BRCA1 and BRCA2 Are Necessary for the Transcription-Coupled Repair of the Oxidative 8-Oxoguanine Lesion in Human Cells

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ABSTRACT

The breast and ovarian cancer susceptibility genes, BRCA1 and BRCA2, are likely to participate in DNA lesion processing. Oxidative lesions, such as 8-oxoguanine, occur in DNA after endogenous or exogenous oxidative stress. We show that deficiency for either BRCA1 or BRCA2 in human cancer cells leads to a block of the RNA polymerase II transcription machinery at the 8-oxoguanine site and impairs the transcription-coupled repair of the lesion, leading to a high mutation rate. Expression of wild-type BRCA1 from a recombinant adenovirus fully complements the repair defect in BRCA1-deficient cells. These results represent the first demonstration of the essential contribution of BRCA1 and BRCA2 gene products in the repair of the 8-oxoguanine oxidative damage specifically located on the transcribed strand in human cells. This suggests that cells from individuals predisposed to breast and/or ovarian cancer may undergo a high rate of mutations because of the deficiency of this damage repair pathway after oxidative stress.

INTRODUCTION

Germ-line mutations in either the BRCA1 or the BRCA2 gene are responsible for the majority of hereditary breast cancers (1). Although the precise biological activities of these two genes are still unknown, it is now widely accepted that they belong to a group of molecules that participate in the monitoring and/or the repair of DNA lesions (2, 3). The relaxation of this monitoring caused by mutations of either of these two genes leaves unrepaired events that lead to the accumulation of mutations and ultimately to cancer. BRCA1 appears to play a role in two unrelated repair processes. On the one hand, the capacity of the BRCA1 protein to interact with hRAD51 and to promote homologous recombination are strong indications for a role in recombination repair (4, 5). On the other hand, human BRCA1 and murine brca1 may participate in transcription-coupled DNA repair of some ionizing radiation-induced DNA lesions (6, 7). The role of murine brca2 in DNA repair is also well documented (8).

Basic metabolic pathways produce reactive oxygen species that are potentially mutagenic, and the mammary tissue is particularly exposed to such oxidative stress because of specific hormone metabolism (9, 10). It is likely that the lack of an efficient oxidative lesion repair represents a significant contribution to breast cancer. 8-oxoG, one of the most damaging lesion among the numerous oxidized bases (11), is produced in relatively high quantities and carries a high mutagenic potency because of the formation of a stable bp with adenine (12, 13). We have observed recently that 8-oxoG lesions located on a transcribed strand is repaired in a transcription-coupled fashion requiring the presence of CSB, XPG and TFIIH factors, whereas on the nontranscribed strand, it is repaired by the OGG1 glycosylase (14, 15). Therefore, base lesions induced by ionizing radiation (such as 8-oxoG or thymine glycols) on the transcribed strand, if not repaired, will lead to either transcription blockage and/or mutagenic bypass.

All together these observations have led us to postulate that BRCA1 or BRCA2 may participate in repair of the highly prevalent 8-oxoG lesion and that breast cancer cells in which either of the two genes is inactivated may be defective in this pathway. We have explored this possibility with a shuttle vector assay specifically designed to measure the level of repair of 8-oxoG lesions present on either a transcribed or a nontranscribed strand of the shuttle vector in human cells.

We show that human cancer cells deficient for BRCA1 or BRCA2 are impaired in the TCR of 8-oxoG. Expression of wild-type BRCA1 from a recombinant adenovirus fully complements the repair defect in BRCA1-deficient cells.

MATERIALS AND METHODS

Cells. MRC5SV1 is a normal human fibroblast cell line transformed by SV40. MCF7 is a breast tumor-derived cell line (BRCA1+/+, BRCA2/+, and p53+/+). HCC 1937 is a cell line derived from a breast tumor in a woman carrying a 5382 insC germ-line mutation of BRCA1. The tumor cells have retained the mutant allele and lost the wild-type allele. It also carries a unizygous mutation of p53 (R306Stop) and a deletion of the PTEN gene (16). CAPAN-1 cells are derived from a pancreatic carcinoma from an individual presumably carrying a germ-line mutation in BRCA2 (6174delI). The cell line is unizygous for this mutation (17).

Plasmids. PS189-ΔSVori has been described previously (18). The vector used in the SV40 promoter region, pSI89-ΔSVS, was obtained by deletion of the HpaIII-BamHI fragment. Monomodified plasmids based on pSI89-ΔSVori or pSI89-ΔP34 were constructed using a [γ-32P]ATP-modified, 19-mer oligonucleotide carrying a unique 8-oxoG in the sequence GATGCGGGCGGCGGTGTTG (corresponding to codons 10–14 of the human Hras gene), where the restriction site NcoI is underlined, as already published (14, 18). This sequence, located 3′ of the SV40 Tag gene and upstream of a polyadenylation signal, can be transcribed or not, depending on the vector used (Fig. 1A).

Repair Assay. Repair analysis was performed following a similar experimental protocol as described previously (14). Transfections of nonreplating monomodified plasmids into the different cell lines were mediated using the FuGene procedure (Roche, Meylan, France). Cells were incubated for the indicated times and collected. Efficient elimination of any contaminating DNA input before extracting plasmid DNA from cells was performed by treatment of cell cultures with Dnase I. Extrachromosomal plasmid DNA was recovered by a small-scale alkaline lysis method (19) and used to transform by electro-2

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4 The abbreviations used are: 8-oxoG or GO, 8-oxoguanine; RT-PCR, reverse transcription-PCR; RSV, Rous sarcoma virus; TS, transcribed sequence; NTS, nontranscribed sequence; TCR, transcription-coupled repair; NER, nucleotide excision repair; TAg, T antigen; CS, Cockayne’s syndrome; XP, xeroderma pigmentosum; βgal, β-galactosidase.
The ratio between NgomV-sensitive plasmids to total progeny plasmids normalized to the frequency of vectors sensitive to NgomV digestion after direct transfection in bacteria (without passage through human cells) of the pS189-ΔSVori/GO:C.

Transcription Analysis of the 8-oxoG-carrying Template. Twelve h after transfection with the pS189-ΔSVori/GO:C, transcripts spanning either the coding sequence of the SV40 TAg (300 bp) or the sequence around the 8-oxoG (270 bp), were amplified by RT-PCR analysis performed on total RNA. The primers used for multiplex PCR analysis permit the detection of transcripts from a 300-bp fragment hybridizing inside the SV40 TAg gene and from a 270-bp fragment spanning the 8-oxoG lesion (Fig. 1A). The primers used for the two amplifications are, respectively: 5'−GACCTTTAAATCCTG-TAGG−3' and 5'−TTTATACGAGTAGTTGGACTG−3' for the 300 bp; and 5'−CTTGAGGCTGATTGTTTGCG-3' and 5'−GAACGAAATTTTTTGGAG-3' for the 270-bp fragment.

Expression of Wild-Type BRCA1 in Recombinant AdRSVBRCA1-infected Cells. AdRSVBRCA1 is an E1/E3-defective and nonreplicative recombinant adenovirus of the Ads serotype. A full-length cDNA fragment encoding the 1863 amino acids of human wild-type BRCA1 was subcloned between the Sall and EcoRV sites of pAdRSVβgal plasmid behind the long terminal repeat of the RSV to generate the pAdRSVBRCA1 adenoviral shuttle plasmid. AdRSVBRCA1 was constructed in 293 cells by homologous recombination between pAdRSVBRCA1 recombinant shuttle vector and Clul-restricted AdRSVβgal viral DNA, amplified, and purified as described previously (20). Viral infections of HCC 1937 cells were performed with AdRSVβgal (control) or AdRSVBRCA1 for 4 h at a multiplicity of infection of 200 (determined as the best compromise between efficiency of infection and cytopathic effect). Whole-cell lysates were prepared at day 1 (D1) and day 2 (D2) after infection. Fifty μg of total protein extracts were separated on 6% SDS-PAGE, transferred on nitrocellulose filter, and immunoblotted with an anti-BRCA1 antibody (OP 92; Oncogene Research Products, Cambridge MA) as described (21). The zero time for the kinetics of 8-oxoG repair corresponds to the 24-h time after infection by the recombinant adenovirus.

RESULTS

The Lack of Functional BRCA1 or BRCA2 Impairs the Capacity to Remove 8-oxoG. To explore the role of the BRCA1 gene in the removal of 8-oxoG in human cells, we choose a shuttle vector assay described previously (14, 18). Results obtained with shuttle plasmids in mutagenesis and repair analysis are highly reproducible and were always validated by genomic DNA data when available (22). Briefly, the vector is a double-stranded DNA plasmid carrying a unique 8-oxoG residue within a specific sequence (the human Ha-ras codons 10−14 region containing a unique NgomV restriction enzyme site) that has been inserted 3' of the SV40 TAg gene transcription unit. The 8-oxoG-containing sequence is located either on a TS driven by the SV40 TAg promoter or on a NTS in a derivative plasmid in which transcription does not occur because the SV40 early promoter has been deleted. The presence of a 8-oxoG within the recognition sequence activity prevents NgomV restriction enzyme activity. The efficiency of 8-oxoG removal was quantified by measuring the yield of plasmid DNA recovered from transfected human cells, amplified in 8-oxoG repair-deficient bacteria, that has regained the sensitivity to NgomV restriction enzyme. The DNA fraction that remains resistant to the enzyme represents plasmids carrying mutations at the site of the original 8-oxoG. Direct DNA sequencing has shown that these mutations are predominantly G to T transversions (13). The demonstration of the preferential repair of the 8-oxoG in human cells is presented in Fig. 2A, which shows that during the first 4 h, the kinetics of 8-oxoG removal is about twice faster when it is located on a TS versus a NTS sequence in both normal human fibroblasts and in MCF-7 breast cancer cells. Within 12 h, the removal of the 8-oxoG located on the NTS is complete in all cell lines tested (Fig. 2B). When located on the TS, the 8-oxoG is completely removed within 8 h in cell lines carrying wild-type BRCA1 or BRCA2 alleles (SV40-trans-
formed normal fibroblast cell line MRC5SV1 and tumor breast cell line MCF7 (Fig. 2C). By contrast, the removal of the 8-oxoG is totally impaired in BRCA1-mutated HCC 1937 cells and ~90% deficient in BRCA2-mutated CAPAN-1 cells (Fig. 2C). It is tempting to propose that this very clear repair deficiency is attributable to the lack of BRCA1 or BRCA2 function.

Blockage of RNA Transcription by 8-oxoG in BRCA1 and BRCA2 Mutated Cells. It is commonly accepted that the deficient TCR of UV-induced lesions in some DNA-repair deficient cells is attributable to sterical prevention of the NER enzymes to have access to the lesion by stalled RNA polymerase II (23). To investigate the possibility that the deficiency in repair of 8-oxoG lesion observed in BRCA1- and BRCA2-defective cells may reflect an extended block of transcription because of stalled RNA polymerases, transcripts of two regions of the plasmid template were studied by RT-PCR. Twelve h after transfection of the 8-oxoG-containing plasmid, transcripts of the SV40 TAg gene occurred (Fig. 1B). This observation implies that the transcription machinery is blocked by the 8-oxoG itself or by protein complexes recognizing the lesion, and that the RNA polymerase II complex cannot proceed through the lesion in the absence of repair. For the BRCA2-mutated cells, the 270-bp band was detected but at a level corresponding to <20% of control cells (Fig. 1B). This result agrees with the low level of 8-oxoG repair in this cell line (Fig. 2C).

Wild-Type BRCA1 Gene Product Rescues the Deficiency in Repair of 8-oxoG Lesion in HCC 1937 Cells. A biologically active wild-type BRCA1 protein was restored in HCC 1937 cells by infection with a recombinant adenovirus. High-titer adenovirus stocks give high yields of infection, allowing the study of a large cell population expressing BRCA1. Infection of HCC 1937 cells by the adenovirus expressing the β-galactosidase gene leads to gene transfer and expression in 80% of the infected cells (data not shown). Cells infected by the recombinant adenovirus (AdRSVBRCA1) express stable wild-type BRCA1 protein for at least 48 h (Fig. 3). Twenty-four h after infection of HCC 1937 cells by the AdRSVBRCA1 virus, the shuttle vector containing the 8-oxoG lesion was transfected. The kinetics of repair of the lesion over the subsequent 12 h shows a complete recovery of the 8-oxoG repair (Fig. 2D), suggesting that in these cells, the repair defect can be fully complemented by the restoration of BRCA1-associated functions. The infection by an adenovirus vector, which expresses the bacterial β-galactosidase gene (AdRSVβGal), does not complement the repair deficiency (Fig. 2D).

These results clearly link the deficiency in repair of 8-oxoG observed in HCC 1937 cells to the absence of active BRCA1. Furthermore, it shows that BRCA1 is necessary only for the pathway, which is fully linked to the transcription machinery, whereas the oxidative lesion present on the NTS is repaired by a BRCA1-independent pathway because of the hOGG1 glycosylase, as recently shown using ogg1−/− mouse embryo fibroblasts (15). A recombinant adenovirus expressing full-length BRCA2 is not available to carry complementation.
tation experiments; because of its large size, the BRCA2 cDNA cannot be stably inserted in adenovirus vectors.

DISCUSSION

By our present study, we have shown that BRCA1- or BRCA2-deficient cells are unable to repair oxidative 8-oxoG lesions specifically located on the TS. This is the first demonstration of the lack of TCR of such a lesion in human tumor cell lines. The complete recovery of repair activity upon expression of the wild-type protein indicates that the defect is specifically linked to the absence of a functional BRCA1 protein. This result agrees with the lack of TCR of ionizing radiation-induced lesions (7), including thymine glycol (6), observed in HCC 1937 cells or in cells derived from brca1 knock-out mice. Moreover, the BRCA2 gene had never been shown to be involved in any TCR pathway in human cells yet.

HCC 1937 cells are unisegous for a mutant allele of the p53 gene (16). The lack of 8-oxoG repair in these cells is unlikely to be linked to the absence of a functional p53 protein because expression of BRCA1 is sufficient to fully restore the repair to the wild-type level. Furthermore, SV40-transformed MRC5SV1 cells, in which p53 is sequenstered by the SV40 Tag, displays a normal repair pattern (Fig. 2C).

TCR has been described essentially for UV-induced DNA lesions (23, 24) because of the blockage of RNA polymerase II transcription. HCC 1937 cells exhibit normal survival after UVC (from 0 to 20 J/m2) and UVB (from 0 a to 500 J/m2) irradiation as well as normal unscheduled DNA synthesis.5 This result indicates that the BRCA1 protein is not directly involved in the TCR pathway specific for UV-induced DNA lesions and that the TCR pathway specific for 8-oxoG involves different components.

We already have demonstrated (14) that the removal of 8-oxoG on TS is fully dependent on an active TCR process. This specific removal is fully inhibited in CS cells as well as in cells from patients exhibiting both CS and XP diseases (13). These latter patients belong to XP group B, D, or G. This result indicates that CSB, XPG, XBP, and XPD proteins are essential in the process of 8-oxoG TCR to prevent the blockage of RNA polymerase II by the 8-oxoG or its removal from the template. As shown in Fig. 1B, the transcription machinery of BRCA1 and BRCA2 mutant cells is also blocked by the 8-oxoG lesion. The effect of oxidative damage on transcription has received little attention, particularly in mammalian cells. In prokaryotes, in vitro studies led to conflicting conclusions, because 8-oxoG lesions cause the termination transcription by T7 polymerase (25) but not by E. coli polymerase (26). Nonetheless, the data presented here demonstrate that in cells unable to repair 8-oxoG, RNA polymerase II is unable to bypass the lesion, whereas it is able to do so in cells fully proficient for 8-oxoG repair. The presence of stalled RNA polymerase molecule at the lesion site in these defective cells provides a likely explanation for the inability to remove it by the repair enzymes. It is possible that binding of mismatch recognition proteins to 8-oxoG blocks the progress of RNA polymerase and thus provides a signal for preferential repair. Consistent with this scenario is the known requirement for MSH2 in TCR of oxidative damage in both human cells and yeast (27, 28) and the recognition of 8-oxoG:C and 8-oxoG:A mispairs by MSH2-MSH6 in Saccharomyces cerevisiae (29). Using a two-hybrid assay, Bertrand et al. (30) reported that MSH2 exists in a complex with NER proteins in S. cerevisiae. Recent data from our laboratory showed slower removal of 8-oxoG in the transcribed strand of the shuttle vector in human tumor cells lacking hMSH2 than in normal cells but no difference in rate of removal between the two cell lines when the 8-oxoG is in a NTS.6 The existence of an 8-oxoG-specific DNA binding protein in human cells was inferred from the inhibition of activity of purified hOGG1 on an 8-oxoG-containing oligonucleotide in vitro by addition of whole-cell extract (31). Altogether these data provide a rationale for a complex between mismatch repair proteins involved in the blockage of RNA polymerase II. Recent data (32) demonstrating interaction between BRCA1 and several DNA repair proteins including mismatch repair partners support this hypothesis.

In this context, BRCA1 and BRCA2 proteins should intervene in synergy with the five repair proteins at steps yet unknown. The reported interactions between BRCA1 protein and RNA polymerase II, and the basal transcription factors TFIIF, TFIIE, and TFIH (33, 34) may imply protein-protein interactions at the site of RNA polymerase blockage. It is worthwhile recalling that the XPD and XPB helicases are present in the TFIH complex (35), leading to the hypothesis of a physical interaction between BRCA1, BRCA2, XPD, and XPB as part of the regulation of the TCR process specific for oxidative lesions. The putative interactions between CSB, CSA, and XPG proteins have not been established yet.

The understanding of breast oncogenesis in the context of BRCA1 or BRCA2 deficiency should now incorporate the observation that these proteins play a role in the repair of 8-oxoG and probably other oxidative lesions but not in classical NER such as UV-induced DNA lesion repair. Radical oxygen species, and particularly the hydroxyl radical OH·, or ionizing radiation can indeed oxidize DNA bases, giving rise to 8-oxoG, 8-oxo-adenine, Fapy derivatives, and thymine glycols (11). Among these lesions, the 8-oxoG is probably the most abundant and mutagenic damage. A significant high level of these oxidized bases has been reported in several cancer tissues and particularly in breast carcinoma (9, 36). The origin of this high oxidation status of cancer cells is still not known but may be linked to the normal metabolism of estrogens. For example, a future metabolism of 17β-estradiol has been shown to produce hydrogen peroxide and probably singlet oxygen (10). The relation between the oxidation metabolism of estrogens and breast cancer has been related in some epidemiological studies, where a given cytochrome P4501A1 gene polymorphism was associated with increased susceptibility to estrogen-related breast cancer in the African-American population (37). Outside the induction of oxidized bases in DNA, the oxidative stress in breast cells can modulate the estrogen receptor functions and therefore participate in some type of tumor promotion (38).

In conclusion, several studies reported a high level of oxidative stress in breast tissue that may be linked to estrogen metabolism and hormonal status. This state can produce oxidized DNA lesions such as the 8-oxoG. In the absence of a full error-free repair of this damage, mutations will occur at a very high frequency. We already observed that in cells from Cockayne’s patients unable to repair 8-oxoG, this unique residue gives rise to 30–40% mutations (14), which confirms a high tendency of replication enzymes to pair this lesion with adenine (12). We can therefore hypothesize that a breast cell already mutated on the BRCA1 or BRCA2 gene will be more susceptible to accumulate point mutations at a high frequency eventually on proto-oncogenes or tumor suppressor genes, leading to malignancy. The BRCA1 and BRCA2 mutations, carried by the HCC 1937 and the CAPAN-1 cells, respectively, are among the most prevalent mutations linked to familial breast and ovarian cancers (39). The deficiency in the repair of 8-oxoG linked to the genetic status reported here thus represents a model highly relevant to the hereditary predisposition to breast and ovarian cancers.

5 A. Sarasin, unpublished observation.
6 F. Le Page and A. Sarasin, unpublished data.
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