ABSTRACT

Known gene mutations account for approximately 50% of the hereditary risk for breast cancer. Moderate and low penetrance variants, discovered by genomic approaches, account for an as-yet-unknown proportion of the remaining heritability. A truncating mutation c.325C>T:p.Arg109* (R109X) in the ATP-dependent helicase ERCC3 was observed recurrently among exomes sequenced in BRCA wild-type, breast cancer–affected individuals of Ashkenazi Jewish ancestry. Modeling of the mutation in ERCC3-deficient or CRISPR/Cas9-edited cell lines showed a consistent pattern of reduced expression of the protein and concomitant hypomorphic functionality when challenged with UVC exposure or treatment with the DNA alkylating agent IlludinS. Overexpressing the mutant protein in ERCC3-deficient cells only partially rescued their DNA repair–deficient phenotype. Comparison of frequency of this recurrent mutation in over 6,500 chromosomes of breast cancer cases and 6,800 Ashkenazi controls showed significant association with breast cancer risk (ORBC = 1.53, ORER = 1.73), particularly for the estrogen receptor–positive subset (P<0.007).

SIGNIFICANCE: A functionally significant recurrent ERCC3 mutation increased the risk for breast cancer in a genetic isolate. Mutated cell lines showed lower survival after in vitro exposure to DNA-damaging agents. Thus, similar to tumors arising in the background of homologous repair defects, mutations in nucleotide excision repair genes such as ERCC3 could constitute potential therapeutic targets in a subset of hereditary breast cancers. Cancer Discov; 6(11):1267–75. ©2016 AACR.
INTRODUCTION

Genetic susceptibility to breast cancer has been shown to be strongly associated with rare coding gene mutations and common noncoding genomic variants (1). Loss-of-function mutations in well-characterized genes, such as those in BRCA1/2 and other members of the homologous recombination pathway, are routinely used for clinical risk assessment in cancer, but account for a small fraction of excess familial risk (2). Common single-nucleotide polymorphisms (SNP) are yet to demonstrate clinical utility, but over 90 SNPs account for over 37% of familial relative risk (3). An as-yet-to-be-defined proportion of the remaining familial risk is accounted for by variants of “moderate” risk, including coding or noncoding variants in the DNA repair pathways such as nucleotide excision repair (NER), mismatch repair (MMR), and base excision repair (BER), which play an important role in checkpoint and genomic integrity maintenance (4, 5). In addition to serving as candidate cancer susceptibility genes, members of the NER pathway also play a critical role in DNA-damage repair caused by chemotherapeutic agents and radiation exposure. ERCC3 is an ATP-dependent DNA helicase that is part of the TFIIH transcription factor complex. In this report, we characterize the functions of a recurrent founder mutation in ERCC3 and demonstrate its association with breast cancer risk.

RESULTS

Identification of a Recurrent ERCC3 R109X Mutation in Ashkenazi Probands with BRCA1/2 Wild-Type Breast Cancer

Exome sequencing was carried out on DNA extracted from peripheral blood/saliva of 46 early-onset (<45 years) and 13 familial BRCA wild-type (WT) breast cancer probands (33 with known ER-positive status) of Ashkenazi Jewish ancestry. We filtered for rare protein truncating variants using public data sources such as the Exome Sequencing Project (ESP), 1000 Genomes, and the Exome Aggregation Consortium (ExAC) without The Cancer Genome Atlas. We further filtered for recurrent mutations in the DNA-repair genes with low background mutation load using the smallest residual variation intolerance score percentage (6) amongst 70 genes, calculated based on the ExAC data. We observed three individuals in the same BRCA WT kindred (Fig. 1) with the same protein truncating mutation, R109X in ERCC3 (HGMD: c.325C>T; p.R109*; chr2:128050332; rs34295337). Sanger sequencing was used to confirm the next-generation sequencing findings (shown in Supplementary Fig. S1). Two of the siblings were affected with ER-positive breast cancer, and the third was a male with breast cancer. The same mutation was also observed in two other individuals at relatively young age (<40 years), from unrelated kindreds with multiple cases of breast cancer. Details of the families and cancer incidence are described in Supplementary Table S1. The variant is almost absent in other world populations, and relatively rare in Caucasians in public data sources such as the ExAC Consortium (Non-Finnish EUR MAF = 0.0008; ESP-EUR MAF = 0.0007). Analysis of the structure of the first 300 amino acids of ERCC3 shows that R109 is most likely part of a right-handed alpha helix (Supplementary Fig. S2). However, no crystal structure is available for high confidence prediction.

Phenotype Rescue by Complementation Assay

To understand whether this variant has a deleterious effect on the gene function, we carried out a series of in vitro functional assays. Using a previously well-characterized ERCC3-deficient cell line derived from a patient with combined xeroderma pigmentosum/Cockayne syndrome (XP/CS; ref. 7), phenotype rescue after DNA damage was assessed by cell viability assay. The parental XPCS2BA cell line and derived lines stably overexpressing the ERCC3 R109* mutant (R109X) or the WT ERCC3 (Fig. 2A and B) showed varying degrees of sensitivity to DNA damage–inducing agents such as UVC and a fungal sesquiterpene IlludinS. We observed that cells overexpressing ERCC3 R109X showed significantly higher cell death compared with the ERCC3 WT, whereas the untransfected cells all
ERCC3 R109X Mutation and Breast Cancer

**Host Cell Reactivation Assay**

Using an exogenous source of DNA, namely a luciferase reporter plasmid, we measured the ability of intact live cells to repair DNA damage. Through measurement of the reporter, the extent of reactivation of the damaged plasmid is quantitated and is a readout of DNA-repair activity. Relative luminescence measured after cotransfection of the reporter plasmid (exposed to 600 J/m²) showed a 1.5-fold difference between mutant and WT overexpression cell lines, suggesting a markedly reduced reporter reactivation in the R109X (Fig. 2E; P < 0.0001). We observed almost no reactivation in the parental cell line at this dose.

**Activation of the DNA-Damage Response Pathway Following UVC Irradiation**

DNA-damage response is marked by activation of H2AX (γH2AX) and leads to the induction of cell-cycle checkpoint initiation by activation of CHK1. To explore the ability of ERCC3 R109X to trigger the checkpoint cascade in response to DNA damage, we examined γH2AX and phosphorylated CHK1 following UVC-mediated DNA-damage induction in XPCS2BA cells. UV-induced phosphorylation of H2AX is reduced in cells deficient in the nucleotide excision repair pathway (9). In agreement with this, we found lower levels of activated H2AX in the XPCS2BA cell line compared with cells where WT ERCC3 expression had been restored. In the mutant, we observed lower γH2AX levels, almost as low as in the parental cell line. Activation of the checkpoint kinase CHK1 was also strongly reduced in the mutant as compared with the WT-overexpressing cells, and similar to the untransfected XPCS2BA (Fig. 2F).

**Functional Characterization of Genome-Edited ERCC3 Mutants in Human Mammary Epithelial Cells**

Overexpression of ERCC3-mutant constructs does not recapitulate physiologic levels of protein in a germline heterozygous mutation state within cells. Therefore, using CRISPR/Cas9, we engineered several heterozygous mutations in the mammary epithelial cell line HMLE that mimic the site of the originally discovered recurrent mutation in individuals...
with breast cancer (Supplementary Table S2; Supplementary Fig. S3). Quantitative real-time PCR of ERCC3 transcripts (exons 1, 2 and exons 9, 10) showed relative transcript levels reduced by half in these cells when compared with the parental HMLE cell line (Fig. 3A). Western blotting also showed the reduction in total ERCC3 protein levels (Fig. 3B), suggesting that in the CRISPR clones, ERCC3 is transcribed mainly from the remaining WT allele. Because we did not observe any homozygous mutations amongst the surviving CRISPR clones, we are unsure if a homozygous mutation is viable. The ERCC3 CRISPR clones generally showed substantial reduction in relative cell viability 72 hours after exposure to IlludinS (Fig. 3C). At 2 ng/mL IlludinS, all the parental HMLE cells survived, whereas the CRISPR edited cell lines showed significantly reduced survival (P < 0.0001). These data suggest that the mutants function at a much lower efficiency compared with WT and hence may be classified as hypomorphs. To demonstrate that the observed effect of IlludinS on the viability of the CRISPR-edited HMLE is specific to the resulting ERCC3 deficit, we performed rescue experiments by overexpressing WT ERCC3 in these cells. We observed that the ERCC3 CRISPR cell lines, after stable overexpression of WT ERCC3, showed the same response to IlludinS as the WT HMLE cell line (Fig. 3D), thereby showing complete rescue of the phenotype, confirming that this phenotype was indeed a result of the engineered ERCC3 deficiency.

To assess the induction and removal of phosphorylated H2AX in response to DNA damage, we performed immunostaining and flow cytometric analysis of γH2AX-positive cells following treatment with IlludinS. Although this led to a similar fold induction of γH2AX-positive cells in the WT and CRISPR clones (not shown), the reduction in γH2AX was significantly lower in the ERCC3 CRISPR mutants, indicating less efficient DNA-damage repair in these cells (P < 0.05; Fig. 3E).

**ERCC3 R109X as a Risk Allele for Breast Cancer in Ashkenazim**

Using breast cancer–affected individuals and controls from the Memorial Sloan Kettering Cancer Center (MSKCC), the University of Pennsylvania, and the Clalit National Israeli Cancer Control Center, we performed a matched case–control association of the ERCC3 variant using TaqMan genotyping and sequencing across 3,286 cases and 2,716 controls. A third group of 705 controls was sourced from The Ashkenazi Genome Consortium (TAGC) project (ref. 10 and in preparation) control resource (Table 1A). All control groups showed similar allele frequencies. A total of 101 heterozygote carriers were present in the combined data. A 1.53-fold increased risk was observed for breast cancer (OR = 1.53, lower CI = 1.07; P = 0.023) after examining over 6,500 chromosomes in cases and 6,800 chromosomes in controls (Table 1A). A stronger
association was observed with the ER-positive subtype (OR = 1.73, lower CI = 1.19; P = 0.007; Table 1B). The majority of the tumors in the carriers had an ER-positive status. These data show that the ERCC3 c.325 T allele is a moderate risk factor for breast cancer in individuals of Ashkenazi ancestry. Unphased haplotype analyses from the TAGC heterozygote carriers suggested a founder mutation (Supplementary Fig. S4). In the chromosomal region 2q14.3, a 3.4 cM-long haplotype was observed in the carriers which was significantly longer than in noncarriers (P < 10^{-14}).

**DISCUSSION**

Here, we identified a truncating germline mutation in the first putative helicase domain of the DNA-repair gene ERCC3 and report a strong genetic association with risk of breast cancer in individuals of Ashkenazi ancestry. The R109X variant, seen in 1.83% of Ashkenazi Jewish individuals with breast cancer and 2.06% in the ER-positive subset, confers a moderate risk (OR = 1.73). The same mutation was also observed twice as a somatic event in a breast carcinoma and a soft-tissue sarcoma (Supplementary Fig. S5A). In general, ERCC3 is seldom disrupted in somatic tissue by genomic integrity loss such as amplification or deletions (22). In light of the rarity of known mutations in ERCC3, there have thus far been no studies elucidating cancer susceptibility in heterozygous carriers. The only mouse model that has been described modeled the XP/CS syndrome (23, 24), whereas heterozygous knockout mice also showed elevated frequency of spontaneous genotoxic events. Importantly, ERCC3 transcript expression was shown to be lower in the proband’s cells compared with a non-mutation carrier (Supplementary Fig. S6). Dosage dependency of key molecular components of the DNA-damage repair pathway, such as holoinsufficiency, has been previously demonstrated (16–20), leading to tumorigenesis in *in vitro* and *in vivo* models of H2AX, BLM, and CHEK1.

Hypomorphic mutations in the DNA-damage repair gene *NBN* cause the autosomal recessive chromosomal instability disorder Nijmegen breakage syndrome (NBS) in homozygous individuals, but have also been shown to lead to increased cancer incidence in heterozygous relatives of patients with NBS, thus providing a plausible example of a hypomorphic mutation that is also a susceptibility gene (14, 15). Similarly, ERCC3 R109X behaves as a hypomorph in our functional assays. In experiments using an ERCC3-deficient cell line, R109X was partially successful at phenotype rescue, with the cells exhibiting intermediate repair capability, suggesting that they are more likely to harbor a second hit following mutagenesis. Importantly, ERCC3 transcript expression was shown to be lower in the proband’s cells compared with a non-mutation carrier (Supplementary Fig. S6). Dosage dependency of key molecular components of the DNA-damage repair pathway, such as holoinsufficiency, has been previously demonstrated (16–20), leading to tumorigenesis in *in vitro* and *in vivo* models of H2AX, BLM, and CHEK1.

ERCC3 homozygous knockout mice have been shown to be embryonic lethal, indicating that the gene is necessary for development (21). Additionally, very few ERCC3 mutations have been reported, even amongst patients with XP, suggesting the gene is intolerant to common mutational mechanisms. Analysis of gene-based mutability from the ESP and ExAC exome data has shown that quantitative metrics that predict gene conservation and mutation tolerance rank ERCC3 as bearing low background mutational load (22). In light of the rarity of known mutations in ERCC3, there have thus far been no studies elucidating cancer susceptibility in heterozygous carriers. The only mouse model that has been described modeled the XP/CS hereditary DNA repair deficiency syndrome (21). Animals with heterozygous knockout of the NER genes XPC and XPE/DB2 showed increased cancer incidence after exposure to UV irradiation (23, 24), whereas heterozygous XPC knockout mice also showed elevated frequency of spontaneous...
mutations as a function of age (25). Also, a mouse model harboring a mutation in the other helicase of the TFIIH complex, XPD, showed a strongly increased cancer incidence in response to UV exposure (26).

This is the first study that shows a specific truncating mutation in ERCC3 conferring increased risk of breast cancer. However, polygenes in DNA repair and/or other pathways, acting epistatically with ERCC3, are likely contributors to and modifiers of the heritable risk of breast cancer, complicating attempts to demonstrate cosegregation of single variants in multiplex kindreds. We have previously demonstrated a modestly elevated breast cancer risk associated with specific mutations of CHEK2 and APC in the Ashkenazi Jewish genetic isolate (27, 28), and other recurring mutations of CHEK2, HOUX13, PALB2, and RAD51C have been variably associated with breast cancer risk in diverse populations (29–32). Although ERCC3 R109X is more frequent in Ashkenazi Jews, we have observed it as well as other ERCC3 mutations in non-Ashkenazi individuals (data not shown), but because of the rarity of these ERCC3 mutations, further population-based studies will be required to assess their role as breast cancer susceptibility alleles. Although the relative risks of 1.5 to 2.0 shown here are higher than those associated with most genome-wide association study SNPs, the clinical utility of variants associated with risks in this range remains to be established. Nonetheless, these discoveries provide insight into molecular pathways of breast cancer pathogenesis and potentially identify therapeutic targets (33–36). The enhanced susceptibility of ERCC3-mutant cells to reagents of the fungal sesquiterpene class, demonstrated here, suggests additional in vivo studies of the therapeutic efficacy versus toxicity of these compounds against breast tumors in an ERCC3-mutant genetic background.

METHODS

Next-Generation Sequencing of Breast Cancer Probands and Controls

One microgram of germline DNA extracted from peripheral blood was used for whole-exome capture using the Agilent SureSelect 38-Mb paired-end sequencing with the Illumina HiSeq 2000. Sequence reads in the form of FASTQ files were aligned to the human decoy reference (GRCh37) to generate BAM files using Burrows-Wheeler Aligner (BWA) v0.7.12. Picard tools were used for quality metric calculation and marking duplicate reads. The Genome Analysis Tool Kit (GATK) version 3.30-g37228af was used for variant calling using the haplotype caller algorithm. Variant quality score recalibrated (VQSR) data were used for filtering variants. Variant-level and interval-level annotations were performed using SnpEff, ANNOVAR, and CAVA programs. Downstream analysis consisted of filtering out low-quality variant calls and those already reported as common in public databases.

The TAGC has sequenced the whole genomes of 128 and 577 individuals of Ashkenazi Jewish ancestry using the Complete Genomics Center, respectively. The paired-end libraries were generated using the TruSeq DNA Nano Kit, sequenced to an average depth of 30, and reads were aligned using a standard pipeline involving BWA version 0.7.8 and GATK version 3.2.2. Extensive quality control is performed on all whole-genome sequencing samples, including alignment rates (>97%), median and mean library insert size (>350bps), percent duplication (typically <20%), mean genome coverage (>30x) and uniformity of coverage, TiTv and Het-Hom ratios. An automated concordance check is also performed against the SNP array genotyping data, to ensure against sample swap at any step during the sequencing process, and to further validate the quality of the single-nucleotide variant calls from the sequencing data.

All research participants provided written consent to an Institutional Review Board–approved research protocol to allow for the collection and use of biospecimens.

Haplotype Analysis

Using only high-quality biallelic SNPs, the haplotype lengths carrying the ERCC3 R109X mutation were calculated to either side of the mutation in the TAGC carriers as the length until an opposite homozygous genotype. This is an overestimate, due to the lack of accurate phasing information. The mean length was 3.4 cm, estimated by using the HapMap recombination rates for the region. The mean lengths of haplotypes shared between noncarriers were also computed. The significance of the difference between the distributions of haplotype lengths at the carriers and at 100 random noncarriers was calculated using the Kolmogorov-Smirnov test.

Sanger Sequencing

Sanger sequencing of the ERCC3 R109X variant was performed using the following primers:

ERCC3_F1: CAGGGAGGATCTGCTATT

ERCC3_R1: CTGGAACATCTGTTCTTGT

TagMan Genotyping

The allelic discrimination assay C__25963434_10 for genotyping was done using TaqMan (Life Technologies). The assay was run on an ABI HT7900 machine and automatically clustered and manually reviewed. Confirmed heterozygotes were run as positive controls, and duplicate concordances were checked per plate.

Statistical Analyses

Allele counts were tabulated from the TaqMan genotyping after QC. Statistical analysis was performed using the R statistical package using the fisher.test module. Because the truncating genetic variant was hypothesized and shown by functional assays to lead to increased risk associated with reduced DNA repair efficiency, we report one-sided Fisher exact test results. Nevertheless, two-sided tests for both breast cancer and ER status were also significant at the 5% level (breast cancer overall: \( P = 0.036, OR = 1.53, 95\% CI = 1.01–2.34; ER^+ : P = 0.011, OR = 1.73, 95\% CI = 1.11–2.70)."

Plasmids

The pENTR221 plasmid containing human ERCC3 ORF was purchased from TransOMIC. The ERCC3 ORF was cloned into the pLX302 lentiviral expression plasmid, a gift from David Root, Broad Institute of MIT and Harvard (Addgene # 25896). The ERCC3 R109X mutant was generated from the WT ERCC3 plasmid using the QuickChange II XL Site-Directed Mutagenesis Kit (Agilent). pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang, Broad Institute of MIT and Harvard (Addgene # 48138). Guide RNAs were designed using the CRISPR Design Tool (37). The 24-mer oligonucleotides were synthesized (ref. 37; Integrated DNA Technologies), annealed, and cloned into PX458.

Cell Culture and Transfections

The HMLE cell line, established in the laboratory of Robert A. Weinberg at the Whitehead Institute for Biomedical Research, Cambridge, MA, was grown in mammary epithelial growth medium (MEGM) and supplements as recommended by Lonza. The XPCSBA-sv40 cell line was grown in RPMI-1640–HEPES medium (Invitrogen), supplemented with 10% FBS and 1% penicillin-streptomycin and glutamate. HEK293T cells (ATCC; CRL-3216) were maintained in...
DMEM supplemented with 10% FBS and 1% penicillin-streptomycin and glutamine. The HMLE and HEK293T lines were kindly provided by Robert Benzer (MSKCC), and the XPCS2BA-sv40 cell line was a generous gift from Jan Hoeijmakers (Erasmus MC, Rotterdam, the Netherlands). The cell lines were not further authenticated. Cell cultures were maintained in a humidified incubator at 37°C in 5% CO2 and tested for *Mycoplasma* on a monthly basis.

Transfections were carried out with the Amaxa Cell Line Nucleofector Kit V (Lonza) and Nucleofector IIb device according to the manufacturer’s instructions. Viral vectors, cotransfected with pSPAX2 and pseudotyped with VSV-G, were produced in HEK293T cells using the Lipofectamin2000 transfection reagent. The virus supernatant was concentrated by centrifugation for 90 minutes at 20,000 RPM at 4°C, and pellets were dissolved in OptiMEM (Gibco). Transduction of cells with virus supernatant was carried out in the presence of 8 μg/mL polybrene. Stably transfected cell lines were generated by selection with 0.5 μg/mL puromycin.

Cells cotransfected with pmaxGFP (Lonza) and the pX458-sgRNA plasmids, containing guide RNAs targeting the ERCC3 locus in close proximity to the ERCC3 mutation site (chr2: 128050332) with or without a repair template (ssODN) containing the specific mutation, were sorted as single cells into 96-well plates based on GFP fluorescence using a BD FACSAria cell sorter.

**Screening of CRISPR/Cas9-Edited Cell Lines**

Single-cell clones generated from the transfected cells were screened and expanded and genomic DNA extracted using the Quick-Extract DNA Extraction Solution (Epicentre). The genomic region surrounding the target site was amplified using the following primer sequences:

ERCC3_SURV F, 5′-TGTGGTGGTGCCAGCTTAT-3′;
ERCC3_SURV R, 5′-ACACCTCACTTTGGGTGTG-3′

The purified PCR products were subjected to Sanger sequencing.

**Real-Time PCR**

RNA was extracted 24 hours after transfection using the RNeasy Mini Kit (Qiagen) and reverse transcribed with the ReadyScript First Strand kit (Epicentre). Real-time PCR analyses were performed on a ABI PRISM 7900HT Sequence Detection System using the Power SYBR Green PCR Master Mix (Life Technologies) according to the manufacturer’s instructions. Analysis was performed based on the comparative CT method. Values reported are mean of triplicate experiments. The following primer sequences were used:

RPL32 F, 5′-AACTCGTCTTGCAATGGCCTC-3′;
RPL32 R, 5′-ACCCTGTTGTCAATGCTC-3′

**Western Blotting**

Protein lysates were prepared in RIPA buffer (Pierce). Samples were run on 4% to 12% gradient Bis-Tris SDS-PAGE gels (Invitrogen), transferred onto PVDF membranes (Bio-Rad), and probed with antibodies against ERCC3 (ARP37963_P050; 1:2,500; Abcam Systems Biology), phospho-Histone H2AX (Ser139) (1:1,000; Cell Signaling Technology), phospho-CHK1 (Ser345; 1:1,000; Cell Signaling Technology), and phospho-Histone H2A.X (Ser139) (1:1,000; Cell Signaling Technology). The membranes were washed twice with PBS/0.5% BSA and incubated with anti–phospho-Histone H2A.X (Ser139) antibody for 45 minutes at room temperature. Cells were rinsed twice with PBS and incubated with Alexa-488-conjugated secondary antibody for 45 minutes at room temperature. For each condition, 20,000 cells were recorded, and data from two replicate experiments were analyzed using the Flowjo software (V10.1).

**Flow Cytometry**

For flow cytometric analysis, 24 hours after seeding, cells were either left untreated or were treated with 8 ng/mL IlludinS for 1 hour. Treated cells were subsequently washed with PBS and supplemented with drug-free medium. The cells were harvested at different time points after treatment and fixed with 4% paraformaldehyde for 10 minutes at room temperature. Cells were quickly chilled on ice, and ice-cold MeOH was added to a final concentration of 90%. The cells were incubated on ice for 30 minutes and stored at −20°C. For immunostaining, the cells were washed twice with PBS/0.5% BSA and incubated with anti–phospho-Histone H2AX (Ser139) antibody (1:250; Cell Signaling Technology) for 1 hour at room temperature, washed again and incubated with Alexa-488-conjugated secondary antibody for 45 minutes at room temperature. For each condition, 20,000 cells were recorded, and data from two replicate experiments were analyzed using the Flowjo software (V10.1).

**Disclosure of Potential Conflicts of Interest**

M. Robson is a consultant/advisory board member for AstraZeneca. No potential conflicts of interest were disclosed by the other authors.

**Disclaimer**

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, the U.S. Army, or the Department of Defense. The funding institutions had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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**Cell Viability Assays**

For viability assessment following treatment with IlludinS (Cayman Chemical), cells were seeded into 96-well plates 24 hours prior to treatment. For post-UV viability assays, cells were exposed to the appropriate doses of UV irradiation and subsequently seeded into 96-well plates. Cell viability was measured after 72 hours using the CellTiter-Glo Luminescent Cell Viability Assay (Promega).

**Host Cell Reactivation Assay**

The pl50 plasmid, derived from the pGL3-control vector containing the Firefly luciferase gene (Promega) and the pl52, a derivative from the pRL-SV40 vector (Promega) containing the Renilla luciferase gene (as an internal transfection control), were used for transfection of cells. The pl50 vector was treated with increasing doses of UV radiation. These vectors were cotransfected into the XPCS2BA and stable ERCC3 WT and R109X-overexpressing cell lines using the Eugene 6 Transfection Reagent (Promega). Forty-eight hours after DNA transfection, the luciferases’ activity was measured with the Dual-Glo Luciferase Assay System (Promega) and a GloMAX Luminometer (Promega).
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