Cancer cells are often associated with secondary chromosomal rearrangements, such as deletions, inversions, and translocations, which could be the consequence of unrepaired/mis-repaired DNA double strand breaks (DSBs). Nonhomologous DNA end joining is one of the most common pathways to repair DSBs in higher eukaryotes. By using oligomeric DNA substrates mimicking various endogenous DSBs in a cell-free system, we studied end joining (EJ) in different cancer cell lines. We found that the efficiency of EJ varies among cancer cells; however, there was no remarkable difference in the mechanism and expression of EJ proteins. Interestingly, cancer cells with lower levels of EJ possessed elevated expression of BCL2 and vice versa. Removal of BCL2 by immunoprecipitation or protein fractionation led to elevated EJ. More importantly, we show that overexpression of BCL2 or the addition of purified BCL2 led to the down-regulation of EJ. Further, we found that BCL2 interacts with KU proteins both in vitro and in vivo. Hence, our results suggest that EJ in cancer cells could be negatively regulated by the anti-apoptotic protein, BCL2, and this may contribute toward increased chromosomal abnormalities in cancer.

Efficient repair of DNA double strand breaks (DSBs) is critical for the maintenance of genomic stability in every cell of an organism. DSBs can be generated by both intrinsic (physiologic processes, such as replication, V(D)J recombination, class switch recombination, meiosis, and generation of free radicals during oxidative metabolism) and extrinsic (ionizing radiations and chemotherapeutic drugs) agents (1, 2). There are two major modes of repair of DSBs: homologous recombination and non-homologous end joining (NHEJ) (3, 4). Homologous recombination requires a minimum of 100-bp homology, whereas NHEJ requires limited or no homology (5, 6). Although homologous recombination can repair DSBs in higher eukaryotes, it is restricted to the late S and G2 phases of the cell cycle, whereas NHEJ is active throughout the cell cycle (7, 8). A less efficient alternative NHEJ has been discovered recently and is implicated in the generation of chromosomal translocations in cancer (9, 10).

The mechanism of NHEJ has been elucidated in the past several years. NHEJ involves a complex network of proteins, which include Ku70/80, DNA-PKcs, XRCC4, LIGASE IV, ARTEMIS, and XLF (3, 4, 11, 12). Ku proteins, which act as a heterodimer, consist of Ku70 and Ku80; they recognize and bind to the DSBs and subsequently recruit DNA-PKcs to the site (13–15). The DNA ends are processed by ARTEMIS or a DNA-PKcs-ARTEMIS complex to create ligatable ends (16, 17). The error-prone DNA polymerases, polymerase μ and λ, carry out DNA synthesis during the joining process (18, 19). The modified ends are then ligated by XLF, XRCC4, and LIGASE IV complex (20–22). Because NHEJ does not depend on a homologous partner to repair the breaks, in many instances, it can be error-prone. Deletions and insertions are the most common modifications seen at the NHEJ junctions (12, 17, 23). Given that unrepaired DSBs in organisms can lead to chromosomal translocations and genomic instability, resulting in cancer or apoptosis, the quick sealing mechanism, NHEJ, is crucial for maintaining the genomic integrity (24). However, it is possible that the end joining observed in in vitro assays using crude cell extracts could also be accounted for by single strand annealing (SSA) and alternative NHEJ, besides the classical NHEJ.

Studies on various cancer cells have revealed the presence of chromosomal abnormalities, including deletions and chromosomal translocations (25–28). Based on this, it has been suggested that cancer cells may have either impaired (29–33) or, in some instances, elevated repair activity (34).

BCL2, an anti-apoptotic protein, located in the inner mitochondrial membrane, upon activation can promote cell proliferation and tumorigenesis (35). It has been shown that chromosomal translocations, such as (t14;18) juxtapose the BCL2 gene to the immunoglobulin enhancer, leading to overexpression of BCL2 in B lymphocytes (36, 37). Such translocations could lead to deregulation of apoptotic pathways, culminating into neoplasia (25). Recent studies have suggested the plausible role of BCL2 in genomic instability and development of cancer (38, 39). In another study, it was shown that BCL2 can interact with Ku proteins through its BH1 and BH4 domains and decrease the efficiency of Ku binding to DNA ends (40). In a recent study, it has been suggested that multipotent hair follicle bulge stem cells are more radiosensitive due to higher levels of BCL2.
BCL2 Down-regulates DNA End Joining in Cancer Cells

and enhanced DNA repair activity, leading to an attenuated p53 response (41).

In this study, we show that human cancer cell lines repair different DSBs with varying efficiency, although the mechanism of EJ is comparable between cancer cells. We further show that the cancer cells with higher expression of BCL2 possessed lower EJ activity, whereas the ones with lower BCL2 expression showed higher EJ. Removal of BCL2 from cancer cell lines by protein fractionation or immunoprecipitation enhanced the EJ activity, whereas the overexpression or addition of purified BCL2 led to down-regulation of EJ. Finally, we show that although BCL2 is a mitochondrial membrane protein, it is also present in the nucleus at lower levels and interacts with KU proteins, which could be one of the mechanisms by which BCL2 down-regulates EJ in cancer cells.

EXPERIMENTAL PROCEDURES

Enzymes, Chemicals, and Reagents—Chemical reagents were obtained from Sigma and Amresco. Restriction enzymes and other DNA-modifying enzymes were purchased from New England Biolabs (Beverly, MA). Radioisotope-labeled nucleotides were purchased from BRIT (Hyderabad, India). Culture media were from Sera Laboratory International Ltd. (West Sussex, UK), and FBS was from Invitrogen.

Cell Culture—Human leukemia cell lines CEM and K562, Burkitt’s lymphoma cell lines RAJI and DAUDI, cervical cancer cell line HeLa, and lung cancer cell line A549 were purchased from the National Center for Cell Science (Pune, India). Lymphoblastoid cell line GM00558B and B-cell leukemia cell lines REH and NALM6 were kind gifts from Dr. Michael Lieber. Cells were grown in RPMI 1640, DMEM, Ham’s media, or minimal essential medium containing 10% FBS and antibiotics in appropriate conditions.

Preparation of Cell-free Extracts—Cell-free extracts were prepared as described earlier with minor modifications (42, 43). Briefly, cancer cell lines of interest were cultured in bulk and washed in PBS. Approximately 8 × 10^7 cells were resuspended in 4 ml of hypotonic buffer (Buffer A: 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 5 mM DTT, and 0.5 mM PMSF) and incubated for 20 min. Cells were homogenized in the presence of protease inhibitors (1 μg/ml each of aprotinin, leupeptin, and pepstatin), and an equal volume of ice-cold buffer B (50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 2 mM DTT, 0.5 mM PMSF, 25% sucrose, and 50% glycerol) was added, followed by 1 ml of neutralized, saturated ammonium sulfate solution. The resulting lysate was stirred gently and centrifuged for 3 h at 32,000 rpm in an SW41 rotor in a Beckman ultracentrifuge (model L8-70M) at 2 °C. Proteins were precipitated using ammonium sulfate (0.33 g/ml) from the supernatant, pelleted, dissolved, and dialyzed in buffer C (25 mM HEPES-KOH (pH 7.9), 0.1 M KCl, 12 mM MgCl2, 1 mM EDTA, 2 mM DTT, and 17% glycerol). The clarified extract was aliquoted, quick frozen in liquid nitrogen, and stored at −80 °C until use. Protein concentration was determined by Bradford’s colorimetric assay and was generally between 3 and 5 mg/ml for each of the extracts.

Oligomers—The oligomers listed were gel-purified as described (44) (supplemental Table 1). The 5’-end labeling of the oligomeric DNA was done using T4 polynucleotide kinase and [γ-32P]ATP, purified, and stored at −20 °C until use (44).

Preparation of DNA Substrates—The oligomeric DNA substrates (75 bp) containing 5’-end-compatible overhangs were prepared by slow annealing of [γ-32P]ATP-labeled TSK1 with cold complementary oligomer TSK2 (1:3 ratio) in the presence of 100 mM NaCl and 1 mM EDTA (43). Similarly, double-stranded DNA containing 5’-‘5’ and 5’-3’ noncompatible overhangs were prepared by mixing [γ-32P]ATP-labeled TSK1 with cold complementary oligomers VK11 and VK13, respectively. Blunt-ended substrate was prepared by annealing radiolabeled VK7 with cold VK8.

EJ Assay—EJ reactions were performed as described earlier with modifications (43, 45). Reactions were done in a volume of 10 μl by mixing 4 nm end-labeled DNA substrate in EJ buffer containing 30 mM HEPES-KOH (pH 7.9), 7.5 mM MgCl2, 1 mM DTT, 2 mM ATP, 50 μM dNTPs, 2 mM EDTA, 0.1 μM cold strand and 0.1 μg of BSA at 37 °C for 2 or 4 h. 5 μg of cell-free extracts was used per reaction, unless mentioned otherwise. The EJ reactions were terminated by the addition of EDTA (10 mM) and proteinase K. The reaction products were deproteinized by phenol/chloroform extraction, followed by ethanol precipitation in the presence of glycogen. The pellet was resuspended in 10 μl of Tris (10 mM), EDTA (1 mM), resolved on 8% denaturing PAGE, dried, exposed to a PhosphorImager screen, and scanned using an FLA9000 phosphor imager (Fuji). The resulting image was analyzed using Multi Gauge (version 3.0) software. For quantitation, the area (rectangle) covering the DNA band of interest was selected, and the intensity was calculated and expressed as photo-stimulated luminescence (PSL) units. The same sized rectangle was used to determine background, which was subtracted. The intensity measured from each band in a lane, resulting from EJ, was added and plotted.

Preparation of Cytoplasmic and Nuclear Extracts—Cytoplasmic and nuclear extracts were prepared as described earlier (46). Briefly, K562, CEM, and REH cells were washed with PBS and lysed in lysis buffer (10 mM Tris–HCl (pH 7.5), 2 mM MgCl2, 3 mM CaCl2, and 320 mM sucrose supplemented with protease inhibitors and 1 mM DTT). The cytoplasmic proteins were separated by centrifugation. Nuclear proteins were extracted using buffer (20 mM HEPES (pH 7.7), 1.5 mM MgCl2, 420 mM NaCl, 0.2 mM EDTA, and 25% glycerol along with protease inhibitors and 1 mM DTT). The extracts were stored at −80 °C until use. The purity of cytoplasmic and nuclear extracts was tested by immunoblotting using the markers α-tubulin and histone 3, respectively.

T7 Exonuclease and XhoI Digestion—T7 exonuclease digestion was performed by incubating purified EJ products with either increasing concentrations or 5 units of T7 exonuclease (New England Biolabs) at 25 °C for 2 h. In some cases, a fraction of EJ products was digested with XhoI (4 units) (37 °C for 4 h) prior to T7 exonuclease digestion. The products were then resolved by 8% denaturing PAGE, which was dried and exposed.

PCR Amplification, Cloning, and Sequencing of EJ Junctions—The EJ products of interest were cut out from PAGE, and DNA was eluted in a solution containing Tris (10 mM), EDTA (1 mM) and NaCl (0.5 M). The purified DNA was then used for PCR
amplification of EJ junctions using the primers VK24 and SS3. Gel-purified PCR products were cloned into TA vector, and the presence of insert was verified by restriction enzyme digestion and DNA sequencing (Macrogen Inc.).

**Immunoblotting**—For immunoblotting analysis, ~20 μg of protein was resolved on 8–10% SDS-PAGE (47). Following gel electrophoresis, proteins were transferred to PVDF membrane (Millipore), blocked with 5% skimmed milk powder, and probed with appropriate primary antibodies against KU70, Ku80, DNA-PKcs, XRCC4, LIGASE IV, ARTEMIS, polymerase λ, pATM, p53, MRE11, RAD50, and NBS1 and secondary antibodies as per standard protocol. The blots were developed using chemiluminescent detection solution (Immobilon™ Western, Millipore) and scanned by a gel documentation system (LAS 3000, Fuji).

**Immunoprecipitation**—Protein G-agarose beads (Sigma) were activated by immersing in water, followed by incubation in immunoprecipitation buffer (0.5 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.2% Nonidet P-40) for 30 min on ice. The activated beads were incubated with anti-BCL2 or anti-Ku80 antibody at 4 °C for 5–8 h to generate antibody-bead conjugate. It was then pelleted, washed, and incubated with cell-free extract at 4 °C overnight. The supernatant was collected, and the immunoprecipitation was verified by Western blot analysis. BCL2-depleted cell-free extracts were used for the EJ assay.

**Fractionation of Cell-free Extracts**—For fractionation of cell-free extracts by size exclusion chromatography, a Bio-Gel P100 column (Bio-Rad) was used. Approximately, 2 mg of CEM cell extracts were used for fractionation as per the manufacturer’s protocol. The column was washed and equilibrated with dialysis buffer. The protein was loaded and allowed to separate based on molecular size. Fractions were collected, concentration of protein was estimated, and fractions were stored at −80 °C.

Fractions containing partially purified BCL2 protein (fractions 15–17) were mixed in a ratio of 1:1:1, and increasing concentrations were added (20, 40, and 60 μg) to K562 (2.5 μg) cell-free extracts (incubated for 30 min on ice). EJ assay was performed on compatible and noncompatible ends as described above.

**Cloning, Overexpression, and Purification of BCL2 Protein**—BCL2 was amplified from mammalian expression vector, NIH_MGC_92 (Open Biosystems) using primers MS50 and MS51 (supplemental Table 1). The fragment was digested with Nhel and XhoI, gel-purified, and ligated to pET28a following digestion with same enzymes. The clone was sequenced, and deletions of the C-terminal 70 amino acids was confirmed. For BCL2, the C-terminal truncation is necessary for the protein to be in the soluble fraction.

Human BCL2 was expressed in *Escherichia coli*. In brief, plasmid pET28a-His<sub>6</sub>-BCL2 was transformed into *E. coli*, BL21(DE3) and induced to express His<sub>6</sub>-BCL2 fusion protein at 37 °C with 0.2 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h. The cells were harvested and resuspended in lysis buffer (50 mM phosphate buffer (pH 8), 0.3 mM NaCl, 0.1% Triton X-100) and incubated on ice for 30 min following the addition of 1 mg/ml lysozyme and PMSF. The cells were sonicated on ice and centrifuged at 12,000 × g for 30 min at 4 °C. The supernatant was collected and loaded on Ni<sup>2+</sup>-NTA resin (Novagen). The protein was eluted with elution buffer (0.33 M imidazole in 0.3 M NaCl and 20 mM Tris-HCl (pH 7.9)). The elute was collected and dialyzed (25 mM HEPES-KOH (pH 7.9), 0.1 mM KCl, 12 mM MgCl<sub>2</sub>, 1 mM EDTA, 2 mM DTT, and 17% glycerol). The purity of recombinant protein was checked by PAGE, and the concentration was determined by the Bradford method. Finally, the purified recombinant protein was stored at −80 °C.

**BCL2 Overexpression in K562 Cells**—Approximately 20 μg of BCL2 expression vector (NIH_MGC_92) was transfected by electroporation using a Gene Pulser X-Cell (Bio-Rad) into 3 × 10<sup>6</sup> cells at 875 V and 50 microfarads. Following transfection, the cells were grown in RPMI containing 10% FBS. Cells were harvested after 48 h, and cell-free extracts were prepared. The EJ assay was carried out with 5 μg of protein on 5’ overhangs and 5’-5’ noncompatible ends, as mentioned above.

**Immunostaining**—Cells were washed with PBS, fixed in 4% paraformaldehyde (5 min), and permeabilized with 0.1% Triton X-100 (10 min) at room temperature. BSA (0.1%) was used for blocking (30 min) and subsequently incubated with appropriate primary antibody at 4 °C overnight. FITC- and phycoerythrin-conjugated secondary antibodies raised in goats and mice, respectively, were used for detection of the signal. After washing, the cells were stained with DAPI, mounted, and observed under a fluorescence microscope using specific filters for fluorescein (NIKON). In selected cases, slides were observed under a confocal laser-scanning microscope (Olympus).

**RESULTS**

Efficiency of EJ Varies among Cancer Cell Lines—Cell-free extracts were prepared from various cancer cell lines derived from patients of chronic myelogenous leukemia (K562), T-cell leukemia (CEM), B-cell leukemia (REH and NALM<sub>6</sub>), Burkitt’s lymphoma (RAJI and DAUDI), lung cancer (A549), and cervical cancer (HeLa). GM00558B, a lymphoblastoid cell line derived from a healthy individual, was used as control.

The different steps involved during the EJ assay and four different substrates used for assaying EJ are outlined (supplemental Fig. 1). Integrity of the substrates was confirmed by PI nuclease digestion (supplemental Fig. 2). To compare EJ efficiency in different cell lines, protein concentration was estimated by Bradford assay and SDS-PAGE (supplemental Fig. 3,A–D). The overall protein profile in all cell lines was comparable. For the end joining assay, 5 μg of cell-free extracts was used, based on the protein titration assay (data not shown). Time-kinetics studies showed that the optimum joining activity was at around 2 h (compatible ends) or 4 h (noncompatible and blunt ends) of incubation (data not shown).

First, we checked the joining efficiency of compatible ends using the cell-free extracts from different cancer cell lines. For this, we incubated a 75-bp double-stranded oligonucleotide containing 5’ overhangs with different cell extracts for 2 h in EJ buffer. Upon analysis of purified joined products on a denaturing PAGE, we found that the extracts catalyzed end-to-end joining of the substrates, resulting in dimerization, trimerization, and other levels of multimerization (Fig. 1A). In addition to the above joined products, bands were also visible between substrate and dimer bands, which were identified as circular products (see below) (Fig. 1A). The overall efficiency of joining varied among the different cancer cell extracts (Fig. 1, E and I).
Similar to normal cell extracts, K562, DAUDI, and RAJI cells showed higher levels of joining (Fig. 1, E and I). CEM, REH, and RAJI cells on DNA substrates containing 5' compatible ends (A), blunt ends (B), 5'-3' noncompatible ends (C), and 5'-3' noncompatible termini (D). In all panels, M represents [γ-32P]ATP-labeled 50-nt ladder. E–L, bar graphs showing quantitation of the end-joined products resulting from various cancer cell lines. EJ products from multiple experiments were quantitated using MultiGauge and plotted. Error bars, S.E. The intensity of bands is expressed as PSL units. Cell lines used are indicated. Shown is a comparison of EJ efficiency based on multiple experiments for GM00552B, K562, CEM, REH, and RAJI cells on DNA substrates containing 5' compatible ends (E), blunt ends (F), 5'-5' noncompatible ends (G), and 5'-3' noncompatible termini (H). The bar graphs show EJ efficiency in A549, DAUDI, HeLa, and NALM6 on 5' compatible ends (I), blunt ends (J), 5'-3' noncompatible ends (K), and 5'-3' noncompatible termini (L).

**FIGURE 1.** Comparison of EJ of 5' overhang blunt and noncompatible ends in different cancer cell lines. For the EJ assay, 5 μg of extracts were incubated with respective [γ-32P]ATP-labeled oligomeric DNA substrate for 2 h or 4 h at 37°C. The reaction products were deproteinized, precipitated, dissolved, and resolved on 8% denaturing PAGE. In the case of substrate alone, DNA was incubated in the reaction buffer and loaded. A–D, comparison of the EJ profile of GM00552B, K562, CEM, REH, and RAJI cells on DNA substrates containing 5' compatible ends (A), blunt ends (B), 5'-3' noncompatible ends (C), and 5'-3' noncompatible termini (D). In all panels, M represents [γ-32P]ATP-labeled 50-nt ladder. E–L, bar graphs showing quantitation of the end-joined products resulting from various cancer cell lines. EJ products from multiple experiments were quantitated using MultiGauge and plotted. Error bars, S.E. The intensity of bands is expressed as PSL units. Cell lines used are indicated. Shown is a comparison of EJ efficiency based on multiple experiments for GM00552B, K562, CEM, REH, and RAJI cells on DNA substrates containing 5' compatible ends (E), blunt ends (F), 5'-5' noncompatible ends (G), and 5'-3' noncompatible termini (H). The bar graphs show EJ efficiency in A549, DAUDI, HeLa, and NALM6 on 5' compatible ends (I), blunt ends (J), 5'-3' noncompatible ends (K), and 5'-3' noncompatible termini (L).

Similar to normal cell extracts, K562, DAUDI, and RAJI cells showed higher levels of joining (Fig. 1, E and I). CEM, REH, NALM6, and HeLa extracts exhibited poor joining (Fig. 1, E and I). Thus, studies using 5' compatible termini showed that all cancer cell extracts were able to catalyze joining, albeit with different efficiencies.

We have also used oligomeric DNA substrates containing blunt or noncompatible ends to mimic the endogenous DSBs within cells (supplemental Fig. 1B). Noncompatible termini cannot be joined by simple ligation because the overhangs at both ends of the substrates are nonligatable; hence, joining of such ends can take place only by EJ. In the present study, we have used two types of noncompatible ends, 5'-5' and 5'-3' overhangs (supplemental Fig. 1B). Results showed that cell-free extracts of GM00552B, K562, CEM, and RAJI were capable of joining the blunt ends (Fig. 1B, lanes 2–6). However, in all the cases, joining was weak and resulted only in dimer, of which REH showed least joining (Fig. 1, B and F). Similarly, the efficiency of blunt end joining was weak, even when A549, DAUDI, HeLa, and NALM6 cell extracts were used for the study. However, among the cell lines studied, DAUDI showed the highest blunt end joining activity (Fig. 1J). Thus, results suggest that
blunt end joining catalyzed by cancer cells is less efficient as compared with 5’ compatible overhangs (Fig. 1, compare A, E, and I with B, F, and J), which was also consistent with the joining activity exhibited by T4 DNA ligase (data not shown). Further, restriction enzyme digestion analysis showed that most of the joined junctions were recleavable (data not shown), suggesting that joining occurred with minimum or no modifications.

DNA end joining of 5’-5’ and 5’-3’ noncompatible overhangs was tested by incubating the respective [γ-32P]ATP-labeled substrates with cancer cell extracts. Results showed that overall efficiency of joining was lower in the case of noncompatible ends as compared with compatible ends (Fig. 1, compare A with C with D). Comparison of joining efficiency in cell lines using both of these substrates indicated that GM00558B, K562, RAJI, A549, and DAUDI cell-free extracts possessed higher EJ activity (Fig. 1, G, H, K, and L). Interestingly, the efficiency of joining was less in the case of CEM, REH, HeLa, and NALM6 (Fig. 1, G, H, K, and L). Hence, these results suggest that joining efficiency of compatible ends was higher compared with noncompatible or blunt ends in all of the cancer cell extracts. Further, the efficiency of joining varied among cancer cell lines irrespective of the termini.

Cell-free Extracts from Cancer Cell Lines Catalyze DNA Circularization—We observed 1–2 additional bands between DNA substrates and 150-bp dimer products (Fig. 1A). To identify the nature of the bands, we carried out T7 exonuclease digestion of these reaction products (Fig. 2A). T7 exonuclease is known to act in the 5’–3’ direction on a linear DNA but not on circular DNA. End joining reaction products resulting from K562 extracts were treated with increasing concentrations (0.1–10 units) or 5 units of T7 exonuclease at 25 °C for 2 h and resolved on an 8% denaturing PAGE. Results showed that T7 exonuclease was able to digest the bands corresponding to the substrate and linear dimer DNA but not the one in between (Fig. 2B, lanes 2 and 3) (data not shown), suggesting the circular nature of the products. In order to confirm the results, we first linearized the joined products by Xhol digestion and then subjected them to T7 exonuclease treatment. Results showed that following digestion with Xhol, even the circularized band was sensitive to T7 exonuclease (Fig. 2B, lanes 4 and 5). Interestingly, one of the circular bands (Form I) was sensitive neither to Xhol nor to T7 exonuclease, possibly because of inherent distortion of each nucleotide due to circularization of apparently a 75-mer (Fig. 2B, lanes 4 and 5). Therefore, the results suggest that extracts were able to catalyze circularization of DNA substrates along with multimerization with different efficiencies (Fig. 2B).

To check whether the efficiency of joining could be enhanced by the addition of a phosphate group to the oligomers (commercially synthesized oligomers possess an OH group at both the 3’- and 5’-ends), TSK1 was labeled with [γ-32P]ATP, whereas TSK2 was phosphorylated using cold ATP. Both TSK1 and TSK2 were then annealed and used for the study. Upon incubation with extracts of cancer cell lines and GM00558B, we could not find any difference in the joining efficiency between phosphorylated and unphosphorylated substrates (data not shown).

Amplification, Cloning, and Sequencing of EJ Junctions—In order to characterize the EJ junctions derived from noncompatible ends from different cancer cell lines, we PCR-amplified, cloned, and sequenced the joined junctions (Fig. 3). Sequencing results showed a number of modifications at the junctions in both types of termini used, based on which clones were categorized as M1, M2, M3, or M4 (supplemental Fig. 4).

The results showed that in the case of K562 cells, among 18 EJ junctions of 5’-5’ noncompatible termini analyzed, all had deletions (Fig. 3A). Whereas all clones showed deletions from the left side of the DSB (three had longer deletions of 27 and 28 bp), 11 showed a deletion from the right side (eight clones with longer deletions) (Fig. 3A, M1 and M4). Three of the junctions utilized 2-nt microhomology (Fig. 3A, M2). These results suggest that 5’-5’ noncompatible end joining in K562 cells took place with various modifications from both termini. In the case of the EJ junction resulting from 5’-3’ ends, seven of eight clones had deletions, and in all those cases, a deletion was seen from the right side, of which four had longer deletions (Fig. 3A, M1, M2, and M4). Three of eight clones joined with 2- or 1-nt microhomology (Fig. 3A, M2). Three clones were with novel insertions (Fig. 3A, M1 and M2). In one of the clones, joining occurred without any deletions (Fig. 3A, M3).

In the case of EJ junctions resulting from CEM cells, the 5’-5’ noncompatible substrates showed deletions in all 13 clones sequenced (12 and 9 each had deletions from the left and right side, respectively); however, the longer deletions were rare (Fig. 3B). Microhomology-mediated joining was seen in six of 13 clones (Fig. 3B, M2, and supplemental Fig. 4, M2). Seven of 12 clones joined with deletion but did not use any microhomology (Fig. 3B, M4). The M1 mode of joining was observed in two
clones (Fig. 3B, M1). None of the clones showed any insertion in the case of junctions resulting from 5’-3’ overhangs, although end filling of one of the termini was seen in all clones (Fig. 3C, M3). This indicates that single strand ligation of overhangs, followed by gap filling, could be the mechanism of EJ in these junctions (supplemental Fig. 4, M3).

In the case of EJ junctions of 5’-5’ noncompatible ends derived from REH cells, deletions were seen in all cases (14 of 14 and six of 14 had deletions from the left and right side, respectively) (Fig. 3C). End filling was seen at one of the termini (eight of 14 molecules); however, we did not find any novel insertions at all (Fig. 3C, M1). Five of 14 molecules joined using a micro-homology-mediated mechanism (Fig. 3C, M2). In the case of 5’-3’ end joining junctions, four of nine molecules had at least one end deleted (M2 and M1). Five of nine molecules joined using the M3 mode of EJ (Fig. 3C and supplemental Fig. 4, M3).

In the case of junctions resulting from 5’-5’ noncompatible ends of GM00558B cells, deletion of one of the overhangs was noted in all of the clones, whereas three of 11 had deletions from other termini (Fig. 3D). Eight of 11 clones used the M1 mode of joining, whereas others used M2 or M4 (Fig. 3D).

**Expression of NHEJ Proteins Do Not Correlate with Altered EJ Efficiency**—In order to understand the mechanism by which various cancer cell lines differ in their joining efficiency, we tested the expression of NHEJ proteins in respective cell extracts by Western blotting following equalization of the amount of protein (Fig. 4A). We have used GM00558B, K562, CEM, REH, and RAJI cells for the study. First, we checked the expression of DNA end-binding proteins, KU. Interestingly, we find that expression of both KU70 and KU80 was comparable in all of the cancer cell lines; however, we did not find any novel insertions at all (Fig. 3C, M1). Five of 14 molecules joined using a micro-homology-mediated mechanism (Fig. 3C, M2). In the case of 5’-3’ end joining junctions, four of nine molecules had at least one end deleted (M2 and M1). Five of nine molecules joined using the M3 mode of EJ (Fig. 3C and supplemental Fig. 4, M3).

In the case of junctions resulting from 5’-5’ noncompatible ends of GM00558B cells, deletion of one of the overhangs was noted in all of the clones, whereas three of 11 had deletions from other termini (Fig. 3D). Eight of 11 clones used the M1 mode of joining, whereas others used M2 or M4 (Fig. 3D).
GM00558B cells. In all of the cell lines except GM00558B, pATM was detectable, although with varying intensities. Expression of p53 was undetectable in the case of K562, whereas it was ∼2.5-fold higher in other cancer cells compared with GM00558B. We have also checked the expression of MRN complex (MRE11, RAD50, and NBS1), which is known to act as a nuclease during both EJ and homologous recombination. Results showed that GM00558B and K562 express higher levels of MRN complex compared with other cancer cells. The expression of MRE11, RAD50, and NBS1 was low in the case of REH, RAJI, and CEM cells (Fig. 4, A and B). The above results show that although there is some variation in the expression levels of the repair proteins between cancer cells, no direct correlation can be drawn between EJ efficiency and expression of these proteins.

Expression of Anti-apoptotic Protein, BCL2, Differs between Cancer Cell Lines—In a recent report, it was shown that BCL2 protein interacts with KU70 and KU80, and it was proposed that such an interaction may affect NHEJ (40). Therefore, we wondered whether the expression of BCL2 in cancer cells could be correlated with the EJ efficiency. Interestingly, we found that CEM, REH, and NALM6 had the highest BCL2 expression, followed by RAJI and HeLa (Fig. 5, A and B). K562, A549, DAUDI, and GM00558B had very low levels of BCL2 (Fig. 5, A and B). Therefore, our results indicate that the levels of BCL2 expression may be a factor affecting the efficiency of EJ because the cancer cell lines with higher BCL2 expression showed lower EJ efficiency (Fig. 5, A–C).

BCL2 Expression Is Detectable in the Nuclear Membrane—To unravel the possible mechanism by which BCL2 could be involved in EJ, expression of BCL2 at the subcellular level was assessed. Because EJ operates in the nucleus, it was obvious to check for the expression of BCL2 in the nucleus and its interaction, if any, with EJ protein KU70 or KU80. Because BCL2 expression was highest in CEM cells, we chose it for further studies. To compare the expression of BCL2 in different subcellular compartments, cytoplasmic and nuclear extracts were prepared from CEM cells, concentration was determined, and proteins were resolved on SDS-PAGE. Results showed that the protein profile differed among the extracts, although overall the protein loaded was comparable (Fig. 5D). Western blot analysis showed differential localization of BCL2 in the whole cell, cytoplasmic, and nuclear extracts (Fig. 5, E and F). Interestingly, we observed the presence of BCL2 in the nuclear extracts, although it was several-fold lower (Fig. 5, E (lane 3) and F). When the same blot was stripped and used for hybridization with anti-KU70, we found that expression of KU70 was mostly in the nuclear extract, as expected (Fig. 5E, lane 3). Sim-
ilar experiments were also repeated with K562 and REH cells (Fig. 5G and H). Results showed a consistent lower expression of BCL2 in the nuclear fraction compared with its abundance in the cytoplasmic extracts (Fig. 5G). In the case of K562, where BCL2 expression was low, its presence in the nucleus was also undetectable (Fig. 5G). Histone 3 and α-tubulin were used as nuclear and cytoplasmic loading controls, respectively (Fig. 5H). Thus, the results confirmed the presence of BCL2 in the nucleus.

We further performed immunostaining to confirm the presence of BCL2 in the nucleus of cells. Based on the above data we selected K562, CEM, and REH cell lines for the study. Results showed that BCL2 expression was greater in CEM and REH cells as compared with K562 cells (Fig. 5I). BCL2 expression was also seen in the nucleus, mostly localized to the nuclear membrane, besides the cytoplasm. The cells with
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FIGURE 6. EJ in fractionated extracts of CEM cells. A, SDS-PAGE profile of fractionated extracts. Approximately 2 mg of cell-free extract was separated by size exclusion chromatography on a Bio-Gel P100 column. The fractionated samples were resolved on a 10% SDS-polyacrylamide gel, stained, and visualized. B, Western blot analysis for BCL2 and different EJ proteins in the fractions described in A. Fractions 10–17 were used for further study along with the whole cell extract. Refer to the Fig. 4 legend for further details. C–F, bar graph showing the efficiency of EJ (quantitated and presented in PSL units) in various fractions when oligomeric DNA with different DSBs were used. C, EJ of 5’ compatible ends; D, blunt ends; E, 5’-5’ noncompatible ends; F, 5’-3’ noncompatible ends. An EJ assay was performed using 1 μg of protein from fractions 10–17 and whole cell extracts (C).

secondary antibody alone were used as control (Fig. 5I). Confocal microscopy studies on CEM cells further confirm the presence of BCL2 in the nuclear membrane, although at a low level (data not shown). Thus, data show that BCL2 is also present in the nucleus, which is consistent with the previous studies.

**EJ in Fractionated Cell-free Extracts of CEM**—In order to check whether BCL2 expression in cancer cells interferes with EJ, depletion of BCL2 from the extracts was carried out by using two different methods, fractionation and immunoprecipitation. Because the BCL2 protein is 25 kDa and EJ proteins are 37 kDa or above, size exclusion chromatography was used for the separation of BCL2. Protein fractions of CEM cells were collected and resolved on SDS-PAGE to confirm the separation based on molecular weight (Fig. 6A). Results showed that most of the proteins were eluted between fractions 10 and 17 (Fig. 6A) (data not shown). The presence of EJ proteins in the fractions was analyzed by Western blotting. Results showed that all of the EJ proteins studied were eluted out between fractions 10 and 13 (Fig. 6B). Fractions 15–17 showed the presence of BCL2, which can be explained because it is a small protein (Fig. 6B). We could also observe low levels of BCL2 in fractions 10 and 11 (Fig. 6B), which is not very surprising based on the report that BCL2 can form a complex with KU70 and KU80 (40).

Further, we used CEM fractions 10–17 for the EJ assay. All four DNA substrates (5’ overhang, blunt ends, 5’-5’, and 5’-3’ noncompatible ends) were used for comparing the EJ. In each case, ~1 μg of protein was used, and whole cell extracts were used as control. Results showed that fractions 10–15 were able to catalyze joining of compatible ends (Fig. 6C). Interestingly, in the case of fraction 12, where BCL2 was undetectable, maximum efficiency of joining was observed, and it was 40% higher than the control (Fig. 6C). An assay with blunt ends showed that the fractions from 11 to 14 catalyzed the end joining, and maximum efficiency was shown by fraction 11, which showed more than a 50% increase when compared with the whole cell extract (Fig. 6D). Similar results were found in the case of 5’-5’ overhangs (Fig. 6E). However, in the case of 5’-3’ overhangs, we could not find such an enhancement in activity after fractionation (Fig. 6F). Hence, our studies suggest that depletion of BCL2 protein from cancer cell lines could lead to elevated levels of EJ.

Immunoprecipitation was also used to deplete BCL2 protein from cell-free extracts of CEM. The removal of BCL2 was confirmed by Western blot analysis (Fig. 7A) and was used for an EJ assay on compatible and noncompatible ends. Results showed that immunodepletion of BCL2 led to an increase in the efficiency of joining in the case of 5’ overhangs, further confirming the above results (Fig. 7, B (lanes 1–3) and C). We could also observe such enhancement in the joining in 5’-5’ and 5’-3’ noncompatible termini (Fig. 7, B (lanes 4–9) D, and E). However, when similar experiments were performed with K562 extracts, we did not find such a difference in the EJ efficiency (data not shown). Therefore, various lines of experimentation suggest that, depending on the BCL2 expression, the EJ efficiency could be regulated in cancer cells.

**Addition of Partially or Completely Purified BCL2 Proteins** Down-regulated EJ in K562 and DAUDI Cell Extracts—We were interested in testing whether the addition of partially purified fractions of BCL2 protein from CEM cells to K562 cell-free extracts could inhibit the EJ. In order to test this, we pooled fractions 15–17 (Fig. 6B, lanes 7–9), and increasing concentrations of it were mixed with K562 cell-free extracts and incubated with compatible and 5’-5’ noncompatible ends. Interestingly, we found that the addition of BCL2 inhibited the joining process in both cases, although the circularization was unaltered in the case of the compatible ends (Fig. 8, A and B). This is understandable, given that circularization of compatible ends could be achieved by DNA ligase alone. These results show...
that the addition of partially purified BCL2 could decrease the joining efficiency of K562 cells. T7 exonuclease and XhoI digestion suggested that the band that was unaffected following the addition of BCL2 with compatible ends was indeed circular (Fig. 8C).

We have also used BCL2 protein, purified to homogeneity, to assess its role in EJ (supplemental Fig. 5). DAUDI cells, null for BCL2 protein, were used for this study. Increasing concentrations of purified BCL2 protein (5, 10, 25, 50, and 100 ng) were incubated with DAUDI cell extracts and used for EJ reaction on 5/-compatible and 5/-5’ noncompatible ends (supplemental Fig. 6). Results showed a concentration-dependent reduction of joining activity in the case of 5/-5’ noncompatible ends (Fig. 8E), whereas the effect was restricted to 25–100 ng when the compatible end was used as the substrate (Fig. 8D). Thus, the results suggest that the addition of BCL2 can indeed down-regulate the end joining activity in the cells where BCL2 is not present.

**Overexpression of BCL2 in K562 Cells Leads to Down-regulation of EJ**—The aforementioned results prompted us to test whether overexpression of BCL2 in vivo can affect EJ. K562 cells were transfected with BCL2 expression constructs by electroporation and harvested after 48 h, and cell-free extracts were prepared. Overexpression of BCL2 was confirmed by Western blot analysis (Fig. 9A, lanes 1 and 2). CEM extracts were used as positive control for BCL2 expression (Fig. 9A, lane 3). An EJ assay following BCL2 overexpression on 5’ overhangs and 5’-5’ noncompatible ends showed that efficiency of joining was reduced significantly upon BCL2 expression compared with control (Fig. 9B, lanes 2, 3, 5, and 6). In the case of compatible ends, joining efficiency decreased considerably, whereas for noncompatible ends, it decreased by 3-fold (Fig. 9, C and D). Thus, our results further demonstrate that BCL2 overexpression can down-regulate EJ even in those cells where otherwise an efficient EJ was present.

**BCL2 and KU Proteins Interact in CEM Cells**—Because the above studies have shown that BCL2 down-regulates EJ in CEM cells, we wondered whether BCL2 interacts with KU proteins. First, we tested for the presence of KU70/80 in CEM cells by immunostaining. Results showed that both KU70 and KU80 were expressed mostly in the nucleus of CEM cells (data not shown). In order to study the interaction between KU70 and BCL2 at the intracellular level, we performed colocalization experiments in CEM cells. We found that both BCL2 and KU70 colocalized, suggesting possible interaction between them (Fig. 10A). Further, we analyzed the interaction between BCL2 and KU70 proteins in CEM extracts by immunoprecipitation of BCL2, followed by probing with antibodies against KU. Results showed the presence of the KU70 in the pellet-containing beads, suggesting its interaction with BCL2 (Fig. 10B). This was further confirmed by reverse immunoprecipitation, wherein we could observe presence of BCL2 in the beads, when KU80 was pulled down (Fig. 10C). In addition to BCL2, we could also observe the presence of KU70 in the KU80 pulled-down pellet (Fig. 10C). Therefore, our data suggest that BCL2 interacts with KU proteins and down-regulates EJ in cancer cells.
DISCUSSION

Cancer Cell Lines Catalyze Inter- and Intramolecular Joining of DNA Substrates with Different End Structures—Among different DNA damage described in the literature, DSBs are considered the most deleterious because they affect the continuity of the genome (2). Therefore, failure of repair of DSBs can lead to genomic instability and apoptosis of the cells. Alternatively, it can also lead to chromosomal rearrangements, resulting in deletions, insertions, or chromosomal translocations, culminating in cancer (48). However, how DSBs are repaired when they occur in a cancer cell is a question of great importance. Most of the studies in different cancers have reported an impaired EJ (29–33). The mechanism that leads to the impairment of EJ is, however, unknown. There are also reports showing enhanced EJ activity in cancer cells (49), indicating that different cancer cells could have different levels of EJ.

We were interested to know whether cancer cells could have similar DNA end joining efficiency. Using an oligomer-based cell-free DSB repair system, in which joining of different DSBs could mimic in vivo breaks, we found that leukemia and lymphoma cell extracts catalyzed intermolecular end-to-end joining, resulting in dimer, trimer, tetramer, and different levels of multimers. Two of the carcinoma cell extracts tested also showed end joining, although the efficiency was poor. The majority of the joined products observed by us were consistent with earlier reports (23, 43, 50). In contrast to some of the earlier reports, we could also see the intramolecular circularization (23, 45, 51, 52). Although, by using T7 exonuclease, we could unambiguously prove formation of circular products (Fig. 2) (43), their identity of being circular monomer, dimer, or trimer is not quite clear. Interestingly, when T4 DNA ligase was used, we could not find such circularization on substrates with sticky or blunt ends (data not shown). This indicates that circularization on a 75-mer may need extra DNA bending factors, such as HMGB1, which are present in the extracts.

Efficiency of EJ Varies between Cancer Cell Lines, but the Mechanism Is Comparable—An EJ assay performed using various cancer cell lines showed that the efficiency of the joining
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**FIGURE 9. BCL2 overexpression in K562 cells and EJ assay.** A, Western blot showing BCL2 overexpression. Lane 1, K562; lane 2, BCL2-overexpressed K562; lane 3, CEM. α-Tubulin is used as a loading control. B, EJ assay following BCL2 overexpression. Protein extract was prepared from control and BCL2-overexpressed K562 cells. EJ assay was done with 5′ overhangs (lanes 1–3) and 5′-5′ noncompatible ends (lanes 4–6). C and D, bar graph showing the quantification of gels shown in B. +BCL2, BCL2 was overexpressed in K562 cells. Error bar, S.E.

BCL2 overexpression in cancer cells showed that BCL2 expression down-regulates EJ. This was observed in K562 and DAUDI cells, which overexpressed BCL2 protein. Surprisingly, we found that K562 and DAUDI, having higher EJ efficiency, had low levels of BCL2 (it is known that endogenous expression of BCL2 is less in K562 (61, 62); DAUDI is null for BCL2 (63)), whereas CEM, REH, and NALM6 cells, where BCL2 expression was very high, showed minimal EJ. Hence, our data showed that EJ efficiency is low in cancer cells where BCL2 is highly expressed (Fig. 5, A–C).

Because cancer cells are known to have higher levels of BCL2 (58–60), we wondered whether the observed difference in the efficiency of EJ could be attributed to the expression levels of BCL2 protein. Surprisingly, we found that K562 and DAUDI, having higher EJ efficiency, had low levels of BCL2. This was consistent with earlier studies (43, 53–55). Generally, the end processing at noncompatible ends could include exonuclease/endoendonuclease resection of ends, gap filling, and ligation by EJ proteins (56).

Sequence analysis of joined junctions from noncompatible termini indicated that the joining indeed utilized EJ because we could see the processing of ends and DNA synthesis at the junctions (Fig. 3). Extended deletions were rare, indicating that alternative NHEJ may not be a major mechanism in these neoplastic cell lines (9, 10). Microhomology-mediated end joining has also been reported (57). Junctional sequence analysis of 5′-5′ noncompatible termini revealed three independent mechanisms of repair (supplemental Fig. 4A, M1, M2, and M4). In one case, we could observe deletion of one overhang, followed by end filling of the second overhang and blunt end ligation (supplemental Fig. 4A, M1). In the second mechanism, a 1–3 nt microhomology region was exposed by exonuclease action, and DNA was synthesized, followed by processing of the flap region, possibly by ARTEMIS, finally leading to ligation (supplemental Fig. 4A, M2). In the third mechanism, joining occurred after deletion, however, no microhomology region was utilized (supplemental Fig. 4A, M4). Four independent mechanisms were observed based on DNA sequence analysis in the case of 5′-3′ noncompatible termini junctions (supplemental Fig. 4B).

In the first case, deletion of one overhang, followed by end filling of the second overhang and blunt end ligation, was the mechanism (supplemental Fig. 4B, M1). The second mechanism involves microhomology, as described above (supplemental Fig. 4B, M2). In the third mechanism, joining occurred after deletion, and the flaps were processed by gap filling and ligation (supplemental Fig. 4B, M3). In addition, joining followed by microhomology-independent deletion was also seen in the case of 5′-3′ noncompatible ends (supplemental Fig. 4B, M4).

**BCL2 Expression Down-regulates EJ in Neoplastic Cells**—We wondered whether the observed difference in the EJ could be accounted for by differential expression of DSB repair proteins in different cancers. However, the Western blot analysis showed that there is no consistent difference in the expression levels of major EJ proteins that can explain the observed differential joining in the different cancer cell lines studied (Fig. 4).

Because cancer cells are known to have higher levels of BCL2 (58–60), we wondered whether the observed difference in the efficiency of EJ could be attributed to the expression levels of BCL2 protein. Surprisingly, we found that K562 and DAUDI, having higher EJ efficiency, had low levels of BCL2 (it is known that endogenous expression of BCL2 is less in K562 (61, 62); DAUDI is null for BCL2 (63)), whereas CEM, REH, and NALM6 cells, where BCL2 expression was very high, showed minimal EJ. Hence, our data showed that EJ efficiency is low in cancer cells where BCL2 is highly expressed (Fig. 5, A–C). However, it is important to point out that BCL2 is largely a membrane protein. Hence, the “active” concentration of BCL2 in cancer extracts is difficult to determine or define, and it may be influenced by the amount of lipid or other amphipathic surfaces in the extracts.

We also observed that immunoprecipitation of BCL2 from the CEM cells led to enhanced EJ activity (Fig. 7). An EJ assay of...
fractionated cell-free extracts also showed that the fractions containing high molecular weight proteins, in which BCL2 was excluded, were proficient in end joining (Fig. 6). Interestingly, we also found that overexpression of BCL2 in K562 cells, which show higher EJ, led to down-regulation of joining. This finding was further strengthened when partially purified BCL2 proteins were added to K562 cell extracts or purified protein was added to DAUDI cell extracts, leading to a decrease in joining. This finding further indicates that BCL2 down-regulates the efficiency of EJ. Thus, our study suggests that BCL2 can regulate the EJ pathway in cancer cells, which could lead to increased genomic instability. Previous studies on T-cell and B-cell leukemia have shown that such cancers possess elevated levels of various secondary chromosomal abnormalities (64, 65).

It is believed that the cell-free extract catalyzed EJ may account for classical NHEJ, alternative NHEJ, and SSA. Hence, the observed interesting BCL2 effect on EJ could be due to a cumulative effect on these pathways, within the extracts. However, the cancer cell lines studied only utilized a maximum of 3 nt of microhomology, which suggest that most of the end-joined junctions could be the result of NHEJ itself. The other two mechanisms, even if present, could account only for a limited number of junctions. It is also important to point out that the DNA substrates used in the study could be a limitation for exploring the effect of BCL2 on SSA or alternative NHEJ, which needs to be further investigated.

It has also been shown that overexpression of BCL2 in the H1299 cell line along with RAG expression vectors leads to down-regulation of V(D)J recombination, presumably due to failure of NHEJ (64, 65). In contrast, another study showed that overexpression of BCL2 in pro-B cell lines had no effect on the V(D)J recombination efficiency or fidelity (66). The observed discrepancy was attributed to either the different cells and species used, the nature of BCL2 expression, or other factors (66). Interestingly, in our studies, we find a low level of EJ in the pre-B cell line, REH, which expresses higher endogenous levels of BCL2. However, more studies are required to link the EJ efficiency and V(D)J recombination in these cells. More recently, it has been shown that one of the mechanisms to increase resistance to DNA damage-induced cell death in multipotent hair follicle bulge stem cells is by higher expression of the anti-apoptotic gene BCL2, which in turn results in an up-regulation of DNA-PKcs-mediated EJ (41). Hence, it appears that BCL2 may play contrasting roles in cancer cells and stem cells, which need to be explored further.

**BCL2 Interacts with KU Proteins to Regulate EJ in Cancer Cells**—Earlier, using purified proteins, it was shown that BCL2 could interact with KU proteins, and it was suggested that this may prevent binding of KU to the DNA ends (40). Because BCL2 is an inner mitochondrial membrane protein, we wondered how BCL2 would interact with KU proteins, which are abundantly present in the nucleus. Western blot analysis showed that BCL2 is present in the nuclear extract, although only at low levels. In addition, expression of BCL2 was also detectable in the nuclear membranes by immunostaining in cancer cell lines where EJ was weaker. Recent reports also showed that BCL2 could be translocated into the nucleus after irradiation of cells (40). By using colocalization experiments and coimmunoprecipitation studies, we further showed that BCL2 and KU proteins can interact within the cells. Therefore, our study indicates that BCL2 is present in the nucleus even when cells are not exposed to exogenous DNA-damaging agents.

In conclusion, our study showed that the efficiency of EJ in cancers could be regulated by the anti-apoptotic protein BCL2. However, it may not affect the mechanistic aspect of EJ. BCL2 instead may interfere with EJ by sequestering KU and preventing it from binding to DNA ends. Alternate pathways by which BCL2 regulates EJ need to be explored.

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