Lymphoblasts of Women with BRCA1 Mutations Are Deficient in Cellular Repair of 8,5‘-Cyclopurine-2‘-deoxynucleosides and 8-Hydroxy-2‘-deoxyguanosine †

ARTICLE *in* BIOCHEMISTRY · APRIL 2007

Impact Factor: 3.02 · DOI: 10.1021/bi062022p · Source: PubMed

Lymphoblasts of Women with *BRCA1* Mutations Are Deficient in Cellular Repair of 8,5′-Cyclopurine-2′-deoxynucleosides and 8-Hydroxy-2′-deoxyguanosine†

Henry Rodriguez,‡,§,|| Pawel Jaruga,‡,⊥,# Dennis Leber,|| Simon G. Nyaga,¶ Michele K. Evans,¶ and Miral Dizdaroglu*,||

Chemical Science and Technology Laboratory, and Statistical Engineering Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311, Department of Chemical and Biochemical Engineering, University of Maryland Baltimore County, Baltimore, Maryland 21250, Department of Clinical Biochemistry, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland, and Laboratory of Cellular and Molecular Biology, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224

Received September 28, 2006; Revised Manuscript Received December 11, 2006

ABSTRACT: Mutations in breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2* predispose women to a high risk of these cancers. Here, we show that lymphoblasts of women with *BRCA1* mutations who had been diagnosed with breast cancer are deficient in the repair of some products of oxidative DNA damage, namely, 8-hydroxy-2′-deoxyguanosine and 8,5′-cyclopurine-2′-deoxynucleosides. Cultured lymphoblasts from 10 individuals with *BRCA1* mutations and those from 5 control individuals were exposed to 5 Gy of ionizing radiation to induce oxidative DNA damage and then allowed to repair this damage. DNA samples isolated from these cells were analyzed by liquid chromatography/mass spectrometry and gas chromatography/mass spectrometry to measure 8-hydroxy-2′-deoxyguanosine, (5′-*S*)-8,5′-cyclo-2′-deoxyadenosine, (5′-*R*)-8,5′-cyclo-2′-deoxyguanosine, and (5′-*S*)-8,5′-cyclo-2′-deoxyguanosine. After irradiation and a subsequent period of repair, no significant accumulation of these lesions was observed in the DNA from control cells. In contrast, cells with *BRCA1* mutations accumulated statistically significant levels of these lesions in their DNA, providing evidence of a deficiency in DNA repair. In addition, a commonly used breast tumor cell line exhibited the same effect when compared to a relevant control cell line. The data suggest that BRCA1 plays a role in cellular repair of oxidatively induced DNA lesions. The failure of cells with *BRCA1* mutations to repair 8,5′-cyclopurine-2′-deoxynucleosides indicates the involvement of BRCA1 in nucleotide-excision repair of oxidative DNA damage. This work suggest that accumulation of these lesions may lead to a high rate of mutations and to deleterious changes in gene expression, increasing breast cancer risk and contributing to breast carcinogenesis.

Breast cancer is the second leading cause of cancer deaths among women. Inherited mutations that affect a single allele of the breast cancer 1 and 2 genes (*BRCA1* and *BRCA2*) predispose women to a high risk of breast and ovarian cancers, although the magnitude of this risk is controversial (reviewed in ref *1*). Hereditary breast cancers account for approximately 5−10% of all breast cancers among women, whereas other breast cancers are considered to be sporadic (*2*−*4*). On the other hand, 30−60% of familial breast cancers result from inherited mutations on *BRCA1* and *BRCA2* (*4*−*6*). *BRCA1* plays an important role in maintaining the genome integrity, at least in part, through its role in the repair of DNA damage (reviewed in refs *3* and *7*−*10*). Thus, the *BRCA1* product, BRCA1 is involved at multiple steps in the cellular response to DNA damage as it associates and co-localizes with RAD51, a DNA recombinase, and therefore participates in the repair of DNA double-strand breaks by homologous recombination (*11*−*13*). Further evidence shows that BRCA1 plays a role in the repair of other types of oxidative DNA damage because *BRCA1*-deficient cells exhibit chromosomal abnormalities and are hypersensitive to oxidative damage caused by DNA-damaging agents, such as ionizing radiation and hydrogen peroxide (*3*, *14*, *15*).

Epidemiological studies and those with animal models strongly suggest that free radicals generated by oxidative stress may be involved in breast carcinogenesis (*16*−*18*). Oxidative damage by free radicals generates a plethora of modifications in DNA, including base and sugar lesions, 8,5′-cyclopurine-2′-deoxynucleosides, DNA−protein cross-links, and strand breaks (reviewed in ref *19*). The majority of DNA lesions are mutagenic and/or lethal lesions and thus may play an important role in a number of disease processes (reviewed in refs *19* and *20*). Among these lesions, modified bases are mainly repaired by base-excision repair (BER)[1] pathways

† This research was supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health.

* To whom correspondence should be addressed: National Institute of Standards and Technology, 100 Bureau Drive, Stop 8311, Gaithersburg, MD 20899-8311. Telephone: (301) 975-4617. Fax: (301) 975-8505. E-mail: miral@nist.gov.

‡ These authors contributed equally to this work.

§ Present address: Office of Technology and Industrial Relations, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

|| National Institute of Standards and Technology.

⊥ University of Maryland Baltimore County.

Nicolaus Copernicus University.

¶ National Institute on Aging.

10.1021/bi062022p CCC: $37.00
Published on Web 02/09/2007

Table 1: Cell Lines Used in This Work and Their Characteristics as Listed on the Website of CCR (http://ccr.coriell.org/nigms/charmut/catbrca1.html)

repository number	age (year)	age at diagnosis (year)	exon	codon	*BRCA1* mutation	race (all females)
control						
GM01078	30	N/A				Caucasian
GM02152	40	N/A				Caucasian
GM14452	46	N/A				Caucasian
GM02148	54	N/A				Caucasian
GM09015	74	N/A				Caucasian
BRCA1 mutated						
GM14093	30	27	11	401	1323delG	African American
GM13709	32	32	11	672	2135delA	Caucasian
GM16105	35	35			IVS8 +2 T>A	Caucasian
GM14090	43	34	2	23	185delAG	Caucasian
GM14094	44	43	11	392−405	1294del40	Caucasian
GM14637	46	43	13	1443	Arg1443ter	Caucasian
GM13707	50	39	11	392−405	1294del40	Caucasian
GM13715	56	43	20	1755	5382insC	Caucasian
GM13708	68	62	16	1563	Tyr1563ter	not listed
GM13711	75	74	11	1040	Ser1040Asn	Caucasian

(reviewed in refs *21*−*23*). On the other hand, nucleotide-excision repair (NER) may also be involved in the repair of modified bases as reported for the repair of 8-hydroxyguanine (8-OH-Gua) and thymine glycol (*24*, *25*). Furthermore, NER is the only known pathway for the repair of 8,5′-cyclopurine-2′-deoxynucleosides (*26*−*29*). BRCA1 participates in NER of oxidative DNA damage, especially in transcription-coupled repair (TCR) subpathway (*9*, *15*, *25*, *30*−*32*). There is also evidence that BRCA1 binds directly and strongly to DNA as part of its mechanism of action and enhances global genomic repair, a subpathway of NER (*31*, *33*). BRCA1 contributes to the TCR of 8-OH-Gua in human cells (*25*), whereas 8-OH-Gua on the nontranscribed strand is repaired by the human BER enzyme OGG1 and subsequent BER pathways (*34*). On the other hand, little is known about the participation of BRCA1 in BER pathways (reviewed in ref *7*).

Oxidatively induced DNA lesions such as 8-OH-Gua, 8-hydroxyadenine, and 5-(hydroxymethyl)uracil accumulate in malignant and nonmalignant breast tissues and in the blood of breast cancer patients (*35*−*41*). Furthermore, elevated levels of several oxidatively induced DNA lesions observed in the breast connective tissue stroma of women between 33 and 46 years of age commensurate with a known sharp increase in breast cancer incidence in this age group (*42*−*44*). All of these findings point to DNA-repair deficiency as a risk factor for breast cancer development and support the hypothesis that oxidative DNA damage plays a causative role in breast carcinogenesis.

It is well-established that BRCA1 plays a significant role in the repair of DNA damage by NER and perhaps also by BER. However, little is known about the ability of human cells with *BRCA1* mutations to repair different DNA lesions resulting from oxidative damage. In the present work, we investigated the cellular repair of several oxidatively induced DNA lesions in lymphoblasts of women with *BRCA1* mutations in comparison to those of women with no detectable *BRCA1* defficiency.

MATERIALS AND METHODS

Materials.[2] All lymphoblast cell lines with *BRCA1* mutations and control lymphoblasts were purchased from Coriell Cell Repositories (CCR) (Camden, NJ). The details of each cell line can be found on the website of CCR (http://ccr.coriell.org/nigms/charmut/catbrca1.html) and are given in Table 1. *BRCA1*-deficient lymphoblasts were from women who had germline *BRCA1* mutations and had been diagnosed with breast cancer at a known age (Table 1). Control lymphoblasts were from women with no known *BRCA1* mutations and were specifically recommended by CCR to match *BRCA1*-deficient cells in age, gender, and race. HCC1937 and AG10097 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). HCC1937 is a primary breast carcinoma cell line and is homozygous for the *BRCA1* 5382insC mutation that causes a deletion of the C terminus of BRCA1 (*45*). AG10097 is a control cell line with no known *BRCA1* mutations. The cell culture medium RPMI 1640, L-glutamine, and heat-inactivated fetal bovine serum were purchased from Sigma (St. Louis, MO). Nuclease P1 (from *Penicillium citrinum*) was purchased from United States Biological (Swampscott, MA). Snake venom phosphodiesterase was obtained from Sigma (St. Louis, MO). Alkaline phosphatase was purchased from Roche Applied Science (Indianapolis, IN). Acetonitrile (HPLC grade) was from Burdick and Jackson (Muskegon, MI). Biomax5 ultrafiltration membranes (5 kDa molecular-mass cutoff) from Millipore (Bedford, MA) were used to filter hydrolyzed DNA samples. Water (HPLC grade) for liquid chromatography/mass spectrometry (LC/MS) analyses was from J.T. Baker (Phillipsburg, NJ). Water purified through a Milli-Q system (Millipore, Bedford, MA) was used for all other applications. *N*,*O*-bis(trimethylsilyl)trifluorora-

[1] Abbreviations: ANOVA, analysis of variance; BER, base-excision repair; CCR, Coriell Cell Repositories; *S*-cdA, (5′-*S*)-8,5′-cyclo-2′-deoxyadenosine; *R*-cdG, (5′-*R*)-8,5′-cyclo-2′-deoxyguanosine; *S*-cdG, (5′-*S*)-8,5′-cyclo-2′-deoxyguanosine; GC/MS, gas chromatography/mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; NER, nucleotide-excision repair; 8-OH-Gua, 8-hydroxyguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; SIM, selected ion monitoring; TCR, transcription-coupled repair; TMS, trimethylsilyl.

[2] Certain commercial equipment or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

cetamide containing 1% trimethylchlorosilane was obtained from Pierce Chemicals (Rockford, IL).

Cell Culture. Cells were cultured according to the protocol given by CCR. Six independent batches of each cell line were cultured.

Irradiation of Cells and Isolation of DNA. Three independently cultured batches of each cell line were used for each data point. Tubes containing each batch of cells in phosphate-buffered saline (PBS) buffer were placed in an ice bath at 4 °C and irradiated in a ^{60}Co γ source at a dose of 5 Gy (dose rate of 24 Gy/min). Irradiated cells were centrifuged, collected, then placed in fresh medium, and incubated for 1 h at 37 °C to allow for the cellular repair of DNA damage. Non-irradiated cells were treated in the same manner. The medium was removed from cells by centrifugation, and DNA from non-irradiated and irradiated cells was isolated by a salting out procedure (*46*, *47*). Isolated DNA pellets were washed twice with 70% ethanol and centrifuged. After the removal of ethanol, pellets were dried in a SpeedVac under vacuum and then dissolved in water for 24 h at 4 °C. The UV spectrum of each DNA sample was recorded by absorption spectrophotometry between the wavelengths of 200 and 350 nm to ascertain the quality of DNA and an accurate quantification of the DNA concentration. The absorbance at 260 nm was used to measure the DNA concentration of each sample (absorbance of 1 = 50 μg of DNA/mL). Aliquots (50 μg) of DNA samples were dried in a SpeedVac under vacuum.

Analysis by LC/MS and Gas Chromatography/Mass Spectrometry (GC/MS). LC/MS with isotope dilution was used to identify and quantify 8-hydroxy-2′-deoxyguanosine (8-OH-dG) in DNA under the experimental conditions described elsewhere (*48*). The LC column was a Zorbax SB-Aq analytical column (3.1 × 150 mm, 3.5 μm) from Agilent Technologies (Rockville, MD). A stable isotope-labeled analogue of 8-OH-dG, i.e., 8-OH-dG-$^{15}N_5$, was purchased from Cambridge Isotope Laboratories (Cambridge, MA) and used as an internal standard. Aliquots (50 μg) of DNA samples were supplemented with an aliquot of 8-OH-dG-$^{15}N_5$, hydrolyzed to nucleosides with nuclease P1, snake venom phosphodiesterase, and alkaline phosphatase for 24 h, and then analyzed by LC/MS as described (*49*). Selected-ion monitoring (SIM) was used to monitor the characteristic ions of 8-OH-dG (*m*/*z* 168 and 306) and 8-OH-dG-$^{15}N_5$ (*m*/*z* 173 and 311) at the appropriate retention time periods during LC/MS analyses (*50*). The quantification of 8-OH-dG in DNA was achieved using integrated areas of the signals of these ions.

(5′-*S*)-8,5′-Cyclo-2′-deoxyadenosine (*S*-cdA), (5′-*R*)-8,5′-cyclo-2′-deoxyguanosine (*R*-cdG), and (5′-*S*)-8,5′-cyclo-2′-deoxyguanosine (*S*-cdG) were identified and quantified by GC/MS with isotope dilution under the experimental conditions described elsewhere (*48*). Aliquots (50 μg) of DNA samples were supplemented with stable isotope-labeled analogues of these compounds as internal standards, i.e., *S*-cdA-$^{15}N_5$, *R*-cdG-$^{15}N_5$, and *S*-cdG-$^{15}N_5$, respectively, which had been prepared as described (*48*, *49*). Subsequently, DNA samples were hydrolyzed to nucleosides as described above. Hydrolysates were lyophilized, trimethylsilylated, and analyzed by GC/MS (*48*). SIM was used at the appropriate retention time periods during GC/MS analyses to monitor the characteristic ions of trimethylsilyl (TMS) derivatives

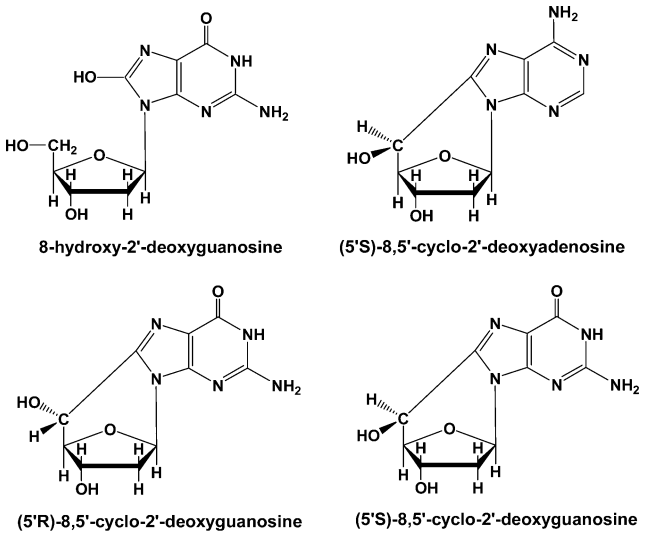

FIGURE 1: Structures of the products identified in this work.

of *S*-cdA (*m*/*z* 309, 450, and 465), *R*-cdG (*m*/*z* 397, 538, and 553), and *S*-cdG (*m*/*z* 397, 538, and 553) and those of their stable-isotope analogues *S*-cdA-$^{15}N_5$ (*m*/*z* 314, 455, and 470), *R*-cdG-$^{15}N_5$ (*m*/*z* 402, 543, and 558), and *S*-cdG-$^{15}N_5$ (*m*/*z* 402, 543, and 558), respectively (*48*, *51*). The quantification of *S*-cdA, *R*-cdG, and *S*-cdG was achieved using integrated areas of the signals of these ions.

Statistical Analyses. Statistical analyses of the data were performed using the software "S-PLUS 7.0 for Windows" through analysis of variance (ANOVA) techniques (*52*).

RESULTS

In the present study, we investigated the formation and repair of some typical oxidatively induced DNA lesions in lymphoblasts of women with mutations in *BRCA1* to test the hypothesis that the repair of oxidative DNA damage may be defective, leading to the accumulation of this type of DNA damage in cells with mutated *BRCA1* when compared to control cells. We assayed 10 lymphoblast cell lines from women diagnosed with breast cancer at some point in their lives and having mutations in *BRCA1* and 5 lymphoblast cell lines from control women who had no detectable mutations in *BRCA1*. Table 1 shows the details of each cell line as given by CCR. Six independent batches of each cell line were cultured. Three batches of the same cell line were exposed to a low dose of γ radiation (5 Gy) to generate oxidative damage and then incubated for 1 h to allow for the cellular repair of this damage. The other three batches were used as non-irradiated controls. DNA was isolated from irradiated and non-irradiated cells, hydrolyzed to nucleosides using three enzymes, and analyzed by LC/MS and GC/MS with isotope-dilution technique to identify and quantify four oxidatively induced lesions, namely, 8-OH-dG, *S*-cdA, *R*-cdG, and *S*-cdG. Figure 1 illustrates the structures of these compounds, which are typical products of reactions of the hydroxyl radical with purine bases in DNA. The mechanistic aspects of their formation can be found elsewhere (reviewed in ref *19*). As an example of measurement, Figure 2 illustrates the ion-current profiles at *m*/*z* 306 of 8-OH-dG and at *m*/*z* 311 of 8-OH-dG-$^{15}N_5$, obtained during LC/MS analysis of the enzyme−hydrolysate of a DNA sample. These ions are the characteristic sodium adduct ions (MNa^+) of both

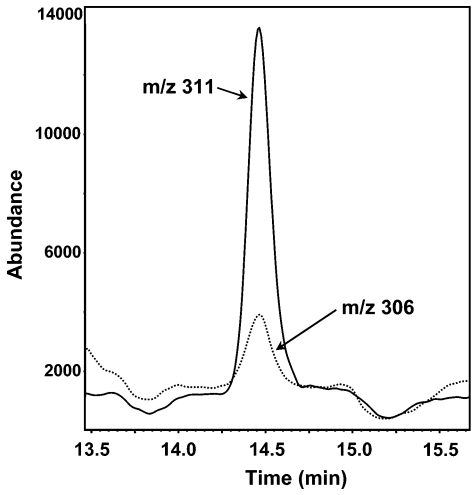

FIGURE 2: Ion-current profiles of the ions at *m*/*z* 306 (MNa^+ ion of 8-OH-dG) and *m*/*z* 311 (MNa^+ ion of 8-OH-dG-$^{15}N_5$) obtained during LC/MS analysis with SIM of the enzymic hydrolysate of a DNA sample.

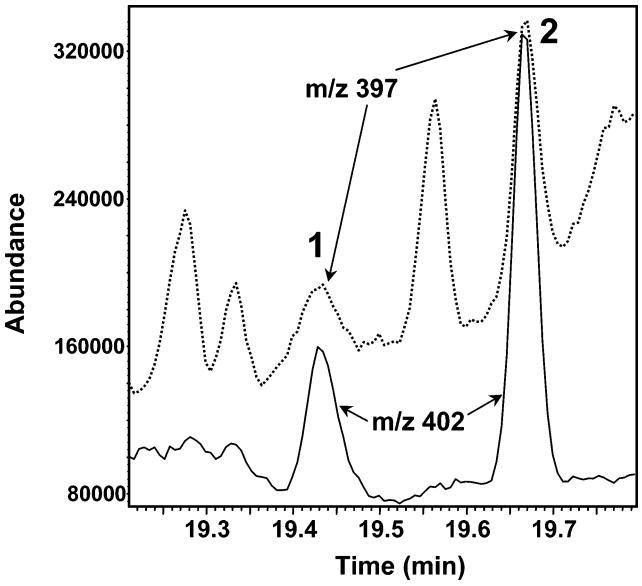

FIGURE 3: Ion-current profiles of the ions at *m*/*z* 397 (a fragment ion of the TMS derivative of *R*-cdG and *S*-cdG) and *m*/*z* 402 (a fragment ion of the TMS derivative of *R*-cdG-$^{15}N_5$ and *S*-cdG-$^{15}N_5$) obtained during GC/MS analysis with SIM of the trimethylsilylated enzymic hydrolysate of a DNA sample. Peak 1, *R*-cdG and *R*-cdG-$^{15}N_5$; peak 2, *S*-cdG and *S*-cdG-$^{15}N_5$.

compounds (*50*). The quantification was achieved by the integration of the signals of monitored ions, such as those in Figure 2, and the calculation of the level of the lesion on the basis of the known amount of the internal standard added to the known amount of DNA samples prior to enzymic hydrolysis. An example of GC/MS measurements of *R*-cdG and *S*-cdG is shown in Figure 3, which illustrates the ion-current profiles of typical fragmentation ions at *m*/*z* 397 of the trimethylsilyl derivatives of *R*-cdG and *S*-cdG and those at *m*/*z* 402 of the trimethylsilyl derivatives of the internal standards *R*-cdG-$^{15}N_5$ and *S*-cdG-$^{15}N_5$ (*48*).

Table 2 shows the mean value (±standard deviation) of the levels of these products in genomic DNA from the measurement of three DNA samples isolated from three independently cultured batches of each cell line. The *p* values from the statistical analyses are also given. In the case of control cells with no *BRCA1* mutations, there were no

Table 2: Levels of the Lesions (Number of Lesions/10^6 DNA Bases) in Genomic DNA from Cell Lines Used in This Work

repository number	compound											
	8-OH-dG			*S*-cdA			*R*-cdG			*S*-cdG		
control	0 Gy	5 Gy	*p* value	0 Gy	5 Gy	*p* value	0 Gy	5 Gy	*p* value	0 Gy	5 Gy	*p* value
GM01078	0.648 ± 0.094	0.597 ± 0.047	0.532	0.564 ± 0.015	0.540 ± 0.016	0.193	0.166 ± 0.004	0.201 ± 0.010	0.008	0.692 ± 0.039	0.657 ± 0.035	0.393
GM02152	0.670 ± 0.026	0.619 ± 0.017	0.094	0.238 ± 0.036	0.196 ± 0.032	0.285	0.133 ± 0.007	0.125 ± 0.015	0.534	0.584 ± 0.030	0.560 ± 0.045	0.564
GM14452	0.541 ± 0.076	0.578 ± 0.046	0.585	0.557 ± 0.038	0.639 ± 0.030	0.076	0.132 ± 0.004	0.136 ± 0.015	0.778	0.613 ± 0.066	0.634 ± 0.055	0.745
GM02148	0.668 ± 0.023	0.698 ± 0.054	0.514				0.115 ± 0.008	0.126 ± 0.009	0.287	0.689 ± 0.022	0.710 ± 0.024	0.411
GM09015	0.687 ± 0.021	0.675 ± 0.062	0.807				0.125 ± 0.007	0.125 ± 0.006	0.940	0.679 ± 0.068	0.653 ± 0.016	0.632
combined value	0.633 ± 0.076	0.625 ± 0.073	0.769	0.475 ± 0.188	0.457 ± 0.190	0.846	0.134 ± 0.018	0.143 ± 0.032	0.407	0.651 ± 0.066	0.643 ± 0.061	0.727
BRCA1 mutated												
GM14093	0.547 ± 0.054	0.718 ± 0.058	0.038	0.371 ± 0.027	0.637 ± 0.114	0.032	0.175 ± 0.008	0.303 ± 0.010	0.0002	0.692 ± 0.054	0.825 ± 0.040	0.049
GM13709	0.507 ± 0.007	0.640 ± 0.061	0.038	0.245 ± 0.063	0.465 ± 0.100	0.058	0.140 ± 0.007	0.158 ± 0.005	0.040	0.541 ± 0.015	0.642 ± 0.036	0.022
GM16105	0.730 ± 0.024	0.911 ± 0.095	0.059	0.568 ± 0.023	0.689 ± 0.048	0.033	0.153 ± 0.011	0.191 ± 0.012	0.028	0.736 ± 0.040	0.862 ± 0.007	0.012
GM14090	0.627 ± 0.008	0.765 ± 0.019	0.001	0.408 ± 0.003	0.497 ± 0.033	0.019	0.108 ± 0.010	0.156 ± 0.012	0.011	0.602 ± 0.016	0.731 ± 0.016	0.001
GM14094	0.855 ± 0.075	1.064 ± 0.042	0.034	0.537 ± 0.026	0.611 ± 0.009	0.019	0.154 ± 0.003	0.183 ± 0.009	0.016	0.642 ± 0.013	0.740 ± 0.019	0.004
GM14637	0.684 ± 0.031	0.833 ± 0.071	0.052	0.364 ± 0.023	0.517 ± 0.034	0.007	0.149 ± 0.008	0.176 ± 0.013	0.064	0.479 ± 0.036	0.568 ± 0.018	0.034
GM13707	0.691 ± 0.026	0.911 ± 0.096	0.035	0.324 ± 0.011	0.375 ± 0.027	0.069	0.128 ± 0.009	0.176 ± 0.007	0.004	0.596 ± 0.018	0.728 ± 0.016	0.002
GM13715	0.444 ± 0.021	0.782 ± 0.087	0.006	0.335 ± 0.025	0.366 ± 0.029	0.314	0.137 ± 0.005	0.200 ± 0.021	0.008	0.639 ± 0.059	0.796 ± 0.054	0.051
GM13708	0.633 ± 0.033	0.759 ± 0.012	0.007	0.354 ± 0.011	0.463 ± 0.004	0.0002	0.147 ± 0.004	0.187 ± 0.006	0.001	0.710 ± 0.018	0.747 ± 0.009	0.059
GM13711	0.669 ± 0.054	0.841 ± 0.067	0.048	0.456 ± 0.028	0.542 ± 0.007	0.014	0.157 ± 0.006	0.189 ± 0.016	0.052	0.747 ± 0.039	0.784 ± 0.158	0.762
combined value	0.639 ± 0.120	0.825 ± 0.128	<0.001	0.396 ± 0.098	0.516 ± 0.115	<0.001	0.145 ± 0.019	0.192 ± 0.041	<0.001	0.638 ± 0.089	0.754 ± 0.116	<0.001

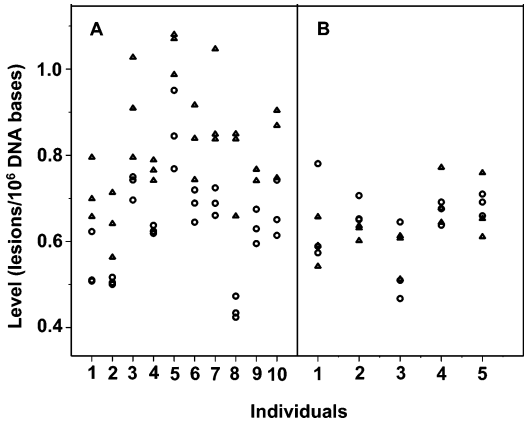

FIGURE 4: Scatter plots of the level of 8-OH-dG in three independently prepared replicates of DNA samples from non-irradiated and irradiated cells. (A) Cells with *BRCA1* mutations. (B) Control cells. (○) Non-irradiated cells and (△) irradiated cells.

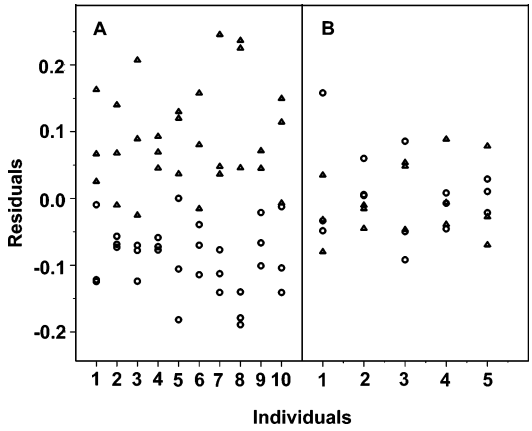

FIGURE 5: Scatter plots of the data of 8-OH-dG after removal of the individual effects using the ANOVA techniques as explained in the text. (A) Cells with *BRCA1* mutations. (B) Control cells. (○) Non-irradiated cells and (△) irradiated cells.

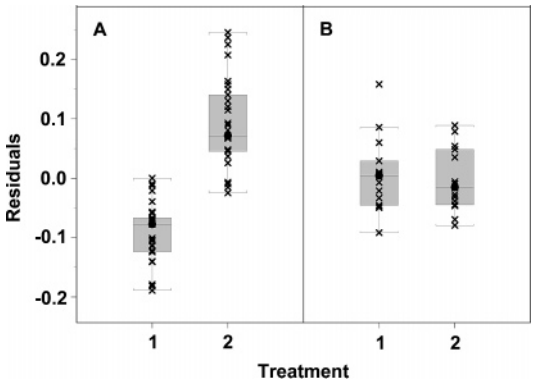

FIGURE 6: Boxplots of the groups A and B in Figure 5 for the 8-OH-dG residual data overlaid on the raw 8-OH-dG residual data points. (A) Cells with *BRCA1* mutations. (B) Control cells. (1) Non-irradiated cells and (2) irradiated cells.

statistically significant differences between the levels of the four lesions before and after irradiation plus incubation time for repair, with one exception in the case of *R*-cdG (30-year-old individual). This indicates that control cell lines were able to repair the DNA lesions measured in this study. In contrast, the majority of the cell lines with *BRCA1* mutations accumulated these lesions in their DNA despite incubation for 1 h following irradiation (Table 2), demonstrating a deficiency in DNA repair. There were some exceptions. In the case of 8-OH-dG in DNA of 35- and 46-year-old individuals and *R*-cdG in DNA of 46- and 75-year-old individuals, no statistically significant difference was observed between the levels before and after irradiation. Similarly, no accumulation of cdA and *S*-cdG was observed in DNA of 32-, 50-, and 56-year-old individuals and in DNA of 56-, 68-, and 75-year-old individuals, respectively. The combined mean values (±standard deviation) of the lesion levels plus *p* values were calculated using all 15 DNA samples from 5 control cell lines and 30 DNA samples from 10 cell lines with *BRCA1* mutations. These values are also given in Table 2. In the case of control cell lines, the measurement of *S*-cdA could be performed with three cell lines only. Control cell lines exhibited no statistically significant differences between the combined levels of the lesions before and after γ irradiation, whereas, in cell lines with *BRCA1* mutations, the combined levels of all four lesions after irradiation plus repair time were statistically greater than those obtained without irradiation.

Figure 4 illustrates the scatter plots of the levels of 8-OH-dG in three independently prepared replicates of DNA samples from non-irradiated and irradiated cells. This plot also displays a statistically significant difference (effect) from individual to individual in the 8-OH-dG measurements in both the *BRCA1*-mutated and control cells. In Figure 5, through ANOVA techniques (*52*), the individual effects were removed from the data for each group shown in Figure 4, leaving the residuals and thus "normalizing" each group. It is important to note that, in the residual data, the absolute differences in the 8-OH-dG data within an individual remain unchanged from the original data, with all individuals now being on the same level within each group of the *BRCA1*-mutated cells and group of the control cells. This approach allows for a more clear analysis because an effect of little interest, the individual effect, has been removed from the data. Figure 6 illustrates the boxplots (*53*) of these groups for the 8-OH-dG residual data overlaid on the raw 8-OH-dG residual data points. The "box" portion of the boxplots displays the interquartile range of the dataset. The median of the dataset is displayed as the horizontal line within the box, with the minimum and maximum data values (excluding outliers) being displayed with the "whiskers" of the plot. Any outliers are shown here or below in other figures simply as a data point beyond the whiskers. The data for each group, the group of the *BRCA1*-mutated cells and the group of the control cells, were divided into two categories, those cells that were exposed to radiation and those that were not (1 and 2 in Figure 3, respectively). Figure 6 clearly displays that a difference does exist in the 8-OH-dG residual measurements and hence the original 8-OH-dG measurements for irradiated versus non-irradiated cells in the group of *BRCA1*-mutated cells. This difference does not exist in the group of control cells. To further support this observation, an ANOVA model was run on both groups to investigate the difference between the control and irradiated cells. It was found that the difference observed between the control and irradiated cells in the group of the *BRCA1*-mutated cells (Figure 6A) is highly statistically significant, with a *p* value of 4.44×10^{-16}, while the difference in the control group (Figure 6B) was not statistically significant, with a *p* value of 0.689. Using the same approach, the boxplots of the data of *S*-cdA, *R*-cdG, and *S*-cdG were also generated and

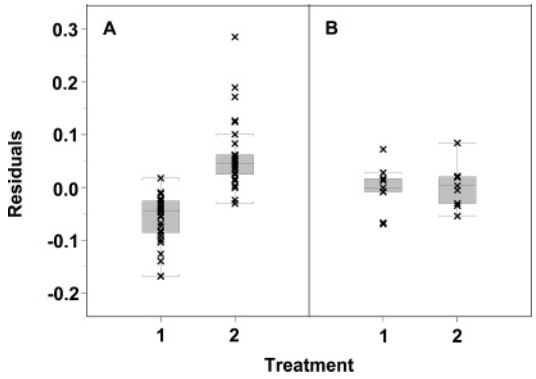

FIGURE 7: Boxplots for the *S*-cdA residual data overlaid on the raw *S*-cdA residual data points. (A) Cells with *BRCA1* mutations. (B) Control cells. (1) Non-irradiated cells and (2) irradiated cells.

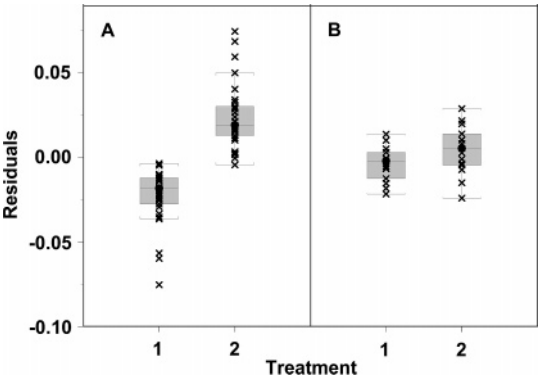

FIGURE 8: Boxplots for the *R*-cdG residual data overlaid on the raw *R*-cdG residual data points. (A) Cells with *BRCA1* mutations. (B) Control cells. (1) Non-irradiated cells and (2) irradiated cells.

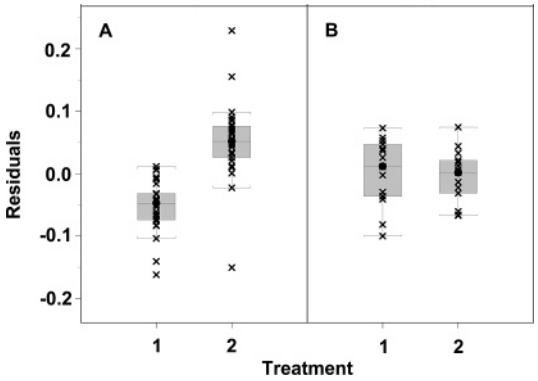

FIGURE 9: Boxplots for the *S*-cdG residual data overlaid on the raw *S*-cdG residual data points. (A) Cells with *BRCA1* mutations. (B) Control cells. (1) Non-irradiated cells and (2) irradiated cells.

displayed in Figures 7−9, respectively. These plots clearly show that a highly statistically significant difference exist in the residual measurements of these three lesions and hence the original measurements for irradiated versus non-irradiated cells in the group of *BRCA1*-mutated cells. The *p* values were 2.85×10^{-11}, 8.45×10^{-7}, and 9.54×10^{-5}, respectively. This difference does not exist in the control groups, with *p* values of 0.799, 0.407, and 0.727, respectively.

Using the same experimental procedures, we also checked the more commonly investigated cell line HCC1937, which has been established from a primary breast carcinoma of a breast cancer patient and is homozygous for the *BRCA1* 5382insC germline mutation (*45*). The AG10097 cell line with no known *BRCA1* mutations was used as a control.

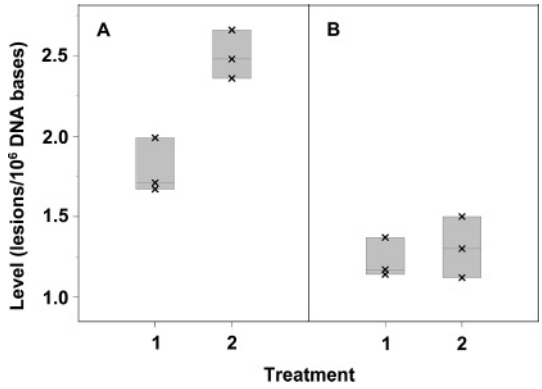

FIGURE 10: Boxplots of the 8-OH-dG levels in DNA from HCC1937 and AG10097 cells. Three independent replicates were used for each data point. (A) HCC1937 cells. (B) AG10097 cells. (1) Non-irradiated cells and (2) irradiated cells.

Figure 10 displays the boxplots of underlying 8-OH-dG measurements of the cells that were exposed to radiation versus those that were not for the HCC1937 (Figure 10A) and AG10097 (Figure 10B) cell lines. These plots clearly show that a difference exists in the 8-OH-dG data for irradiated versus non-irradiated HCC1937 cells, but this is not the case for AG10097 cells. An ANOVA model was run on both cell lines to investigate the difference between the control and irradiated cells. It was found that the difference observed between the control and irradiated cells in the case of HCC1937 cells was statistically significant, with a *p* value of 0.00595, while the difference in the case of AG10097 cells was not statistically significant, with a *p* value of 0.575. Furthermore, the background level of 8-OH-dG in the HCC1937 cell line was significantly greater than that in the control cell line, with a *p* value of 0.0104 (compare the boxplots 1 in parts A and B of Figure 10). Similar results were also observed for *S*-cdA, *R*-cdG, and *S*-cdG (data not shown).

DISCUSSION

The aim of this work was to examine the ability of human cells with *BRCA1* mutations to repair typical products of oxidative damage to DNA after exposure to ionizing radiation and to understand the role of BRCA1 in this process. We used commercially available lymphoblast cell lines with *BRCA1* mutations from female individuals who had previously been diagnosed with breast cancer. Appropriate lymphoblasts with no detectable *BRCA1* mutations served as controls. A low dose of ionizing radiation was used to generate oxidative damage in genomic DNA. The results show that lymphoblasts from female individuals with *BRCA1* mutations are deficient in the cellular repair of four major oxidatively induced DNA lesions following exposure to a low dose of ionizing radiation. There were variations in the levels of the lesions among individuals. These differences might be due to different levels of endogenous oxidative stress and/or repair capacity in different individuals.

We also investigated the cellular repair of the same lesions in genomic DNA of the commonly used HCC1937 primary carcinoma cell line from a breast cancer patient with a homozygous BRCA1 5382insC mutation that causes a deletion of the C terminus of BRCA1. In analogy to lymphoblasts with *BRCA1* mutations, this tumor cell line

exhibited significant accumulation of all four lesions. The lack of repair of 8-OH-dG is in agreement with a previous study that showed a deficiency of the TCR of this lesion in the HCC1937 cell line using assays different from those used in the present work (*25*). Furthermore, elevated background levels of the lesions observed in this tumor cell line compared to the control cell line agree with previous findings that oxidatively induced DNA lesions accumulate in malignant tissues (*35*−*41*, *54*).

The lesions investigated here are the typical hydroxyl-radical-induced products of purine bases in DNA (*19*). 8-OH-dG is formed by the addition of the hydroxyl radical to the C8 position of the purine ring of dG followed by one-electron oxidation of the thus-formed hydroxyl-adduct radical. In contrast, the formation of 8,5′-cyclopurine-2′-deoxynucleosides (*S*-cdA, *R*-cdG, and *S*-cdG) is initiated through H abstraction by the hydroxyl radical from the C5′ position of the sugar moiety. This is followed by intramolecular cyclization and then by oxidation, leading to a covalent bond formation between the C5′ position of the sugar moiety and the C8 position of the base. These compounds are unique in that they represent concomitant damage to both sugar and base moieties of the same nucleoside in DNA. As a consequence, both *R* and *S* diastereomers of cdA and also those of 8,5′-cycloadenosine have been shown to cause an unusual puckering of the sugar moiety and thus significant levels of local distortion in the DNA helix (*55*−*57*). By inference, *R*-cdG and *S*-cdG are also expected to cause considerable distortion of the DNA helix, although no data exist to support this notion.

In human cells, 8-OH-dG is mainly repaired by BER initiated by the removal of 8-OH-Gua by DNA glycosylase OGG1 and subsequent steps (reviewed in refs *21* and *22*). NER and especially TCR may also contribute to the repair of this lesion (*24*, *25*). 8,5′-Cyclopurine-2′-deoxynucleosides cannot be repaired by a DNA glycosylase-initiated BER mechanism because of the presence of the C5′−C8 covalent bond, which would remain intact, even if the glycosidic bond is hydrolyzed. As had been suggested previously (*26*, *27*), recent studies have shown that *R*-cdA and *S*-cdA are indeed repaired by NER and not by either BER or a direct repair mechanism, with the former being repaired more efficiently than the latter (*28*, *29*). Similarly, *R*-cdG and *S*-cdG are also expected to be the substrates of NER rather than BER or any other repair pathway. The role of BRCA1 in NER of oxidative DNA damage is well-established (*9*, *15*, *25*, *30*−*33*). The present study, for the first time, provides the evidence that BRCA1 is involved in the cellular repair of 8,5′-cyclopurine-2′-deoxynucleosides. Because NER is the only repair pathway known for the repair of these lesions, this involvement is highly likely to be via NER, in agreement with the fact that BRCA1 constitutes a major part of the NER pathway (reviewed in ref *23*). This notion is supported by recent evidence that the product of the *XPC* gene, which is a component of NER and is mutated in cancer-prone disease xeroderma pigmentosum (*58*), participates in the repair of 8,5′-cyclopurine-2′-deoxynucleosides (*59*). *BRCA1*-mutant cells of breast cancer patients were also defective in the repair of 8-OH-dG. This agrees with the evidence that BRCA1 contributes to the TCR of 8-OH-dG in human cells (*25*). It is also likely that BRCA1 participates as a cofactor in BER of 8-OH-Gua by stimulating the activity of OGG1. Another component of NER, XPC, has recently been shown to play such a role in human cells (*59*). On the other hand, OGG1 is not required for the TCR of 8-OH-dG (*34*).

Among the lesions measured in this work, 8-OH-dG has long been known to be a highly mutagenic lesion, leading to G → T transversion mutations, and its role in carcinogenesis in general has been discussed extensively for the past 2 decades (reviewed in refs *19* and *60*). Thus, it is likely that this lesion (as well as other lesions) contributes to breast carcinogenesis as well, and evidence supports this notion (*35*−*38*, *41*, *43*). In contrast, little is known about the mutagenic properties of 8,5′-cyclopurine-2′-deoxynucleosides. Recent studies have shown that *S*-cdA is a strong block to transcription and an absolute block to DNA polymerases, including DNA polymerase δ and the bypass polymerase η, and reduces transcription by blocking the transcription-binding factors (*28*, *29*, *61*, *62*). By virtue of being the same class of compounds, *R*-cdG and *S*-cdG are also expected to exhibit similar biological effects. Elevated levels of 8,5′-cyclopurine-2′-deoxynucleosides in genomic DNA *in vivo* regarding cancer incidences in a variety of cases strongly suggest that these lesions may play an important role in carcinogenesis (*42*, *59*, *63*). More specifically, elevated levels of *S*-cdA have been observed in the breast connective tissue stroma of women between 33 and 46 years of age (*42*). This coincides with the known sharp increase in breast cancer incidence in women between 30 and 40 years of age (*44*). *S*-cdA as well as *R*-cdA are well-known to cause significant levels of distortion in the DNA helix because of unusual puckering of the sugar moiety as a consequence of the covalent bond between C5′ and C8 positions (*55*−*57*). Furthermore, *S*-cdA significantly reduces gene expression (*62*). These facts and the slow repairability of *S*-cdA (*28*, *29*) strongly suggest a possible role for this lesion in breast carcinogenesis, perhaps also for *R*-cdG and *S*-cdG because of their similar chemical structures. Furthermore, the accumulation of *S*-cdA in neural cells has been suggested to cause neuronal death in a number of diseases with defective NER (*64*).

In conclusion, we show the accumulation of some major products of oxidative damage to DNA in lymphoblasts from women with *BRCA1* mutations after exposure to oxidative stress generated by ionizing radiation. The data suggest that these cell lines are defective in the repair of oxidatively induced lesions in their genomic DNA and that BRCA1 may play a role in the cellular repair of such lesions. The deficiency in the repair of 8,5′-cyclopurine-2′-deoxynucleosides indicates the involvement of BRCA1 in NER of oxidative DNA damage. The accumulation of mutagenic lesions such as 8-OH-dG may cause predisposed individuals to undergo a high rate of mutations. On the other hand, unrepaired 8,5′-cyclopurine-2′-deoxynucleosides may cause deleterious changes in gene expression by altering transcription. All of these factors may cause an increase in breast cancer risk and contribute to breast carcinogenesis.

ACKNOWLEDGMENT

We are grateful to Dr. Güldal Kirkali of Dokuz Eylul University, Izmir, Turkey, for help with statistical analyses. We thank Althaf Lohani for help in the culture of HCC1937 and AG10097 cell lines.

REFERENCES

1. King, M. C., Marks, J. H., and Mandell, J. B. (2003) Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2, *Science 302*, 643−646.
2. Lakhani, S. R., Jacquemier, J., Sloane, J. P., Gusterson, B. A., Anderson, T. J., van de Vijver, M. J., Farid, L. M., Venter, D., Antoniou, A., Storfer-Isser, A., Smyth, E., Steel, C. M., Haites, N., Scott, R. J., Goldgar, D., Neuhausen, S., Daly, P. A., Ormiston, W., McManus, R., Scherneck, S., Ponder, B. A., Ford, D., Peto, J., Stoppa-Lyonnet, D., Bignon, Y. J., Struewing, J. P., Spurr, N. K., Bishop, D. T., Klijn, J. G., Devilee, P., Cornelisse, C. J., Lasset, C., Lenoir, G., Barkardottir, R. B., Egilsson, V., Hamann, U., Chang-Claude, J., Sobol, H., Weber, B., Stratton, M. R., and Easton, D. F. (1998) Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations, *J. Natl. Cancer Inst. 90*, 1138−1145.
3. Rosen, E. M., Fan, S., Pestell, R. G., and Goldberg, I. D. (2003) BRCA1 gene in breast cancer, *J. Cell. Physiol. 196*, 19−41.
4. Venkitaraman, A. R. (2004) Tracing the network connecting BRCA and Fanconi anaemia proteins, *Nat. Rev. Cancer 4*, 266−276.
5. Ford, D., Easton, D. F., Bishop, D. T., Narod, S. A., and Goldgar, D. E. (1994) Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium, *Lancet 343*, 692−695.
6. Easton, D. F., Ford, D., and Bishop, D. T. (1995) Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium, *Am. J. Hum. Genet. 56*, 265−271.
7. Deng, C. X., and Wang, R. H. (2003) Roles of BRCA1 in DNA damage repair: A link between development and cancer, *Hum. Mol. Genet. 12*, R113−R123.
8. Starita, L. M., and Parvin, J. D. (2003) The multiple nuclear functions of BRCA1: Transcription, ubiquitination and DNA repair, *Curr. Opin. Cell Biol. 15*, 345−350.
9. Somasundaram, K. (2003) Breast cancer gene 1 (BRCA1): Role in cell cycle regulation and DNA repair—Perhaps through transcription, *J. Cell. Biochem. 88*, 1084−1091.
10. Deng, C. X. (2006) BRCA1: Cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution, *Nucleic Acids Res. 34*, 1416−1426.
11. Scully, R., Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T., and Livingston, D. M. (1997) Association of BRCA1 with Rad51 in mitotic and meiotic cells, *Cell 88*, 265−275.
12. Moynahan, M. E., Chiu, J. W., Koller, B. H., and Jasin, M. (1999) BRCA1 controls homology-directed DNA repair, *Mol. Cell 4*, 511−518.
13. Venkitaraman, A. R. (2001) Functions of BRCA1 and BRCA2 in the biological response to DNA damage, *J. Cell Sci. 114*, 3591−3598.
14. Shen, S. X., Weaver, Z., Xu, X., Li, C., Weinstein, M., Chen, L., Guan, X. Y., Ried, T., and Deng, C. X. (1998) A targeted disruption of the murine BRCA1 gene causes γ-irradiation hypersensitivity and genetic instability, *Oncogene 17*, 3115−3124.
15. Abbott, D. W., Thompson, M. E., Robinson-Benion, C., Tomlinson, G., Jensen, R. A., and Holt, J. T. (1999) BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair, *J. Biol. Chem. 274*, 18808−18812.
16. Carroll, K. K. (1992) Dietary fat and breast cancer, *Lipids 27*, 793−797.
17. Welsch, C. W. (1995) Review of the effects of dietary fat on experimental mammary gland tumorigenesis: Role of lipid peroxidation, *Free Radical Biol. Med. 18*, 757−773.
18. Malins, D. C. (1996) Free radicals and breast cancer, *Environ. Health Perspect. 104*, 1140.
19. Evans, M. D., Dizdaroglu, M., and Cooke, M. S. (2004) Oxidative DNA damage and disease: Induction, repair and significance, *Mutat. Res. 567*, 1−61.
20. Wallace, S. S. (2002) Biological consequences of free radical-damaged DNA bases, *Free Radical Biol. Med. 33*, 1−14.
21. Gros, L., Saparbaev, M. K., and Laval, J. (2002) Enzymology of the repair of free radicals-induced DNA damage, *Oncogene 21*, 8905−8925.
22. Dizdaroglu, M. (2003) Substrate specificities and excision kinetics of DNA glycosylases involved in base-excision repair of oxidative DNA damage, *Mutat. Res. 531*, 109−126.
23. Izumi, T., Wiederhold, L. R., Roy, G., Roy, R., Jaiswal, A., Bhakat, K. K., Mitra, S., and Hazra, T. K. (2003) Mammalian DNA base excision repair proteins: Their interactions and role in repair of oxidative DNA damage, *Toxicology 193*, 43−65.
24. Reardon, J. T., Bessho, T., Kung, H. C., Bolton, P. H., and Sancar, A. (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: Possible explanation for neurodegeneration in xeroderma pigmentosum patients, *Proc. Natl. Acad. Sci. U.S.A. 94*, 9463−9468.
25. Le Page, F., Randrianarison, V., Marot, D., Cabannes, J., Perricaudet, M., Feunteun, J., and Sarasin, A. (2000) BRCA1 and BRCA2 are necessary for the transcription-coupled repair of the oxidative 8-oxoguanine lesion in human cells, *Cancer Res. 60*, 5548−5552.
26. Dizdaroglu, M. (1986) Free-radical-induced formation of an 8,5′-cyclo-2′-deoxyguanosine moiety in deoxyribonucleic acid, *Biochem. J. 238*, 247−254.
27. Dizdaroglu, M., Dirksen, M. L., Jiang, H. X., and Robbins, J. H. (1987) Ionizing-radiation-induced damage in the DNA of cultured human cells. Identification of 8,5′-cyclo-2′-deoxyguanosine, *Biochem. J. 241*, 929−932.
28. Brooks, P. J., Wise, D. S., Berry, D. A., Kosmoski, J. V., Smerdon, M. J., Somers, R. L., Mackie, H., Spoonde, A. Y., Ackerman, E. J., Coleman, K., Tarone, R. E., and Robbins, J. H. (2000) The oxidative DNA lesion 8,5′-(*S*)-cyclo-2′-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells, *J. Biol. Chem. 275*, 22355−22362.
29. Kuraoka, I., Bender, C., Romieu, A., Cadet, J., Wood, R. D., and Lindahl, T. (2000) Removal of oxygen free-radical-induced 5′,8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells, *Proc. Natl. Acad. Sci. U.S.A. 97*, 3832−3837.
30. Cressman, V. L., Backlund, D. C., Avrutskaya, A. V., Leadon, S. A., Godfrey, V., and Koller, B. H. (1999) Growth retardation, DNA repair defects, and lack of spermatogenesis in BRCA1-deficient mice, *Mol. Cell. Biol. 19*, 7061−7075.
31. Hartman, A. R., and Ford, J. M. (2002) BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair, *Nat. Genet. 32*, 180−184.
32. Takimoto, R., MacLachlan, T. K., Dicker, D. T., Niitsu, Y., Mori, T., and El-Deiry, W. S. (2002) BRCA1 transcriptionally regulates damaged DNA binding protein (DDB2) in the DNA repair response following UV-irradiation, *Cancer Biol. Ther. 1*, 177−186.
33. Paull, T. T., Cortez, D., Bowers, B., Elledge, S. J., and Gellert, M. (2001) Direct DNA binding by BRCA1, *Proc. Natl. Acad. Sci. U.S.A. 98*, 6086−6091.
34. Le Page, F., Klungland, A., Barnes, D. E., Sarasin, A., and Boiteux, S. (2000) Transcription coupled repair of 8-oxoguanine in murine cells: The ogg1 protein is required for repair in nontranscribed sequences but not in transcribed sequences, *Proc. Natl. Acad. Sci. U.S.A. 97*, 8397−8402.
35. Malins, D. C., and Haimanot, R. (1991) Major alterations in the nucleotide structure of DNA in cancer of the female breast, *Cancer Res. 51*, 5430−5432.
36. Malins, D. C., Holmes, E. H., Polissar, N. L., and Gunselman, S. J. (1993) The etiology of breast cancer: Characteristic alterations in hydroxyl radical-induced DNA base lesions during oncogenesis with potential for evaluating incidence risk, *Cancer 71*, 3036−3043.
37. Malins, D. C., Polissar, N. L., and Gunselman, S. J. (1996) Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage, *Proc. Natl. Acad. Sci. U.S.A. 93*, 2557−2563.
38. Musarrat, J., Arezina-Wilson, J., and Wani, A. A. (1996) Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis, *Eur. J. Cancer 32*, 1209−1214.
39. Djuric, Z., Heilbrun, L. K., Simon, M. S., Smith, D., Luongo, D. A., LoRusso, P. M., and Martino, S. (1996) Levels of 5-hydroxymethyl-2′-deoxyuridine in DNA from blood as a marker of breast cancer, *Cancer 77*, 691−696.
40. Djuric, Z., Heilbrun, L. K., Lababidi, S., Berzinkas, E., Simon, M. S., and Kosir, M. A. (2001) Levels of 5-hydroxymethyl-2′-deoxyuridine in DNA from blood of women scheduled for breast biopsy, *Cancer Epidemiol., Biomarkers Prev. 10*, 147−149.
41. Li, D., Zhang, W., Zhu, J., Chang, P., Sahin, A., Singletary, E., Bondy, M., Hazra, T., Mitra, S., Lau, S. S., Shen, J., and DiGiovanni, J. (2001) Oxidative DNA damage and 8-hydroxy-2-deoxyguanosine DNA glycosylase/apurinic lyase in human breast cancer, *Mol. Carcinog. 31*, 214−223.

42. Anderson, K. M., Jaruga, P., Ramsey, C. R., Gilman, N. K., Green, V. M., Rostad, S. W., Emerman, J. T., Dizdaroglu, M., and Malins, D. C. (2006) Structural alterations in breast stromal and epithelial DNA: The influence of 8,5′-cyclo-2′-deoxyadenosine, *Cell Cycle 5*, 1240−1244.
43. Malins, D. C., Anderson, K. M., Jaruga, P., Ramsey, C. R., Gilman, N. K., Green, V. M., Rostad, S. W., Emerman, J. T., and Dizdaroglu, M. (2006) Oxidative changes in the DNA of stroma and epithelium from the female breast: Potential implications for breast cancer, *Cell Cycle 5*, 1629−1632.
44. American Cancer Society (2003) *Breast Cancer: Facts and Figures 2003−2004*, American Cancer Society, Atlanta, GA.
45. Tomlinson, G. E., Chen, T. T., Stastny, V. A., Virmani, A. K., Spillman, M. A., Tonk, V., Blum, J. L., Schneider, N. R., Wistuba, I. I., Shay, J. W., Minna, J. D., and Gazdar, A. F. (1998) Characterization of a breast cancer cell line derived from a germline BRCA1 mutation carrier, *Cancer Res. 58*, 3237−3242.
46. Miller, S. A., Dykes, D. D., and Polesky, H. F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells, *Nucleic Acids Res. 16*, 1215.
47. Balajee, A. S., Proietti, D. S., Brosh, R. M., Jr., Selzer, R., and Bohr, V. A. (2000) Role of the ATPase domain of the Cockayne syndrome group B protein in UV induced apoptosis, *Oncogene 19*, 477−489.
48. Jaruga, P., Birincioglu, M., Rodriguez, H., and Dizdaroglu, M. (2002) Mass spectrometric assays for the tandem lesion 8,5′-cyclo-2′-deoxyguanosine in mammalian DNA, *Biochemistry 41*, 3703−3711.
49. Jaruga, P., Theruvathu, J., Dizdaroglu, M., and Brooks, P. J. (2004) Complete release of (5′-*S*)-8,5′-cyclo-2′-deoxyadenosine from dinucleotides, oligodeoxynucleotides and DNA, and direct comparison of its levels in cellular DNA with other oxidatively induced DNA lesions, *Nucleic Acids Res. 32*, e87.
50. Dizdaroglu, M., Jaruga, P., and Rodriguez, H. (2001) Measurement of 8-hydroxy-2′-deoxyguanosine in DNA by high-performance liquid chromatography−mass spectrometry: Comparison with measurement by gas chromatography−mass spectrometry, *Nucleic Acids Res. 29*, e12.
51. Dizdaroglu, M., Jaruga, P., and Rodriguez, H. (2001) Identification and quantification of 8,5′-cyclo-2′-deoxyadenosine in DNA by liquid chromatography/mass spectrometry, *Free Radical Biol. Med. 30*, 774−784.
52. Neter, J., Kutner, M. H., Nachtsheim, C. J., and Wasserman, W. (1996) *Applied Linear Statistical Models*, McGraw-Hill, New York.
53. Hoaglin, D. C., Mosteller, F., and Tukey, J. W. (1983) *Understanding Robust and Exploratory Analysis*, John Wiley and Sons, Inc., New York.
54. Olinski, R., Zastawny, T., Budzbon, J., Skokowski, J., Zegarski, W., and Dizdaroglu, M. (1992) DNA base modifications in chromatin of human cancerous tissues, *FEBS Lett. 193*, 198.
55. Haromy, T. P., Raleigh, J., and Sundaralingam, M. (1980) Enzyme-bound conformations of nucleotide substrates. X-ray structure and absolute configuration of 8,5′-cycloadenosine monohydrate, *Biochemistry 19*, 1718−1722.
56. Birnbaum, G. I., Cygler, M., Dudycz, L., Stolarski, R., and Shugar, D. (1981) Comparison of solid state and solution conformations of *R* and *S* epimers of 8,5′-cycloadenosine and their relevance to some enzymatic reactions, *Biochemistry 20*, 3294−3301.
57. Miaskiewicz, K., Miller, J. H., and Fuciarelli, A. F. (1995) Theoretical analysis of DNA intrastrand cross linking by formation of 8,5′-cyclodeoxyadenosine, *Nucleic Acids Res. 23*, 515−521.
58. Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A., and Ellenberger, T. (2005) *DNA Repair and Mutagenesis*, ASM Press, Washington, D.C.
59. D'Errico, M., Parlanti, E., Teson, M., Calcagnile, A., Jaruga, P., Zambruno, G., Stefanini, M., Dizdaroglu, M., and Dogliotti, E. (2006) New functions of XPC in the protection of human skin cells from oxidative damage, *Oncogene 25*, 4305−4315.
60. Grollman, A. P., and Moriya, M. (1993) Mutagenesis by 8-oxoguanine: An enemy within, *Trends Genet. 9*, 246−249.
61. Kuraoka, I., Robins, P., Masutani, C., Hanaoka, F., Gasparutto, D., Cadet, J., Wood, R. D., and Lindahl, T. (2001) Oxygen free radical damage to DNA. Translesion synthesis by human DNA polymerase η and resistance to exonuclease action at cyclopurine deoxynucleoside residues, *J. Biol. Chem. 276*, 49283−49288.
62. Marietta, C., Gulam, H., and Brooks, P. J. (2002) A single 8,5′-cyclo-2′-deoxyadenosine lesion in a TATA box prevents binding of the TATA binding protein and strongly reduces transcription *in vivo*, *DNA Repair 1*, 967−975.
63. Malins, D. C., Anderson, K. M., Stegeman, J. J., Jaruga, P., Green, V. M., Gilman, N. K., and Dizdaroglu, M. (2006) Biomarkers signal contaminant effects on the organs of English sole (*Parophrys vetulus*) from Puget Sound, *Environ. Health Perspect. 114*, 823−829.
64. Brooks, P. J. (2002) DNA repair in neural cells: Basic science and clinical implications, *Mutat. Res. 509*, 93−108.

BI062022P