

Gene Conversion Is Strongly Induced in Human Cells by Double-strand Breaks and Is Modulated by the Expression of BCL-x_L¹

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Abstract

Homology-directed repair (HDR) of DNA double-strand breaks (DSBs) contributes to the maintenance of genomic stability in rodent cells, and it has been assumed that HDR is of similar importance in DSB repair in human cells. However, some outcomes of homologous recombination can be deleterious, suggesting that factors exist to regulate HDR. We demonstrated previously that overexpression of BCL-2 or BCL-x_L enhanced the frequency of X-ray-induced *TK1* mutations, including loss of heterozygosity events presumed to arise by mitotic recombination. The present study was designed to test whether HDR is a prominent DSB repair pathway in human cells and to determine whether ectopic expression of BCL-x_L affects HDR. Using TK6-neo cells, we find that a single DSB in an integrated HDR reporter stimulates gene conversion 40–50-fold, demonstrating efficient DSB repair by gene conversion in human cells. Significantly, DSB-induced gene conversion events are 3–4-fold more frequent in TK6 cells that stably overexpress the antiapoptotic protein BCL-x_L. Thus, HDR plays an important role in maintaining genomic integrity in human cells, and ectopic expression of BCL-x_L enhances HDR of DSBs. This is the first study to highlight a function for BCL-x_L in modulating DSB repair in human cells.

Introduction

Cellular responses to DNA damage include removal or bypass of specific DNA lesions, checkpoint functions to delay cell cycle progression, and the programmed death of damaged cells. When programmed cell death is suppressed, new mutations in critical genomic loci (*e.g.*, tumor suppressor genes) are maintained in the affected cells and their progeny, and this is likely to be important in malignant progression. One DNA lesion that is particularly critical to repair is the DSB.⁴ Mammalian cells have robust nonhomologous DSB repair pathways, which primarily involve NHEJ (1). NHEJ proteins form a complex (DNA-PK) that binds to DNA ends (2). Although DNA-PK is conserved between rodents and humans, its activity has been estimated to be 50-fold more abundant in human cells (3), raising the possibility that DSB repair may differ in some regards between mammalian species. In addition to NHEJ, HDR is a major DSB repair pathway in rodent cells, wherein DSBs are potent inducers of recom-

bination (4). The expectation is that individual DSBs should be potent inducers of recombination in human cells. Nevertheless, the difference in NHEJ raises doubts about this assumption.

Ionizing radiation, which causes DSBs, induces apoptosis in many cell types. Apoptosis is controlled, in part, by proteins of the BCL-2 family. Members of this family share some structural homology but function either to promote cell death or to inhibit cell death. Antiapoptotic proteins include BCL-2 and BCL-x_L (5). The relative abundance of pro- and antiapoptotic members of this protein family determines whether a cell will live or die (6). As might be expected, dysregulation of the network of BCL-2-related proteins contributes to human carcinogenesis. BCL-2 is highly expressed in B-cell follicular lymphoma due to its juxtaposition with the IgH enhancer, and BCL-x_L is up-regulated in a variety of cancers wherein high expression is associated with advanced disease, resistance to genotoxic agents, and poor prognosis (7–10).

We reported previously that human B-cell lines suppressed for apoptosis by overexpression of BCL-2 or BCL-x_L showed significantly elevated levels of radiation-induced *TK1* mutations (11). *TK1* mutations can arise by several mechanisms, including LOH by mitotic interchromosomal recombination (12). Mitotic recombination between homologous chromosomes is presumed to involve homology at the point of crossover, although the fidelity of repair at the breakpoint junctions is usually not assessed. To determine whether human cells can efficiently repair DSBs by HDR and the effect of BCL-x_L overexpression on HDR, we stably transfected TK6-neo and TK6-bcl-x_L cells with a DR-GFP recombination reporter (13). A single DSB is introduced into this reporter via transient expression of the *I-SceI* endonuclease, and when this DSB is repaired by gene conversion, expression of a functional *GFP* gene results. Expression of *I-SceI* greatly stimulated gene conversion in both cell types. In TK6-neo cells, HDR was stimulated 40–50-fold, demonstrating conclusively that DSBs are potent inducers of homologous recombination in wild-type human cells. In addition, DSB-induction led to a 3–4-fold increase in the number of recombinants in TK6-bcl-x_L cells as compared with TK6-neo cells, suggesting a novel role for BCL-x_L in modulating DSB repair in human cells.

Materials and Methods

Cell Culture. TK6-neo and TK6-bcl-x_L cells are human B lymphoblasts that have been described previously (11). Cells were grown at 37°C in suspension cultures and maintained in exponential growth at densities of 1 × 10⁵ to 1 × 10⁶ cells/ml.

Transfection and Recombination Assays. Stable cell lines containing the pDR-GFP reporter construct (13) were generated by electroporation (625 V/cm, 950 μF) using the Bio-Rad Gene Pulser II apparatus (Bio-Rad, Hercules, CA). One million TK6-neo cells or TK6-bcl-x_L cells were transfected in 400 μl of Cytomix buffer using 10 μg of circular plasmid (14). Cells were seeded at 500 cells/well in 96-well plates in standard growth medium supplemented with 0.2 μg/ml puromycin. Puromycin-resistant cell lines were subjected to Southern analysis using *SalI/HindIII* digests to confirm intact integration of the pDR-GFP reporter, and single copy integration was verified

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⁴ The abbreviations used are: DSB, DNA double-strand break; HDR, homology-directed repair; NHEJ, nonhomologous end-joining; GFP, green fluorescent protein; DR-GFP, direct repeat GFP; LOH, loss of heterozygosity; TK, thymidine kinase.

using *Pst*I with a mixture of restriction enzymes that do not cut within the vector (13). The stable cell lines derived from TK6-neo cells are designated TK6-neo-DR-GFP, and the stable cell lines derived from TK6-bcl-x_L cells are designated TK6-bcl-x_L-DR-GFP.

The construct pNZE-CAG, used to monitor plasmid uptake and the response to electroporation in TK6-neo and TK6-bcl-x_L cells, has been described elsewhere (13). Transfectants were monitored by flow cytometry for transient expression of GFP 40–48 h after electroporation.

*I-Sce*I was expressed in transient transfections from the pC β ASce expression vector using the same promoter as described for pNZE-CAG (15). One hundred μ g of the construct were used to transfect 1×10^6 TK6-neo-DR-GFP or TK6-bcl-x_L-DR-GFP cells. Transfected cells were analyzed by flow cytometry 2–7 days after electroporation to measure the percentage of cells expressing GFP.

Flow Cytometry and Fluorescence-activated Cell Sorting. Flow cytometry was performed with a Beckman Coulter EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA) using an argon ion air-cooled laser (emission at 488 nm/15 mW power) and XL Data Acquisition and Display software (version 1.5). Green (FL1) versus orange (FL2) fluorescence plots were performed as described previously (13). GFP+ cells are shifted green-ward off the green/orange diagonal in the direction of increasing FL1. Typically, 5×10^4 to 1×10^5 cells were analyzed in each experiment for a given cell line.

A Beckman Coulter Elite ESP sorter was used to sort GFP+ cells, using a 525 ± 20 nm band pass filter (Omega Optics). A total of 10,000–30,000 GFP+ cells were collected. The sorted cell population was 95–98% GFP+. Sorted cells were expanded and reanalyzed for GFP expression. An aliquot of the expanded culture was frozen, and the remaining 3×10^7 cells were subjected to DNA extraction for analysis of structural changes leading to gene conversion using restriction digests and Southern analysis.

Southern Analysis of Gene Conversion in GFP+ Cells. Restriction analysis was performed using standard methods to confirm that gene conversion had occurred in GFP+ cells containing DR-GFP (13). *SceGFP* is mutated through the incorporation of the *I-Sce*I site at a *Bcg*I restriction site by substituting 11 bp of wild-type GFP sequence with those of the *I-Sce*I site. These substituted bases also introduce two in-frame stop codons. The 5'- and 3'-truncated GFP gene, *iGFP*, can be used to correct the mutation in the

SceGFP gene to result in a GFP+ gene with a restored *Bcg*I site. Molecular analysis confirms that GFP+ cells are generated by noncrossover gene conversion events within DR-GFP, whereas deletional recombination events result in a 3'-truncated GFP gene that cannot express GFP (13).

Results

DSBs Induce HDR by Gene Conversion >50-fold in Human Cells. TK6-neo cells are TK6 cells that contain an integrated copy of the pSFFV-neo vector (11). To test whether human cells undergo homologous recombination at elevated levels on introduction of a chromosomal DSB, we integrated the recombination reporter substrate DR-GFP into the genome of TK6-neo cells (Fig. 1A). Two independent clones containing a single copy of the substrate were used for subsequent analysis.

Clones were examined for both spontaneous and DSB-induced gene conversion within the DR-GFP substrate. When these clones were examined for green fluorescence using flow cytometry, few fluorescent cells were obtained (e.g., 0.01% for mock-transfected cells; Fig. 1B), indicating that spontaneous gene conversion is rare. To examine DSB-promoted gene conversion, the *I-Sce*I endonuclease was transiently expressed from pC β ASce to introduce a DSB in the upstream *SceGFP* gene. GFP+ cells were readily detected upon *I-Sce*I expression. For example, 0.54% of cells were GFP+ in the clone shown in Fig. 1B (middle panel), indicating more than a 50-fold increase over mock-transfected cells.

To prove that GFP+ cells arose by gene conversion, GFP+ cells were separated from GFP- cells in populations electroporated with the *I-Sce*I expression vector. Fluorescence-activated cell-sorted GFP+ cells were expanded in culture for DNA extraction and Southern blot analysis. Before sorting, 0.33–1% of the TK6-neo cells in a given population transfected with *I-Sce*I were GFP+ (e.g., Fig. 1B, middle panel). Generally, sorted cultures had 96–98% GFP+ cells upon immediate reanalysis. After several weeks of growth, the frac-

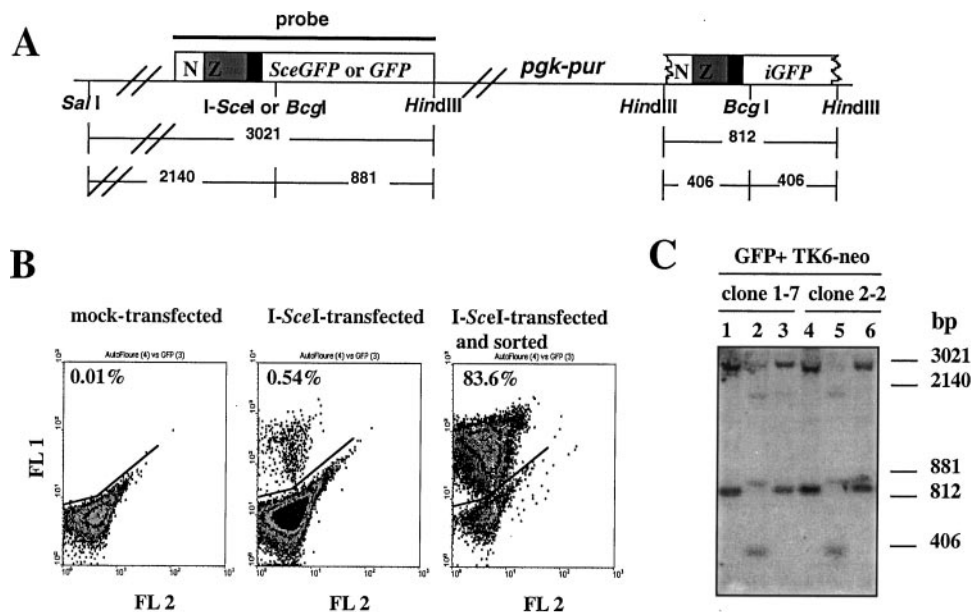


Fig. 1. A, the recombination repair substrate DR-GFP is composed of two differentially mutated GFP genes, *SceGFP* and *iGFP* (14). When the *I-Sce*I endonuclease is expressed in cells containing DR-GFP, a DSB will be introduced at the *I-Sce*I site in the *SceGFP* gene. Repair of the DSB by noncrossover gene conversion with the downstream *iGFP* gene results in the reconstitution of the functional GFP gene. The nuclear localization signal (N) and the zinc finger domain (Z) aid in the nuclear retention of the protein (14). B, representative analysis of GFP fluorescence in TK6-neo-DR-GFP cells (clone 1-7). Two-color fluorescence was performed, with the percentage of green fluorescent cells (FL1) indicated at the top left of each panel. FL1, green fluorescence; FL2, orange fluorescence. Very few GFP+ cells are found in mock-transfected cultures (left panel). In this example, *I-Sce*I expression strongly elicits gene conversion (>50-fold), enhancing the fraction of GFP+ cells to 0.54% (middle panel). GFP+ cells were initially sorted to >96% purity (data not shown). Reanalysis of the sorted GFP+ population several weeks after transfection with *I-Sce*I indicates the stability of GFP expression (83.6% GFP+ cells, right panel). C, physical confirmation of gene conversion after a single *I-Sce*I-induced DSB. A Southern blot is shown revealing the cleavage pattern of genomic DNA extracted from the sorted pools of TK6-neo-DR-GFP cells. In GFP+ cells, the wild-type GFP sequence has been restored, i.e., the *I-Sce*I site has been replaced with the *Bcg*I site. GFP+ cells are resistant to cleavage by *I-Sce*I (Lanes 3 and 6) and susceptible to cleavage by *Bcg*I (Lanes 2 and 5); *Sal*I/*Hind*III, Lanes 1 and 4; *Sal*I/*Hind*III/*Bcg*I, Lanes 2 and 5; *Sal*I/*Hind*III/*I-Sce*I, Lanes 3 and 6.

tion of GFP+ cells in some sorted cultures decreased slightly to 83–96% (e.g., Fig. 1B, right panel), whereas in others the fraction of GFP+ cells did not change at all. The reason for the slight decrease in the fraction of GFP+ cells in some of the sorted cultures during expansion is unclear, although it might be the result of the low number of GFP+ cells in the starting population or occasional epigenetic silencing of the *GFP* gene. After expansion, the level of fluorescence of each population was generally stable for several weeks in culture.

Genomic DNA from the sorted populations was subjected to Southern analysis using the *GFP* coding sequence as a probe (13). If a gene conversion event occurs at the *I-SceI* site, the *GFP* wild-type sequence is restored along with its *BcgI* site, which is detected by restriction digest in conjunction with *SalI* and *HindIII* digests. The gene conversion product was observed in each of the GFP+ sorted populations (Fig. 1C), i.e., the *SalI/HindIII* band of 3021 bp is not cleaved by *I-SceI* (Fig. 1C, Lanes 3 and 6) but is cleaved by *BcgI* to two bands of 2140 and 881 bp (Fig. 1C, Lanes 2 and 5). (Note: because *BcgI* is not a robust restriction enzyme, we frequently do not obtain complete cleavage.) These results demonstrate that the expression of GFP resulted from gene conversion restoring the wild-type *GFP* sequence by HDR.

Ectopic Expression of BCL-x_L Enhances HDR of a Single, Site-specific DSB in Human Cells. We then investigated whether ectopic expression of BCL-x_L affects the ability of human lymphoid cells to undergo HDR. TK6-bcl-x_L cells are TK6 cells containing an integrated copy of pSFFVneo-bcl-x_L and express approximately 80–100-fold higher levels of functional BCL-x_L than TK6-neo cells (11). We constructed four independent clones of TK6-bcl-x_L cells containing a single integrated copy of DR-GFP for use in gene conversion studies.

In mock transfection experiments, very few GFP+ cells were detected in either TK6-neo-DR-GFP or TK6-bcl-x_L-DR-GFP cells (Fig. 2A). The combined results were $0.016 \pm 0.003\%$ GFP+ cells (mean \pm 1 SE) for two TK6-neo-DR-GFP cell lines and $0.010 \pm 0.002\%$ GFP+ cells (mean \pm 1 SE) for four TK6-bcl-x_L-DR-GFP cell lines. Gene conversion was rare in each cell type, indicating that high levels of BCL-x_L did not noticeably enhance gene conversion in the absence of DSB induction. It is not clear whether the very small difference in the number of GFP+ cells detected in the different genetic backgrounds is meaningful, and it is unlikely that fluctuation analysis would clarify this point, given the low spontaneous frequency.

DSB-mediated gene conversion was measured in all cell lines after transient expression of *I-SceI*. To exclude position effects of the integrated DR-GFP, each DR-GFP derivative of TK6-neo and TK6-bcl-x_L was analyzed individually. *I-SceI*-induced DSBs stimulated gene conversion in TK6-neo-DR-GFP cell lines resulting in $0.56 \pm 0.12\%$ and $0.61 \pm 0.08\%$ GFP+ cells for clone 1-7 and clone 2-2, respectively (mean \pm 1 SE; Fig. 2B). Ectopic expression of BCL-x_L strongly enhanced the fraction of GFP+ cells after the induction of the single site-specific DSB. Expression of *I-SceI* in four independent TK6-bcl-x_L-DR-GFP cell lines resulted in $2.32 \pm 0.14\%$, $3.10 \pm 0.56\%$, $2.87 \pm 0.31\%$ and $3.01 \pm 0.25\%$ GFP+ cells (mean \pm 1 SE; Fig. 2B). Three conclusions may be drawn from these data: (a) a single DSB stimulates HDR 40–50-fold in wild-type TK6 cells; (b) one DSB stimulates HDR 200–300-fold in TK6-bcl-x_L cells; and (c) ectopic expression of BCL-x_L enhances HDR when compared with isogenic control cell lines.

To ensure that the increase in DSB-stimulated HDR in TK6-bcl-x_L cells was not an artifact attributable to electroporation and plasmid uptake, we measured the transfection efficiencies in TK6-neo and TK6-bcl-x_L cells using the GFP expression vector pNZE-CAG. We monitored both the fraction of GFP+ cells and the intensity of GFP

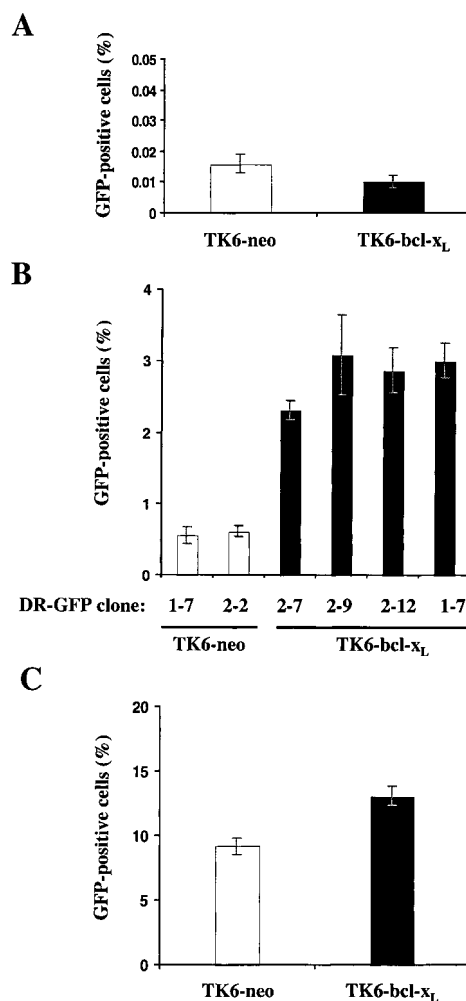


Fig. 2. A, spontaneous gene conversion in TK6-neo and TK6-bcl-x_L cell lines. Cells were transfected with or without a mock plasmid (pDS267; kindly provided by Dr. D. Schild; Lawrence Berkeley National Laboratory) and analyzed for expression of functional GFP by flow cytometry. The results shown indicate the mean \pm 1 SE of the combined data for two TK6-neo cell lines (8 experiments) and four TK6-bcl-x_L cell lines (27 experiments). B, the induction of a DSB strongly induces gene conversion in TK6-neo cells (40–50-fold) and induces gene conversion even more strongly in TK6-bcl-x_L cells (200–300-fold). TK6-bcl-x_L cells have 4–5-fold higher levels of GFP+ cells, indicating enhanced HDR of a DSB by gene conversion with high levels of BCL-x_L. The data represent the mean \pm 1 SE of five experiments for each cell line. C, plasmid uptake and/or expression is slightly elevated (1.4-fold) in TK6-bcl-x_L cells compared with TK6-neo cells. The transfection efficiency for each cell line was measured after transient transfection with pNZE-CAG. The results shown indicate the mean \pm 1 SE of the combined data for two TK6-neo and four TK6-bcl-x_L DR-GFP clones. Ten experiments were carried out using both TK6-neo-DR-GFP clones, and 15 experiments were carried out using the TK6-bcl-x_L-DR-GFP clones.

fluorescence by flow cytometry 40–48 h after transfection. The mean fluorescence intensities for GFP+ cells were similar in each genetic background. Plasmid uptake was slightly higher (1.4-fold) in the TK6-bcl-x_L-derived DR-GFP cell lines (Fig. 2C; $P < 0.001$, *t* test). We detected $9.18 \pm 0.64\%$ (mean \pm 1 SE) GFP+ cells for TK6-neo clones and $13.07 \pm 0.72\%$ (mean \pm 1 SE) GFP+ cells for TK6-bcl-x_L-derived DR-GFP cell lines. When we correct for the small difference in plasmid uptake, ectopic expression of BCL-x_L results in a 3–4-fold enhancement of HDR.

Gene Conversion Is Associated with Loss of the *I-SceI* Site in Human Cells Expressing High Levels of BCL-x_L. To confirm that HDR is associated with loss of the *I-SceI* site in the DR-GFP reporter, genomic DNA was prepared for Southern analysis from sorted GFP+ (e.g., Fig. 3A, right panel) and GFP– TK6-bcl-x_L-DR-GFP cells (13). In cells that remained GFP– after transfection with the *I-SceI* expres-

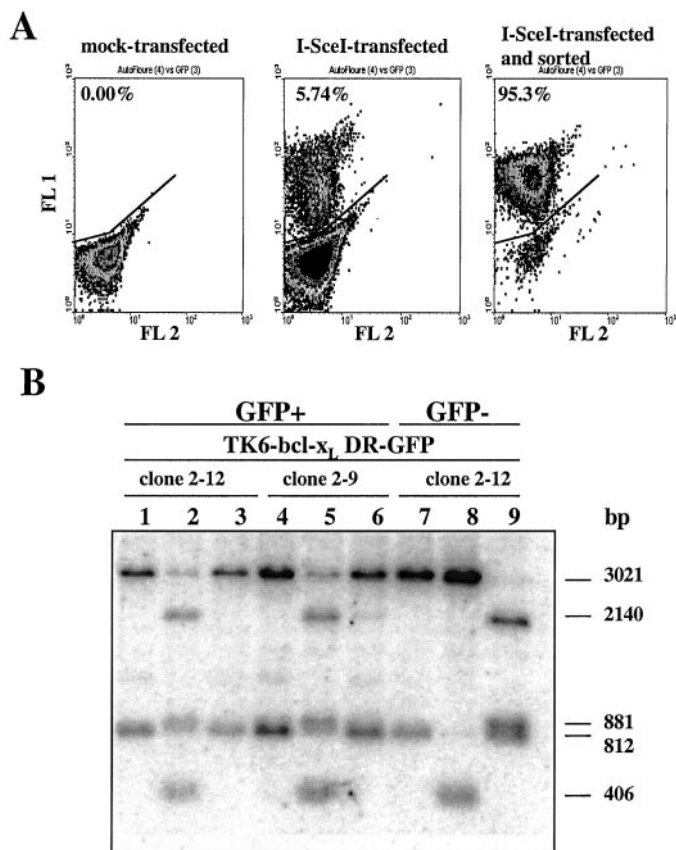


Fig. 3. A, representative analysis of GFP fluorescence in TK6-bcl-x_L-DR-GFP cells (clone 2-9). The percentage of GFP+ cells is indicated in the top left corner of each panel. No GFP+ cells were found in mock-transfected cultures (left panel). In this example, I-SceI expression strongly induces gene conversion and enhances the fraction of GFP+ cells to 5.74% (middle panel). GFP+ cells were sorted to >96% purity (data not shown). Reanalysis of the sorted GFP+ population several weeks after transfection with I-SceI indicates the stability of GFP expression (95.3% GFP+ cells; right panel). Data analyses were performed as described for Fig. 1B. B, physical confirmation of gene conversion after a single I-SceI-induced DSB. A Southern blot is shown revealing the cleavage pattern of genomic DNA extracted from the sorted pools of TK6-bcl-x_L-DR-GFP cells. Data analyses were performed as described for Fig. 1C. GFP+ cells are resistant to cleavage by I-SceI (Lanes 3 and 6) and susceptible to cleavage by BcgI (Lanes 2 and 5). Conversely, in GFP- cultures, the *SalI/HindIII* fragment is cleaved by I-SceI (Lane 9) but not by BcgI (Lane 8). *SalI/HindIII*, Lanes 1, 4, and 7; *SalI/HindIII/BcgI*, Lanes 2, 5, and 8; *SalI/HindIII/I-SceI*, Lanes 3, 6, and 9.

sion vector, the nonrecombined *SceGFP* gene gives bands of 2140 and 881 bp when the *SalI/HindIII* band is digested with I-SceI (Fig. 3B, Lane 9) but maintains the 3021-bp band when digested by BcgI (Fig. 3B, Lane 8). This indicates that the GFP- cell population maintained the I-SceI site, presumably because only a fraction of the cells were successfully transfected. By contrast, in GFP+ cells, the 3021-bp band is not cleaved by I-SceI (Fig. 3B, Lanes 3 and 6) but is cleaved instead by BcgI to produce two bands of 2140 and 881 bp (Fig. 3B, Lanes 2 and 5; note: because BcgI is not a robust restriction enzyme, we frequently did not obtain complete cleavage). Thus, this analysis confirmed that gene conversion had occurred in GFP+ cells.

Discussion

Our results provide evidence that HDR is an important mechanism of DSB repair in human cells. We examined HDR and its induction by DSBs in TK6 cells that carry wild-type *TP53* and manifest an apoptotic response to X-rays that is typical of lymphocytes (16). HDR was stimulated 40–50-fold after the induction of a single site-directed DSB. These results are consistent with earlier work in human cells that suggested that clastogens could

elicit gene conversion, albeit at lower frequencies (17, 18). The present study shows that HDR is a significant DSB repair pathway in human cells, as has been well documented in yeast, chicken, and rodent cells (for review, see Ref. 19).

In mammalian cells, HDR is presumed to require the activity of RAD51 to promote strand exchange and is facilitated by interacting proteins that include XRCC2 and XRCC3 (reviewed in Ref. 19). Hamster cells deficient in either XRCC2 or XRCC3 are defective in error-free HDR of DSBs (13, 20). A deficiency in one of the proteins known to participate in HDR can impair this type of DNA repair by up to 2 orders of magnitude. Several other proteins regulate HDR, presumably through direct or indirect interactions with RAD51. Included among these is BRCA1 (21). Mouse embryonic stem cells containing a hypomorphic *Brca1* allele have 5–6-fold lower levels of HDR of an I-SceI-induced chromosomal DSB (21, 22). Other cells carrying *BRCA2* mutations also have diminished capacities (5-fold to >100-fold) to repair a chromosomal DSB by gene conversion (22). Thus, regulation of HDR appears to involve a complex network of proteins, including known tumor suppressors.

Here we tested derivatives of TK6-bcl-x_L and TK6-neo cells for their ability to undergo error-free HDR. We previously showed that ectopic expression of BCL-x_L or BCL-2 enhanced radiation-induced *TK1* mutagenesis in TK6 cells (11). The enhanced *TK1* mutagenesis is associated with a higher proportion of mitotic recombination.⁵ However, because of the difficulty in analyzing breakpoint junctions, we were not able to investigate the fidelity of the recombination events in our previous systems. The integrated reporter DR-GFP used in the present study specifically addresses this point and indicates 3–4-fold higher levels of error-free HDR in TK6-bcl-x_L cells as compared with TK6-neo cells. One way radiation-induced *TK1* mutagenesis might be enhanced in cells expressing high levels of BCL-x_L or BCL-2 is through the up-regulation of HDR. Although HDR is generally an error-free process, it can occasionally result in a loss of function at a heterozygous locus when the repair event leads to homozygosity of a mutant allele.

One way the apoptotic program may limit HDR is through the cleavage and inactivation of hRAD51 (23). In a preliminary study, we observed that caspase-mediated cleavage of hRad51 is significantly delayed and reduced after X-irradiation of TK6-bcl-x_L cells.⁶ If hRAD51 function is maintained in TK6 cells with both wild-type TP53 and high levels of BCL-x_L, this may contribute to enhanced recombination, as reported in other cells expressing high levels of RAD51, although a new study indicates that ectopic expression of RAD51 can down-regulate HDR in TP53 mutant Chinese hamster ovary cells or in TP53-null HT1080–1885 cells (23–26).

A recent report in mouse L cells expressing wild-type TP53 showed that overexpression of BCL-2 did not alter the frequency of spontaneous gene conversion (27). This agrees with our finding that overexpression of BCL-x_L did not appear to modulate gene conversion in mock-transfected human cells with wild-type TP53. Although the failure to observe an effect of BCL-2 family members on spontaneous gene conversion seems surprising, given our results for DSB-promoted events, this may simply reflect an inability to measure these very rare events with sufficient precision. An alternate possibility is that the initiating events for spontaneous gene conversion (and the proteins that transact the conversion events) differ from the endonuclease-induced DSBs used by us and by Saintigny *et al.* (27). The rodent cell studies did not address the impact of BCL-2 or BCL-x_L

⁵ A. Kronenberg, S. Gauny, W. Liu, C. Cherbonnel-Lasserre, and C. Wiese. Overexpression of BCL-2 or BCL-x_L is associated with enhanced spontaneous and X-ray-induced mitotic recombination in human cells, manuscript in preparation.

⁶ C. Wiese and A. Kronenberg, unpublished results.

expression on DSB-initiated HDR in cells with wild-type TP53. However, their studies using rodent cells with mutated forms of TP53 indicated that BCL-2 or BCL-x_L down-regulated the high levels of HDR in these cells. This raises the possibility of a complex feedback regulation of HDR that includes TP53 and BCL-2 family members.

The mechanism of action of BCL-x_L is not entirely understood. BCL-x_L localizes to the periphery of the mitochondria and the perinuclear membrane (28). Given what is known about the intracellular localization of BCL-x_L, its ability to modulate HDR is most likely through an indirect mechanism, not via direct interaction with the recombination machinery.

BCL-2 was the first antiapoptotic protein implicated in human carcinogenesis. High levels of BCL-2 have been detected in the majority of follicular neoplasms, in diffuse large B-cell lymphomas, and less commonly in other non-Hodgkin's lymphomas (29). The progression from indolent to aggressive disease in these lymphomas can involve LOH on selected chromosomes (30, 31). Mitotic recombination is a major mechanism for the generation of LOH in tumors (32). Our study provides direct evidence that high levels of expression of BCL-x_L elevate HDR. We speculate that this dysregulation of HDR may actively promote malignant progression.

Acknowledgments

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