0.52 ± 0.10 (2)	
AA271494	Myristoylated alanine rich protein kinase Csubstrate	Macs	0.54 ± 0.14 (3)	0.33
AA124332	Phosphodiesterase 8	Pde8	0.57 ± 0.06 (2)	
AA413508	SAPK/Erk/kinase 1	Serk1	0.60 ± 0.01 (2)	
AA396114	NET1 homologue member of Dbl family of Rho A GEFs)	Net1	0.60 ± 0.02 (2)	
Biosynthesis and metabolism				
AA068968	Involved in GPI-anchor biosynthesis	Pig-b	0.51 ± 0.08 (2)	
AA199988	Mannosidase 1, alpha	Man1a	0.60 ± 0.08 (2)	0.29
Growth factor/Cytokine and receptors				
AA020307	Very low density lipoprotein receptor	Vldlr	0.54 ± 0.15 (3)	
AA008515	Transforming growth factor, β receptor III	Tgfbr3	0.56 ± 0.01 (2)	
W53962	Transforming growth factor, β 2	Tgfb2	0.66 ± 0.05 (3)	
Cytoskeleton/Cell adhesion/Cell and organelle structure				
W99951	Neurofilament, light polypeptide (68 kDa)	Nfl	0.40 ± 0.03 (3)	
W13290	Tight junction protein 1	Tjp1	0.45 ± 0.00 (2)	
AA388122	Maternal embryonic message 3 (vacuolar sorting protein 35)	Mem3	0.45 ± 0.05 (2)	
AA009086	Golgi autoantigen, golgin subfamily a, 4	Golga4	0.49 ± 0.03 (2)	
AA021816	Adducin 3 (γ) [membrane skeletal protein]	Add3	0.56 ± 0.08 (3)	
AA060038	Epsin 2 (involved in clathrin-mediated endocytosis)	Epn2	0.58 ± 0.11 (3)	
AA472871	Lysosomal membrane glycoprotein 2	Lamp2	0.59 ± 0.11 (2)	0.31
AA274739	Pinin (desmosome-associated and nuclear mRNA splicing protein)	Pnn	0.62 ± 0.07 (3)	
Extracellular matrix				
AA239404	Nidogen	Nid	0.48 ± 0.07 (2)	
AA059779	Laminin, γ 1	Lamc1	0.60 ± 0.03 (2)	
AA260280	Procollagen, type III, α1	Col3a1	0.68 ± 0.03 (2)	0.12
Apoptosis/Cell death				
AA175651	Caspase 11	Casp11	0.38 ± 0.22 (2)	
AA098139	Caspase 1	Casp1	0.54 ± 0.09 (3)	
Inflammation/Immune and antiviral response/histocompatibility				
AA174447	IFN-activated gene 203	Ifi203	0.54 ± 0.02 (3)	0.13
AA189587	Natural killer tumor recognition sequence	Nktr	0.59 ± 0.02 (3)	

Table 6 *Continued*

Accession no.	Gene name	Symbol	Ratio: Mean ± SE (range)	Ratio: Affymetrix Array ($N = 1$)
Differentiation/Development/Tissue-specific expression or function				
AA200033	Fragile X mental retardation syndrome 1 homologue (RNA binding protein)	Fmr1	0.54 ± 0.08 (2)	
AA174970	Quaking (KH domain, GGA repeat protein; role in myelination)	Qk	0.60 ± 0.03 (3)	
W91526	Homeobox B9	Hoxb9	0.62 ± 0.12 (2)	0.061
Channels/Pore structure/Transport				
AA137859	Rabaptin 5 (RAB5 effector protein, vesicular transport)	Rab5ep-pending	0.35 ± 0.02 (2)	
AA063753	ATP-binding cassette 1 (multidrug transport & scavenger receptor)	Abc1	0.38 ± 0.05 (3)	0.20
RNA or Protein processing/Protein modification and proteolysis				
AA153909	Itchy (E3 ubiquitin protein ligase)	Itch	0.50 ± 0.03 (2)	
AA154542	Tripeptidyl peptidase II (subtilase family serine protease)	Tpp2	0.51 ± 0.08 (3)	
AA466714	YME1-like 1 (yeast) [ATP-dependent metalloprotease, mitochondrial]	Yme1l1	0.51 ± 0.07 (2)	
AA267222	Ubiquitin-conjugating enzyme E3A	Ube3a	0.54 ± 0.05 (3)	
Miscellaneous and function unknown				
AA146494	Kidney androgen regulated protein (function unknown)	Kap	0.50 ± 0.07 (3)	
W11889	Hemochromatosis	Hfe	0.64 ± 0.04 (2)	0.19

than control (Brca1$^{+/+}$) MEFs to H_2O_2 ($P < 0.001$ to 0.01; Fig. 2*C*). The cell viability values shown in Fig. 2*C* are means ± SE of three independent experiment, each of which used 10 replicate wells per dose of H_2O_2. The herbicide paraquat induces cytotoxicity by causing the generation superoxide ions (O_2^-), which are detoxified by a mechanism distinct from H_2O_2. Brca1-deficient MEFs were more sensitive to paraquat than wild-type MEFs ($P < 0.001$ to 0.01; Fig. 2*D*). A DU-145 wtBRCA1 clone was less sensitive than the Neo clone, whereas BRCA1-siRNA conferred increased sensitivity to paraquat ($P < 0.001$ to 0.05; Fig. 2*E*). These findings suggest that exogenous and endogenous BRCA1 protects cells against several distinct forms of oxidative stress.

The assays shown in Fig. 2*A–E* used a 24-hour exposure to H_2O_2 or paraquat. We performed additional studies to rule out the possibility that the effects of BRCA1 are limited to short-term assays. Thus, DU-145 wtBRCA1 or Neo cell clones were incubated with H_2O_2 for different time intervals from T = 16 to 96 hours and then tested for cell viability with MTT assays. Because of the prolonged exposure times, lower doses of H_2O_2 (either 10 or 25 nmol/L) were tested. These studies revealed persistent and significant increases in viability of the wtBRCA1 cell clones. Thus, at the lower dose of H_2O_2, cell viability increases of up to ~20% were observed, whereas at the higher doses, increases of up to 30 to 35% were found ($P < 0.001$ to 0.05, two-tailed *t* tests).

Finally, we tested the effect of transient expression of wtBRCA1 on the response of DU-145 cells to H_2O_2 with a different end point, trypan blue dye exclusion. MTT assays assess the ability of mitochondria to reduce a tetrazolium salt to formazan, a measure of mitochondrial viability, whereas trypan blue exclusion assesses the ability of an intact plasma membrane to exclude the dye. At H_2O_2 doses of 300 to

Table 7 *Comparison of Brca1 Δ exon 11 MEFs versus exogenous wtBRCA1 in DU-145/MCF-7 (this study)*

A. Concordance of BRCA1-regulated gene expression in different cell types

Accession no.	Gene name (symbol)	MEFs	DU-145	MCF-7 (−E2)	MCF-7 (+E2)
T74192	Plasma protein S (α) [PROS1]	↓ in Brca1 Δ exon 11 MEFs	3.3 ± 0.6 (3)		
AA664180	Glutathione peroxidase 3 (plasma) [GPX3]	↓ in Brca1 Δ exon 11 MEFs	2.4 ± 0.5 (2)		
AA063521	E1B 19K/Bcl-2 binding protein Nip3 (BNIP3)	↓ in Brca1 Δ exon 11 MEFs		1.6 (1)	2.8 ± 0.1 (2)
H27379	Transcription elongation factor (S-II)	↓ in Brca1 Δ exon 11 MEFs			2.1 ± 0.5 (2)
N77754	Lysosome-associated membrane protein 2 (LAMP2)	↓ in Brca1 Δ exon 11 MEFs			2.0 ± 0.1 (2)
R24543	Guanine nucleotide regulatory protein (NET1)	↓ in Brca1 Δ exon 11 MEFs			2.0 ± 0.2 (2)
AA496013	Protein serine/threonine kinase (STK2)	↓ in Brca1 Δ exon 11 MEFs			1.9 ± 0.0 (2)
AA482328	Myristolated ala-rich C-kinase substrate (MACS)	↓ in Brca1 Δ exon 11 MEFs			1.7 ± 0.1 (2)
AA233809	Transforming growth factor β2 (TGFB2)	↓ in Brca1 Δ exon 11 MEFs		1.6 ± 0.1 (2)	

B. Comparison of results from this study *versus* previously published data

Gene Name	Published data (reference)	Our results
Concordant findings		
Rho-associated coiled-coil forming kinase 2 (Rock2)	↓ in Brca1-deficient embryonic stem cells (39)	↓ in Brca1-deficient MEFs
Creatine kinase B (Ckb)	↓ in Brca1-deficient embryonic stem cells (39)	↑ in DU-145 wtBRCA1 clones
Quaking (Qk)	↓ in Brca1-deficient embryonic stem cells (39)	↓ in Brca1-deficient MEFs
Multifunctional aminoacyl tRNA synthtase (Syhuqt)	↓ in Brca1-deficient embryonic stem cells (39)	↑ in DU-145 wtBRCA1 clones
Neurofilament, light polypeptide (68 kDa) [Nfl, NF68]	↓ in Brca1-deficient embryonic stem cells (39)	↓ in Brca1-deficient MEFs
26S proteasome regulatory subunit 7 (Psmc2)	↓ in Brca1-deficient embryonic stem cells (39)	↑ in DU-145 wtBRCA1 clones
UDP-*N*-acteylglucosamine transferase (MGAT2)	↑ by wtBRCA1 in 293T cells (37)	↑ in DU-145 wtBRCA1 clones
Cyclin G2 [CCNG2]	↑ by wtBRCA1 in 293T cells (37)	↑ by wtBRCA1 in MCF-7 cells
Follistatin-like 1 [FSTL1]	↑ by wtBRCA1 in 293T cells (37)	↑ by wtBRCA1 in MCF-7 cells
Selenoprotein plasma protein 1 [SEPP1]	↑ by wtBRCA1 in 293T cells (37)	↓ in Brca1-deficient MEFs
Zing finger protein 148 (ZNF148)	↑ by wtBRCA1 in 293T cells (37)	↓ in Brca1-deficient MEFs
Laminin α-3 (LAMA3)	↑ by wtBRCA1 in 293T cells (37)	↑ in DU-145 wtBRCA1 clones
Ectonucleotide phosphodiesterase 2 (ENPP2)	↑ by wtBRCA1 in 293T cells (37)	↓ in Brca1-deficient MEFs
Potassium channel KCNK1 (= TWIK1)	↑ by wtBRCA1 in 293T cells (37)	↑ in DU-145 wtBRCA1 clones
DNA topoisomerase I (TOP1)	↑ by wtBRCA1 in colon cancer cells (38)	↓ in Brca1-deficient MEFs
Superoxide dismutase 1 (Sod1)	↑ by wtBRCA1 in colon cancer cells (38)	↓ in Brca1-deficient MEFs
CD59 antigen (CD59)	↓ by wtBRCA1 in colon cancer cells (38)	↓ in wtBRCA1 clones
Nonconcordant Findings		
Janus kinase 1 (JAK1)	↑ by wtBRCA1 in 293T cells (37)	↓ in DU-145 wtBRCA1 clones
COP9 subunit 3 (COP9S3)	↑ by wtBRCA1 in 293T cells (37)	↓ in DU-145 wtBRCA1 clones
Lim and senescent cell antigen-like domain 1 (LIMS1)	↑ by wtBRCA1 in 293T cells (37)	↓ in DU-145 wtBRCA1 clones
HIV Tat-interacting protein (HTATIP)	↑ by wtBRCA1 in 293T cells (37)	↓ in DU-145 wtBRCA1 clones

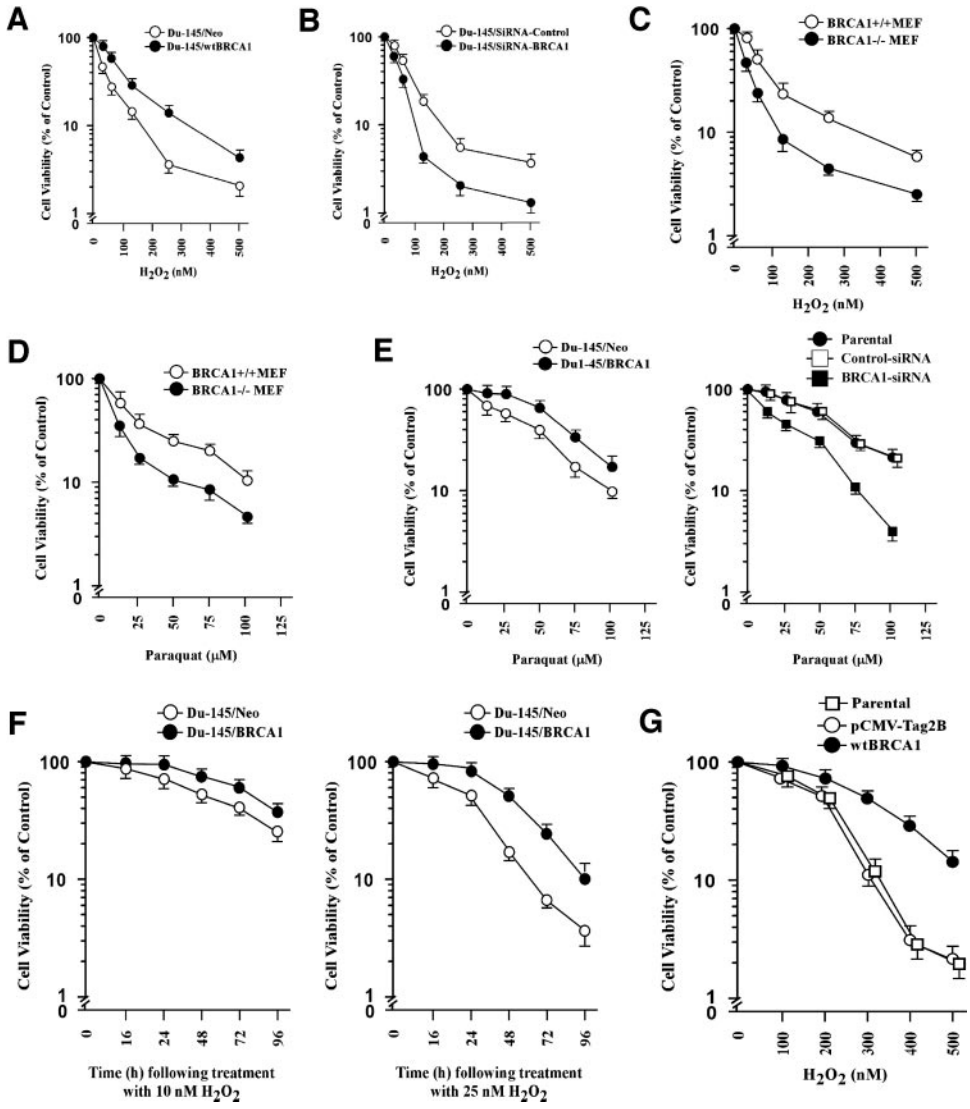

Fig. 2. Exogenous and endogenous BRCA1 confer resistance to oxidative stress. *A*. DU-145 wtBRCA1 clones are resistant to H_2O_2. Two wtBRCA1 and two control (Neo) clones in 96-well dishes were incubated with different doses of H_2O_2 for 24 hours and assayed for cell viability using MTT assays. For each clone and H_2O_2 dose, $n = 10$ wells were tested. Because the two clones of each clonal type behaved similarly, the data were pooled and averaged. Cell viability values are means ± SE (relative to 0 dose control). Comparisons of wtBRCA1 *versus* Neo clones were statistically significant at each dose of H_2O_2 ($P < 0.001$, two-tailed t tests). Note: The data shown in *A* and *B* are representative of $n = 2$ to 3 independent experiments. *B*. BRCA1 knockdown confers sensitivity to H_2O_2. DU-145 cells were incubated with BRCA1 or control siRNA (50 nmol/L × 72 hours), exposed to different doses of H_2O_2 for T = 24 h, and tested for cell viability using MTT assays. Comparisons of BRCA1-siRNA– *versus* control-siRNA–treated cells were significant at all except the lowest dose of H_2O_2 ($P < 0.001$). C. Brca1-deficient MEFs are more sensitive than control MEFs to H_2O_2. Cell sensitivity to H_2O_2 was compared in Brca1 Δ exon 11 ($Brca1^{-/-}$) *versus* wild-type ($Brca1^{+/+}$) MEFs with MTT assays. Values of cell viability are means ± SE of $n = 3$ independent experiments. For each experiment and each dose of H_2O_2, $n = 10$ replicate wells were assayed, and the values were averaged. $Brca1^{-/-}$ MEFs were more sensitive to H_2O_2 at every dose tested ($P < 0.001$ to 0.01). *D*. Brca1-deficient MEFs are more sensitive to paraquat than control MEFs. Cells were treated with different doses of paraquat for T = 24 hours and then tested with MTT assays for their viability. The values of cell viability are means ± SE of $n = 3$ independent experiments. For each experiment and each dose of paraquat, $n = 10$ replicate wells were tested, and the cell viability values were averaged. $Brca1^{-/-}$ MEFs showed reduced survival rates relative to wild-type ($Brca1^{+/+}$) MEFs at each dose of paraquat tested ($P < 0.001$ to 0.01). *E*. wtBRCA1 confers resistance and BRCA1-siRNA confers sensitivity to paraquat. (*left*). DU-145 wtBRCA1 and Neo cell clones were tested with MTT assays for sensitivity to paraquat. Cell viability values are means ± SE of $n = 3$ independent experiments. For each experiment and each dose, $n = 10$ replicate wells were assayed per clone × two clones per clone type = 20 wells per clone type, and the values were averaged. Comparisons of wtBRCA1 *versus* Neo clones were significant at all paraquat doses ($P < 0.01$ to 0.05) (*right*). DU-145 cells were treated with control-siRNA, BRCA1-siRNA (50 nmol/L × 72 hours) or vehicle only (Parental) and then assayed for paraquat sensitivity as above. BRCA1-siRNA–treated cells were more sensitive to paraquat than control-siRNA or vehicle-treated cells ($P < 0.001$ to 0.01). *F*, time course for wtBRCA1-mediated protection against H_2O_2. wtBRCA1 and Neo DU-145 cell clones were exposed to 25 nmol/L (*left*) or 10 nmol/L (*right*) of H_2O_2 for different time intervals ranging from T = 16 to 96 hours and then assayed for cell viability using MTT assays. Cell viability values are expressed relative to untreated cells (T = 0 hour) and represent means ± SE for three independent experiments. At 25 nmol/L H_2O_2, survival rates were higher for wtBRCA1 than Neo clones from T = 24 to 96 hours ($P < 0.01$ to 0.001, two-tailed t tests). At 10 nmol/L H_2O_2, survival was higher in wtBRCA1 cell clones from T = 24 to 96 hours ($P < 0.01$ to 0.05). *G*. Transient expression of wtBRCA1 protects DU-145 cells against H_2O_2, as indicated by trypan blue dye exclusion assays. Subconfluent proliferating cells were transfected overnight with FLAG-wtBRCA1, empty pCMVTag2B vector or no vector (parental), washed, postincubated for 24 hours to allow BRCA1 gene expression, and exposed to different doses of H_2O_2 for T = 24 hours. The cells were then harvested with trypsin, stained with trypan blue dye, and counted. Cell viability was determined by counting the trypan blue dye-excluding cells and expressed relative to untreated control cells. The values plotted are means ± SE of $n = 3$ independent experiments. For each experiment and each dose of H_2O_2, at least 200 cells were counted. At H_2O_2 doses of 300 to 500 nmol/L, cell viability was higher in wtBRCA1-transfected cells than in control cells (empty vector or untransfected; $P < 0.001$).

500 nmol/L, we found significant increases (11 to 40%) in the proportion of wtBRCA1-transfected cells that excluded trypan blue dye, as compared with empty vector transfected cells or untransfected cells ($P < 0.001$; Fig. 2*G*). Values in Fig. 2*G* are means ± SE of three independent experiments. Although there is some variability from experiment to experiment, the cell viability values tended to be higher with the trypan blue assay than the MTT assay. This may reflect the fact that loss of membrane integrity is a late end point; thus, cells that

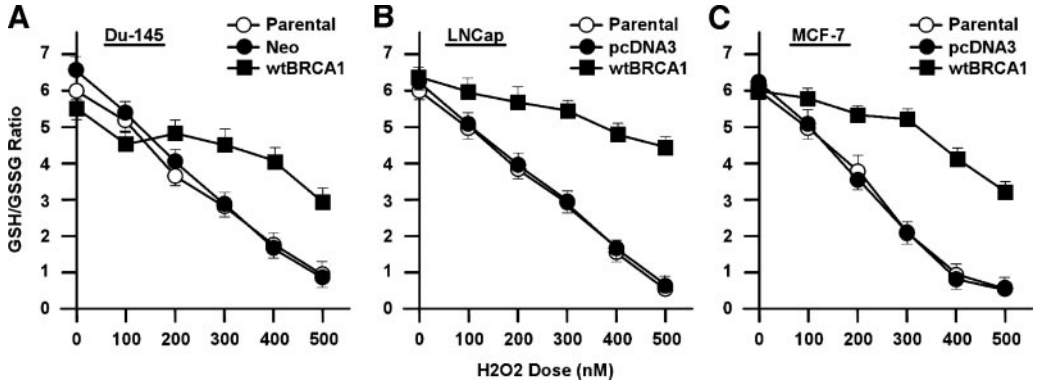

Fig. 3. BRCA1 attenuates loss of GSH in response to oxidative stress. For LNCaP and MCF-7, the cells were transfected overnight with wtBRCA1, empty vector, or vehicle, washed, treated with different doses of H_2O_2 for 24 hours, and assayed for GSH and GSSG. For DU-145, stable wtBRCA1 and Neo cell clones were tested. At doses of $H_2O_2 \geq 100$ nmol/L for MCF-7 and LNCaP and ≥200 nmol/L for DU-145, wtBRCA1-transfected cells had significantly higher ratios of GSH/GSSG than control cells ($P < 0.001$, two-tailed t tests).

have lost mitochondrial function (MTT end point) may not yet have lost their membrane integrity. Regardless, it seems clear that overexpression of BRCA1 (by either stable or transient transfection) protects and inactivation of BRCA1 (by either knockdown or gene deletion) sensitizes cells against oxidative stress.

The response to oxidative stress depends upon the ability of the cell to maintain its redox balance (*i.e.,* the ratio of reduced to oxidized glutathione) in the setting of stress. We examined the effect of exogenous wtBRCA1 on the redox balance of prostate (DU-145 and LNCaP) and breast (MCF-7) cancer cell lines after treatment with different doses of H_2O_2 for 24 hours. The end point was the ratio of GSH to GSSG. BRCA1-transfected cells showed a mostly similar basal redox balance to vector-transfected and untransfected control cells (Fig. 3). H_2O_2 caused a dose-dependent shift in the redox state to increased GSSG and decreased GSH levels. However, wtBRCA1-transfected cells were able to maintain significantly higher ratios of GSH/GSSG than control cells, especially at high doses of H_2O_2 ($P < 0.001$, two tailed t tests). These findings suggest that BRCA1 enhances the production of GSH in response to oxidative stress.

BRCA1 Regulates ARE-driven Transcription. The cytoprotective antioxidant response is mediated, in part, by the nuclear factor (erythroid-derived 2)-like factors NFE2L2 (NRF2) and NFE2L1 (NRF1) via the ARE (29). In this regard, BRCA1 appears to regulate a subset of genes that are known to be regulated by NRF2 [NQO1, MGST2, G6PD, malic enzyme (ME2), and Gsta1/2] and/or that are known to contain AREs in their promoters (NQO1, MGST1/2, and Gsta1/2; refs. 30, 31). This finding suggests that BRCA1 protection against oxidants may be mediated, in part, by NRF2. To test this hypothesis, we performed transient transfection assays using an NRF2-responsive reporter driven by the ARE of NQO1 (NQO1-ARE-Luc).

wtBRCA1 increased the basal activity of the NQO1-ARE-Luc reporter in DU-145, T47D, and MCF-7 cells by 1.6 to 6.6-fold, as compared with empty pcDNA3 vector or no vector (Fig. 4*A*). In these assays, MCF-7 cells showed larger wtBRCA1-induced increases in ARE-Luc activity than DU-145 or T47D cells, but all cell types showed significant increases in ARE-Luc activity ($P < 0.01$). Co-expression of wtBRCA1 with NRF2 caused a modest but significant increase in NRF2-stimulated NQO1-ARE-Luc activity in DU-145 and T47D cells (36 to 50%; $P < 0.01$) but caused a much larger increase in NRF2-stimulated activity (4.3-fold) in MCF-7 cells ($P < 0.001$; Fig. 4*B*). In plasmid dose-response studies of MCF-7 cells, increases in NQO1-ARE-Luc reporter activity were detectable at 10 to 50 ng per well of wtBRCA1 and were half maximal by 100 ng per well (Fig. 4*C*). An 8-fold stimulation of reporter activity was achieved at our standard plasmid dose (0.25 μg per well), and the stimulation reached a maximum of 11-fold at 2.5 μg of plasmid per well.

In contrast to wtBRCA1, BRCA1-siRNA (but not a control-siRNA) significantly decreased basal and NRF2-stimulated NQO1-ARE-Luc activity (Fig. 4, *D* and *E*). Decreases in basal and NRF2-stimulated activity ranged from 60 to 100% ($P < 0.001$). These findings suggest that BRCA1 regulates NRF2 activity through the ARE over a wide range of intracellular BRCA1 protein levels. Next, we determined the BRCA1 structural requirements for stimulation of ARE activity with a series of previously described expression vectors encoding truncated or mutant BRCA1 proteins (Fig. 4*F*; refs. 12, 14, 15). These studies revealed that COOH-terminal truncations (Δ *Bam*HI, Δ *Kpn*I, and Δ *Eco*RI) or mutations (5382insC and C5365G) of BRCA1 retained the ability to stimulate reporter activity, but a point mutation in the NH_2-terminal RING domain (T300G) or a NH_2-terminal truncation abrogated the ability of BRCA1 to stimulate activity (Fig. 4*F*). These findings suggest that the NH_2 terminus of BRCA1 is both necessary and sufficient to stimulate NQO1-ARE-Luc activity.

Fig. 4*G* shows BRCA1 protein levels in MCF-7 and DU-145 cells experimentally manipulated to over- or underexpress BRCA1. As noted earlier (14), DU-145 cells show low basal BRCA1 expression that is significantly increased by stable (Fig. 1*A*) or transient (Fig. 4*G*) expression of exogenous wtBRCA1. In this (Fig. 4*G*) and a prior study (15), basal BRCA1 protein levels in MCF-7 cells were significantly higher than in DU-145 cells were similar to or slightly less than those observed in wtBRCA1-transfected DU-145 cells. By way of comparison, BRCA1 levels in lymphoblastoid cell lines derived from a BRCA1 (185delAG) [R794] and a BRCA2 (6174delT) [R1041] mutation carrier were generally similar to the BRCA1 levels observed in untransfected MCF-7 cells or in wtBRCA1-transfected DU-145 cells (Fig. 4*G*). For both MCF-7 and DU-145 cells, BRCA1-siRNA abolished or nearly abolished BRCA1 protein expression, whereas the control-siRNA had little or no effect on protein expression. The physiologic significance of these findings is considered in the Discussion.

We used a dominant negative NRF2 expression vector (20) to determine whether the endogenous NRF2 protein is required for BRCA1 to stimulate the antioxidant response. Here, we found that co-expression of DN-NRF2 ablated basal NQO1-ARE-Luc activity (some of which is dependent upon endogenous BRCA1), as well as wtBRCA1-stimulated activity ($P < 0.001$; Fig. 5*A*). Consistent with these findings, transient expression of the DN-NRF2 sensitized MCF-7 cells to H_2O_2 and abrogated the ability of wtBRCA1 to protect MCF-7 cells against to H_2O_2 ($P < 0.01$; Fig. 4*B*).

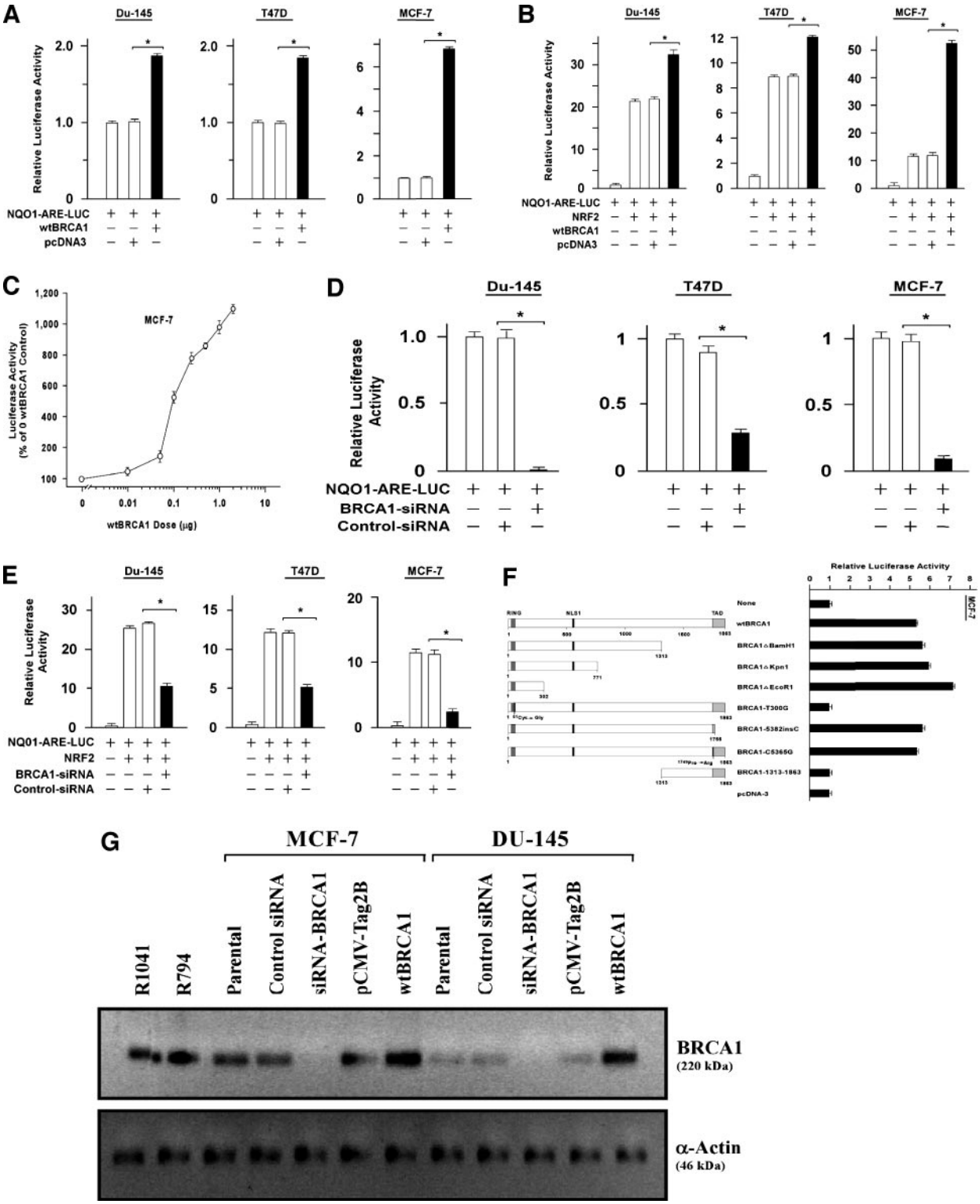

Fig. 4. BRCA1 stimulates NRF2 activity through the AREs. *A* and *B*. wtBRCA1 stimulates basal (*A*) and NRF2-induced (*B*) NQO1-ARE-Luc reporter activity. Cells were transfected with the indicated vector(s), postincubated for 24 hours, and assayed for luciferase activity. The values are expressed relative to the control (reporter only) and are means ± SE of $n = 4$ wells. ∗Represents a significant difference ($P < 0.01$, two-tailed t test). *C*, plasmid dose response for wtBRCA1 stimulation of basal NQO1-ARE-Luc activity. The total transfected DNA content was kept constant by the addition of empty pcDNA3 vector. *D* and *E*. BRCA1-siRNA inhibits basal (*D*) and NRF2-stimulated (*E*) NQO1-ARE-Luc activity. The cells were pretreated with no siRNA (transfection reagent only), control-siRNA, or BRCA1-siRNA for 72 hours, and the transcriptional assays were performed as described above. *F*, structural determinants for stimulation of NQO1-ARE-Luc activity. MCF-7 cells were cotransfected with NQO1-ARE-Luc and a set of expression vectors encoding mutant or truncated BRCA1 proteins, postincubated for 24 hours, and assayed for luciferase activity. The luciferase values are expressed relative to control cells transfected only with NQO-ARE-Luc (= 100%). *G*, BRCA1 protein expression levels in different cell types and in response to experimental manipulation. Proliferating cell cultures were harvested and analyzed for BRCA1 and α-actin by Western blotting. *Lanes 1* and *2* show BRCA1 protein expression in EBV-immortalized peripheral blood lymphocyte-derived cell lines from female BRCA1 (185delAG) [R794] and BRCA2 (6174delT) [R1041] mutation carriers. *Lanes 3* to *12* show BRCA1 protein levels in MCF-7 or DU-145 cells that were untreated (parental), exposed to BRCA1 or control siRNAs (50 nmol/L × 72 hours), or transfected with FLAG-wtBRCA1 or empty pCMV-Tag2B vectors (overnight transfected followed by a 24-hour postincubation).

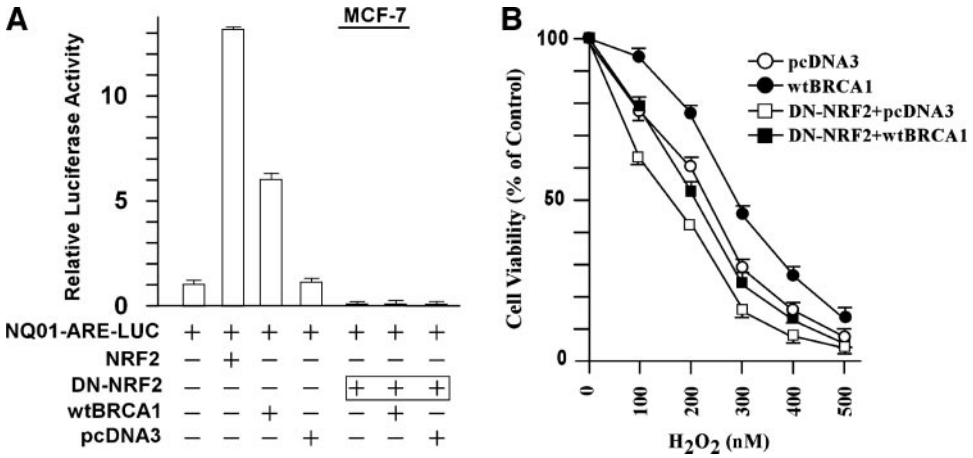

Fig. 5. Inhibition of NRF2 blocks wtBRCA1 stimulation of antioxidant response. *A*. Dominant negative NRF2 (DN-NRF2) inhibits BRCA1-induced NQO1-ARE-Luc activity. Cotransfection of a DN-NRF2 into MCF-7 cells inhibited basal and wtBRCA1-induced NQO1-ARE-Luc activity ($P < 0.001$ for comparisons of cells transfected − *versus* + DN-NRF2). *B*. DN-NRF2 abrogates wtBRCA1-mediated protection of MCF-7 cells against H_2O_2. Cells were transfected ± empty pcDNA3 vector, ± wtBRCA1, and ± DN-NRF2 overnight, washed, and postincubated for 24 hours to allow gene expression. The cells were then analyzed for sensitivity to H_2O_2 using MTT assays, as described above. Control assays in which pcDNA3 vector was omitted (*i.e.,* no vector and DN-NRF2 alone) showed no effect of the pcDNA3 vector and are not shown in the figure for clarity. At 100 to 400 nmol/L, wtBRCA1 protected while DN-NRF2 sensitized cells to H_2O_2 ($P < 0.01$). Addition of DN-NRF2 abolished the protection because of wtBRCA1 ($P < 0.01$).

DISCUSSION

DNA microarray analyses of BRCA1 overexpressing and Brca1-mutant cells identified various categories of genes positively regulated by BRCA1, including genes involved in transcription, stress responses, signal transduction, DNA replication and repair, cell proliferation, metabolism, and other processes. A number of these findings were confirmed by using independent mRNA assays. We identified potential BRCA1-regulated genes consistent with its known functions in DNA repair and cell cycle regulation, *e.g.,* deleted in split hand/split foot syndrome 1 (DSS1) [a BRCA2-interacting protein required for homologous recombination (32)], a DNA cross-link repair gene (Dclre1a, also called SNM1), and several cell cycle regulatory genes [*e.g.,* CDKN2C (p18), G0S2, and Gspt1]. Brca1-deficient cells, which exhibit a defect in centrosome function (16), showed decreased expression of a major centrosome protein, centrosomin A (Csma), three mitotic kinases (Stk2, Stk10, and Clk), and a chromosome segregation gene (Ttc3).

Although the functional categorization of genes is somewhat arbitrary (many genes fit into more than one category), it appeared that overexpression (mutation) of BRCA1 led to increased (decreased) expression of a sizeable group of genes involved in the response to stress, including the antioxidant response, detoxification of xenobiotics, and drug metabolism. Genes up-regulated in BRCA1 overexpressing cells include GSTs and peroxidases (*e.g.,* MGST1/2, GSTT1, GSTZ1, and GPX3), oxidoreductases (*e.g.,* NQO1 and ME2), alcohol and aldehyde dehydrogenases (*e.g.,* ADH5 and ALDH7), a paraoxonase (PON2), an AhR-like protein (MOP2), and other antioxidant proteins.

Consistent with these findings, Brca1-deficient MEFs showed decreased expression of stress-response genes, including Gsta2, Gpx3, Nrf2, Sod1, Ahr, and Sepp1, a selenoprotein that mediates protection against oxidative stress (33). Mice deficient for the major antioxidant response transcription factor Nrf2 exhibited increased susceptibility to hyperoxic lung damage, a reduced expression of several ARE-dependent phase II drug-metabolizing enzymes, increased sensitivity to carcinogens, and decreased protection against carcinogenesis by chemoprevention agents (34, 35). Our findings suggest that BRCA1 regulates the expression of several genes that are known to be regulated by NRF2 and/or to contain AREs in their regulatory regions. A recent study identified Nrf2-regulated genes for which basal and/or inducible expression was increased in the small intestine of $Nrf2^{+/+}$ relative to $Nrf2^{-/-}$ mice (31). BRCA1 increased the expression of some of these Nrf2-regulated genes (NQO1, MGST1/2, Gsta2, G6PD, and ME2). BRCA1 also induced (and Brca1 mutation inhibited) expression of a glutathione peroxidase (GPX3), other isoforms of which are down-regulated in $Nrf2^{-/-}$ cells (31, 34). These results suggest an overlap in the genes regulated by BRCA1 *versus* NRF2.

Consistent with its ability to up-regulate antioxidant gene expression, BRCA1 overexpression conferred resistance, whereas BRCA1 mutation or underexpression conferred sensitivity to two different oxidizing agents (H_2O_2 and paraquat). Because peroxides and superoxide, which is generated by paraquat, are detoxified by distinct enzymatic pathways (*e.g.,* those involving catalase *versus* superoxide dismutase, respectively), these findings suggest that BRCA1 may stimulate more than one antioxidant defense pathway. However, this remains to be demonstrated. BRCA1 is classified as a caretaker gene based on the findings that BRCA1 mutations lead to chromosomal instability and defects in DNA repair (reviewed in ref. 5). The ability of BRCA1 to protect against oxidative stress may contribute to its caretaker function because reactive oxygen species (*e.g.,* H_2O_2, O_2^-, and hydroxyl radicals) generated endogenously in mitochondria and other organelles can cause DNA damage (oxidation). In addition to endogenous reactive oxygen species, which contribute to carcinogenesis (36), many DNA-damaging agents and xenobiotics cause oxidative stress, resulting in DNA damage, protein oxidation, and lipid peroxidation. Some of these lesions are detoxified by BRCA1-regulated genes (*e.g.,* GSTs, GPXs, oxidoreductases, and paroxonases).

Consistent its ability to up-regulate antioxidant genes and protect against oxidants, wtBRCA1 attenuated the loss of GSH due to H_2O_2, thus helping stressed cells to maintain their redox balance. It is not clear how BRCA1 stimulates GSH production under oxidizing conditions. GSH is produced via two processes: (*a*) conversion of GSSG to GSH by glutathione reductase, which requires NADPH; and (*b*) *de novo* synthesis via γ-glutamylcysteine synthetase (31). Both glucose-6-phosphate dehydrogenase (G6PD) and malic enzyme (ME2), which are up-regulated by wtBRCA1, stimulate NADPH formation (process 1). Although γ-glutamylcysteine synthetase was not on the list of BRCA1-regulated genes, γ-glutamylcysteine synthetase in an NRF2/ARE-regulated gene, and BRCA1 stimulates NRF2/ARE activity. Finally, we reported recently that BRCA1 up-regulates the expression

of the small heat shock protein HSP27 (19), which functions to maintain the redox balance, possibly by helping to maintain the activity of cellular redox enzymes (37). Small heat shock proteins such as HSP27 protect cells against oxidative stress, in part, by enhancing G6PD activity (37), which helps to generate the reducing power for conversion of GSSG to GSH. In this regard, our findings suggest that G6PD may be a transcriptional target of BRCA1. The role of HSP27 and other small heat shock proteins in the BRCA1-mediated protection against oxidative and generation of GSH in stressed cells is a subject for additional investigation.

We have established the principle that BRCA1 stimulates ARE signaling and NRF2 transcriptional activity, although the extent of stimulation varied in different cell lines. The stimulation of NQO1-ARE-Luc activity and protection against oxidative stress by wtBRCA1 were ablated by a DN-NRF2, suggesting that NRF2 may be downstream of BRCA1 in an antioxidant response pathway. Although DN-NRF2 also abolished basal ARE-Luc activity and sensitized cells to oxidative stress in the absence of exogenous wtBRCA1, the siRNA experiments suggest that endogenous BRCA1 contributes to basal ARE-Luc activity and resistance to oxidative stress. Hence, some of the effects of DN-NRF2 could be due to pathways downstream of the endogenous BRCA1.

The NH_2 terminus of BRCA1, including the RING domain, was necessary and sufficient to stimulate ARE signaling. A similar pattern (*i.e.,* requirement for the NH_2 terminus but not the COOH-terminus of BRCA1) was observed for stimulation of the HSP27 promoter activity and TERT promoter activity by BRCA1 (15, 19). The siRNA studies suggest the relevance of our findings to sporadic cancers in which BRCA1 expression is reduced, but the implications for BRCA1 mutant cancers are unclear at present because we do not know the extent to which BRCA1 mutant proteins are expressed in human cancers. Although most cancer-associated BRCA1 mutations are protein truncating mutations that should retain the ability to stimulate ARE signaling, the ability to stimulate ARE signaling would be compromised if the mutant BRCA1 proteins are underexpressed or rapidly degraded. Moreover, one cancer-associated BRCA1 mutation, T300G (which affects the NH_2-terminal RING domain), abrogated the ability of BRCA1 to stimulate ARE-Luc activity. Our previous work indicates that the BRCA1-T300G mutant protein is stable and is well expressed (12, 15).

The ability of BRCA1 to protect against oxidant toxicity may be due, in part, to stimulation of antioxidant defenses (*e.g.,* increased expression of antioxidant genes, increased production of GSH, and stimulation of NRF2 transcriptional activity). However, because DNA is a major target of oxidizing agents, the ability of BRCA1 to stimulate DNA repair (5) could also contribute to its cytoprotective activity. The extent to which BRCA1 functions to prevent DNA damage by enhancing detoxification of peroxides and superoxides as opposed to repairing established DNA lesions remains to be determined.

Our studies used three different models to investigate BRCA1 function: (*a*) overexpression (via stable or transient expression of exogenous wtBRCA1); (*b*) underexpression (via RNA interference); and (*c*) inactivation via gene deletion (Δ exon 11, which removes most of the Brca1 protein). Relative to model 1 (overexpression), studies of mice indicate that Brca1 is particularly highly expressed in the mammary gland in proliferating cells undergoing differentiation during puberty and pregnancy (38, 39). It has been suggested that the BRCA1 may play a particularly important role in preventing tumors during specific windows of time (*e.g.,* puberty and pregnancy) in which it is highly expressed. The BRCA1 overexpression model might reflect these time periods when BRCA1is normally overexpressed. This expression pattern may also be reflected *in vitro* because BRCA1 expression is greatly increased when cultured mammary epithelial cells are forced to undergo differentiation (*e.g.,* by the use of a hormonal mixture; refs. 40, 41). It remains to be proved whether these periods in which BRCA1 is highly expressed are directly related to its tumor suppressor function.

The role of endogenous BRCA1 in mediating protection against oxidative stress and/or stimulating NRF2 activity was documented in two different models (deletion of exon 11 in MEFs and knockdown of BRCA1 protein levels with an siRNA). The exon 11 deletion model may reflect the situation in BRCA1 mutant cancers, where the wild-type BRCA1 is usually lost (5, 42). We also note that the BRCA1 Δ exon 11 protein corresponds to a naturally occurring splice variant of BRCA1 in humans and mice (25, 43). As noted earlier, BRCA1 expression is often decreased or absent in sporadic breast cancers that do not exhibit a BRCA1 mutation (3, 4). This loss of BRCA1 expression may be due, in part, to epigenetic causes (hypermethylation of the BRCA1 promoter) and/or haploinsufficiency (loss of one BRCA1 allele; refs. 4, 5, 44). Regardless of the etiology, model 3 (BRCA1-siRNA) may reflect the underexpression of BRCA1 commonly observed in sporadic breast and ovarian cancers. The finding that BRCA1 can modulate various aspects of antioxidant defense over a wide range of BRCA1 expression levels is consistent with a physiologic role for BRCA1 in this pathway.

Taken together, our findings suggest a novel mechanism by which BRCA1 may prevent cancer development by enhancing antioxidant defenses, thereby protecting cells against damage caused by exogenous and/or endogenous reactive oxygen species. However, a definitive linkage between BRCA1-mediated protection against oxidative stress and tumor suppression remains to be demonstrated. They also suggest that in addition to its established roles in the repair of DNA damage, BRCA1 may prevent DNA damage due to ionizing radiation and other sources through the detoxification of reactive oxygen species, although this needs to be proven. Finally, these studies suggest a collaboration between BRCA1 and a transcription factor (NRF2) that functions to mobilize the cell's antioxidant machinery.

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