BCL2 Suppresses PARP1 Function and Nonapoptotic Cell Death


Abstract

BCL2 suppresses apoptosis by binding the BH3 domain of proapoptotic factors and thereby regulating outer mitochondrial membrane permeabilization. Many tumor types, including B-cell lymphomas and chronic lymphocytic leukemia, are dependent on BCL2 for survival but become resistant to apoptosis after treatment. Here, we identified a direct interaction between the antiapoptotic protein BCL2 and the enzyme PARP1, which suppresses PARP1 enzymatic activity and inhibits PARP1-dependent DNA repair in diffuse large B-cell lymphoma cells. The BH3 mimetic ABT-737 displaced PARP1 from BCL2 in a dose-dependent manner, reestablishing PARP1 activity and DNA repair and promoting nonapoptotic cell death. This form of cell death was unaffected by resistance to single-agent ABT-737 that results from upregulation of antiapoptotic BCL2 family members. On the basis of the ability of BCL2 to suppress PARP1 function, we hypothesized that ectopic BCL2 expression would kill PARP inhibitor–sensitive cells. Strikingly, BCL2 expression reduced the survival of PARP inhibitor–sensitive breast cancer and lung cancer cells by 90% to 100%, and these effects were reversed by ABT-737. Taken together, our findings show that a novel interaction between BCL2 and PARP1 blocks PARP1 enzymatic activity and suppresses PARP1-dependent repair. Targeted disruption of the BCL2–PARP1 interaction therefore may represent a potential therapeutic approach for BCL2-expressing tumors resistant to apoptosis. Cancer Res; 72(16): 4193–203. ©2012 AACR.

Introduction

Many tumors harbor genetic, epigenetic, or posttranscriptional alterations that result in overexpression of the antiapoptotic protein BCL2 (1–3). In these tumors, BCL2 inhibits apoptosis from a number of stresses, including DNA damage, microtubule perturbation, and oncogene activation (4), by binding the BH3 (Bcl-2 homology-3) domain of proapoptotic factors. Small molecules that occupy the BH3-binding pocket of BCL2, such as ABT-737 (5), are under development for the treatment of malignancies dependent on BCL2 (6, 7). These agents function by disrupting the interaction between BCL2 and proapoptotic BH3 domain–containing proteins, such as BIM. Upon displacement, certain proapoptotic proteins can initiate oligomerization of the apoptosis effector proteins BAX and BAK.

Cells can overcome dependence on BCL2 in 3 ways (8). First, they can upregulate other antiapoptotic factors through cell-autonomous or non–cell-autonomous processes. For example, long-term selection of BCL2-overexpressing diffuse large B-cell lymphoma (DLBCL) cells in the presence of ABT-737 promotes resistance through upregulation of the antiapoptotic BCL2 family members MCL1 and/or BFL1 (9). Second, cells can downregulate proapoptotic signaling through changes in either gene expression or posttranslational modifications. Third, cells can lose BAX and/or BAK and thereby become incapable of orchestrating intrinsic apoptosis. Each of these mechanisms can confer broad resistance to apoptosis induced by various stimuli, including DNA-damaging agents and microtubule binders (10, 11).

Essentially, all cases of follicular lymphoma and many cases of DLBCL, chronic lymphocytic leukemia (CLL), and other subsets of non–Hodgkin lymphoma depend on BCL2 for survival (8). In the upfront setting, these diseases are broadly sensitive to an array of antineoplastic agents. However, relapse after treatment with one or more therapeutic regimens is commonly associated with resistance to multiple classes of chemotherapy as a consequence of epigenetic, genetic, and/or posttranslational modifications in the intrinsic pathway of apoptosis. Even in cells lacking autonomous resistance to apoptosis, bone marrow and lymphoid node stroma provide attachment sites and surface bound growth factors as survival signals that reduce the apoptotic response to a broad range of chemotherapies (12, 13).
Novel approaches are clearly needed for inducing death in tumor cells with resistance to apoptosis. Here, we identify a novel mechanism for target nonapoptotic cell death that is dependent on PARP1 and suppressed by BCL2.

Materials and Methods
These studies were approved by the Dana-Farber Cancer Institute (DFCI; Boston, MA) Institutional Review Board. All P values were calculated by 2-sided t test using GraphPad software. Additional details are provided in the Supplementary Methods.

Cell culture and reagents
HT, Toledo, and OCI-LY8 were provided by Margaret Shipp. OCI-Ly1-7R, OCI-Ly1-10R, 1863, and 3256 were previously described (14, 15). Cell lines were not authenticated before these studies. N-Methyl-N’-nitro-N-nitosoguanidine (MNNG), etoposide, and hydrogen peroxide were purchased from Sigma. BCL2-DKO cells were generated from a pool of DKO cells transfected with pcDNA3.1-BCL2 and selected in 1 μg/mL G418 (Gold Biotechnology). ABT-737 and ABT-888 were kindly supplied by Abbott Laboratories.

Determination of NAD and ATP concentrations
Mouse embryonic fibroblasts (MEF) were treated 30 minutes with 500 μmol/L MNNG in the presence of ABT-737, ABT-888, DMSO, or combinations. MTT (Sigma) and CellTitre-Glo Luminescent Cell Viability Assay (Promega) were used to determine cellular NAD and ATP, respectively, using SoftMax Pro5.

ELISA
Increasing concentrations of purified glutathione S-transferase (GST)-BCL2 were added to fractionated lysates from HT cells. Similarly, increasing concentrations of ABT-373 were added to fractionated lysates from OCI-Ly1-10R. The protein concentration of each fraction was measured using the bicinchoninic acid method (Thermo-Fisher). PARP1 enzymatic activity on immobilized histones was measured using an ELISA (Trevigen).

Comet assay
OCI-LY1-10R cells were treated with 50 μmol/L MNNG for 15 minutes. Cells were collected before MNNG treatment (pre), immediately after washing out MNNG (post-MNNG), or after washing followed by 3-hour treatment with DMSO, ABT-888 (100 nmol/L), or ABT-737 (100 nmol/L). Cells were subjected to the alkaline comet assay (Trevigen).

Immunofluorescence
Cells were fixed in 4% paraformaldehyde for 30 minutes at room temperature and permeabilized for 10 minutes in 5% bovine serum albumin (BSA)/PBS containing 0.25% Triton X-100. Cells were washed with PBS containing 0.02% Triton X-100 and incubated with antibodies diluted in 2.5% BSA/PBS containing 0.1% Triton X-100 overnight. Images were captured on a Zeiss Axio Observer.A1 fluorescence microscope. Confocal images were captured on a Zeiss 710 laser scanning confocal microscope and analyzed using ImageJ software.

Primary CLL cell culture
The day before the experiment, 5 × 10^4 human stromal cells per well were seeded onto 48-well plates. CLL cells were collected from peripheral blood, isolated by Ficoll separation, and viably frozen in liquid nitrogen. On the day of the experiment, the CLL cells were thawed and added to the stromal culture at 20:1 ratio. For comparison, CLL cells were also cultured in suspension at 3 × 10^6 cells/mL. For assessment of stroma-derived resistance, 50 μmol/L MNNG, 100 nmol/L ABT-737, and/or 200 nmol/L ABT-888 were added 24 hours later. At the indicated time points, CLL cells were collected by gently pipetting off the CLL cells. After appropriate treatments, cells were washed once with PBS and resuspended in PBS containing 1% FBS and FcR blocking reagent. Cells were stained with Pacific blue-CD19 and APC-CXCR4 antibody to differentiate B cells from stromal cells. Cells were resuspended in 50 μL of Annexin-binding buffer and stained with Annexin V-FITC and PI. Cell numbers were quantified after the addition of 2,500 Fluoresbrite-counting beads (Polyscience) per sample. Mitochondrial sensitivity was determined by BH3 profiling, as described (11).

BCL2 transduction colony assay
Cells were plated in 6-well plates (1,000 cells per well) the day before the retroviral infection. The cells were transduced and 24 hours later and were selected in 1 μg/mL G418 (Gold Biotechnology). After 24 hours, cells were washed and incubated in the presence or absence of ABT-737. Cells were fixed in methanol for 1 hour and stained with Giemsa.

Results
Overexpressed BCL2 localizes in the nucleus of lymphoid tumor cells
Multiple previous studies have identified BCL2 within the nucleus of tumor cell lines and fibroblasts with high levels of BCL2 expression (16–19). We first examined BCL2 localization in the DLBCL cell lines OCI-LY1, OCI-LY8, and Toledo. All 3 lines harbor translocation t(14;18), which results in expression of BCL2 under IGH transcriptional control. Immunoblotting of nuclear and cytoplasmic fractions showed BCL2 within the nucleus of all 3 cell lines in the presence or absence of MNNG, ionizing radiation (IR), or ABT-737 (Fig. 1A). Immunofluorescence of OCI-LY8 cells confirmed the nuclear localization of BCL2 (Supplementary Fig. S1). BCL2 also localized to the nucleus in 2 murine B lineage leukemia lines that over-express BCL2 and MYC (15).
To determine whether ectopically expressed BCL2 can localize to the nucleus, we transfected hemagglutinin (HA)-tagged BCL2 into 293T cells. Immunoblotting with anti-HA antibody on cellular fractions revealed that BCL2 was present in the nucleoplasm before and after irradiation (Fig. 1B). irradiation also promoted the recruitment of BCL2 to chromatin (Fig. 1B). Even with ectopic expression, the nuclear and cytoplasmic levels of BCL2 remained lower than those observed in OCI-LY1 and OCI-LY8 cells (Supplementary Fig. S2).

**BCL2 and PARP1 interact in DLBCL cells**

Localization of BCL2 to irradiated chromatin suggested that BCL2 interacts with one or more factors involved in the DNA damage response. To identify proteins in the chromatin fraction that interact with BCL2, we isolated the chromatin fractions from OCI-LY8 cells after irradiation and conducted immunoprecipitation with an anti-BCL2 antibody (Fig. 1C).

Mass spectrometry of a 113-kDa band present only in the irradiated chromatin fraction (Fig. 1C) identified 18 distinct peptides from PARP1 with greater than 99% confidence (Supplementary Table S1).

PARP1 plays a role in several nuclear processes, including base excision repair, transcription regulation, DNA methylation, and chromatin modeling (20). PARP1 responds to DNA damage by using NAD+ to transfer PAR to acceptor proteins, including histones and PARP1 itself.

**The BCL2–PARP1 interaction is disrupted by ABT-737**

To determine whether the BCL2–PARP1 interaction involves the BH3-binding groove of BCL2, we exposed OCI-LY8 cells to irradiation followed by a 30-minute treatment with DMSO, 100 nmol/L ABT-737 or 100 nmol/L of an inactive ABT-737 enantiomer (5). ABT-737 displaced approximately 65% of PARP1 from BCL2 whereas the enantiomer had no effect (Fig. 1D). ABT-737 had little or no effect compared with its inactive enantiomer on the interaction between BCL2 and the nonhomologous end-joining protein KU70 (Fig. 1D), which does not bind within the BH3-binding groove (18).

PARP1 undergoes auto-PARylation in response to DNA damage. This creates a negatively charged scaffold that can mediate nonspecific protein interactions. Thus, the BCL2–PARP1 interaction could involve PAR, rather than PARP1 itself.
Treatment with the PARP1 inhibitor ABT-888 (21) completely blocked MNNG-induced PARylation (Fig. 1E) but had no effect on the BCL2–PARP1 interaction (Fig. 1D), indicating that the interaction with BCL2 is independent of PAR.

**BCL2 interacts directly with PARP1 in vitro**

To clarify whether PARP1 interacts with BCL2 directly or via intermediary proteins, we conducted co-immunoprecipitation on a mixture of purified PARP1 and GST-tagged BCL2. In the absence of DNA and NAD+, glutathione (GSH) beads coated with GST-BCL2 recovered PARP1 (Fig. 2A). In contrast, no PARP1 was recovered when GSH beads were coated with either GST-BCL-xL or GST-BCL-w (Fig. 2A). 2 BCL2 family members that are also bound by ABT-737 (5).

We conducted a fluorescence polarization assay, as previously described (22), to confirm the interaction between full-length PARP1 and BCL2. As expected, full-length ABT-737 avidly interacted with BCL2 (Kᵢ, 24.9 nmol/L) whereas the negative control NOXA failed to interact with BCL2 (Fig. 2B). Consistent with the findings by co-immunoprecipitation, full-length PARP1 interacted with BCL2 (Kᵢ, 77.0 nmol/L; Fig. 2B). The addition of ABT-737 displaced purified PARP1 from GST-BCL2 in a dose-dependent fashion (Fig. 2C), confirming the ability of ABT-737 to directly disrupt the BCL2-PARP1 interaction.

**The PARP1 BRCT domain is required for interaction with BCL2**

Full-length PARP1 includes an N-terminal DNA-binding domain, a C-terminal catalytic domain, and central PADR, BRCT, and WGR domains (Fig. 2D). To map the region that interacts with BCL2, we generated His-tagged C-terminal deletion mutants of PARP1 (Fig. 2D). Immunoblot analysis with antibody to PARP1 confirmed all protein products

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**Figure 2. ABT-737 disrupts the BCL2-PARP1 interaction.** A, purified PARP1 was incubated with GST-tagged BCL2, GST-BCL-xL, or GST-BCL-w. Mixtures were subjected to immunoprecipitation (IP) with anti–GST antibody followed by immunoblotting (IB) for PARP1. Ponceau staining was conducted on immunoprecipitates to confirm equal recovery. B, fluorescence polarization assay of GST-BCL2 mixed with increasing concentrations of ABT-737. NOXA, or PARP1, as previously described (22). Error bars indicate SEM. C, GST-BCL2 and PARP1 were incubated along with increasing concentrations of ABT-737. Immunoprecipitation and immunoblotting were conducted as in A. D, schematic representation of full-length PARP1, deletion constructs, and BRCT domain constructs. F1 and F2 are zinc finger 1 and zinc finger 2 domains, respectively. N indicates the nuclear localization signal. E, bacterially expressed PARP1 constructs were incubated with purified GST-BCL2 followed by immunoprecipitation with anti–His antibody and immunoblotting against BCL2. Purified GST-BCL2 was used as a control for BCL2 immunoblotting. Incubation of the His-ΔBRCT and GST-BCL2 was conducted in the presence or absence of 100 nmol/L ABT-737. G, GST-BCL2 was incubated with brct-only or brctΔBH3 constructs followed by immunoprecipitation and immunoblotting with an antibody specific for the PARP1 BRCT domain. Ponceau staining was conducted to confirm equal loading. WB, Western blotting.
PARP1 function, we measured PARP activity at codon 401. Mutation of both L410 and D406 to alanines in the BRCT domain contains a single BH3 motif (LXXXXD) beginning at codon 401. Mutation of both L410 and D406 to alanines in the BRCT domain did not interact with BCL2 (Fig. 2G). The PARP1 activity relative to untreated cytoplasm (3 hours post-MNNG) was collected before MNNG treatment (pre), immediately after washing MNNG with DMSO, ABT-888, or ABT-737. Cells were treated with MNNG for 15 minutes and MNNG was added to fractionated lysates from OCI-LY8 cells. ABT-737 potentiated PARP1 enzymatic activity in a dose-dependent manner in both nucleoplasm and chromatin fractions (Fig. 3A). Together, these findings indicate that the BRCT–PARP1 interaction blocks PARP1 activity, whereas ABT-737 can displace PARP1 from BCL2 and restore that activity.

Next, we asked whether inhibition of PARP1 function by BCL2 suppresses PARP1-dependent DNA repair. Assaying the effects of BCL2 inhibition on DNA repair is complicated by the dependence of most BCL2-overexpressing cells on BCL2 for survival. We used OCI-LY1 and SU-DHL4 clones previously selected for resistance to ABT-737 by long-term culture in the presence of the drug. These clones maintain BCL2 expression but also upregulated the antiapoptotic BCL2 family members MCL1 and/or BFL1.

To determine whether ABT-737 can increase PARP1 activity by displacing BCL2 from PARP1, we measured the effect of ABT-737 on PARP1 activity in fractionated lysates from OCI-LY8 cells. ABT-737 potentiated PARP1 enzymatic activity in a dose-dependent manner in both nucleoplasm and chromatin fractions (Fig. 3A). Together, these findings indicate that the BRCT–PARP1 interaction blocks PARP1 activity, whereas ABT-737 can displace PARP1 from BCL2 and restore that activity.

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OCI-LY1-10R cells were treated for 15 minutes with MNNG, which is known to activate PARP1-dependent DNA repair. After washing away MNNG, the cells were allowed to repair the damage for 3 hours in the presence of vehicle, ABT-888, or ABT-737. We conducted the alkaline comet assay, which quantifies both single- and double-stranded DNA breaks. Cells treated with ABT-737 had significantly smaller comet tails, indicating more extensive resolution of MNNG-induced DNA damage than in cells treated with MNNG and vehicle (Fig. 3C). The addition of ABT-888 did not further suppress repair, suggesting that most of the PARP1-mediated repair in these cells is inactive (Fig. 3B). Of note, neither ABT-737 nor MNNG affect the nuclear localization of BCL2 (Fig. 1A).

BCL2 blocks PARP1-dependent, nonapoptotic death in DLBCL cells

PARP1 enzymatic activity is required for a form of nonapoptotic cell death previously termed "parthanatos" (25–27). This pathway can be induced by ischemia or treatment with the alkylating agent MNNG, both of which result in DNA damage that requires PARP1-dependent repair. This form of cell death does not involve the intrinsic apoptosis pathway, as wild-type and Bax−/− Bak−/− MEFs have similar sensitivity to MNNG-induced nonapoptotic death (24, 28).

We hypothesized that inhibition of PARP1 enzymatic activity by BCL2 would suppress this form of nonapoptotic cell death. Supporting this hypothesis, previous studies have reported that BCL2 can inhibit nitric oxide–induced, nonapoptotic cell death of PC12 and HeLa cells (29) as well as nonapoptotic death of neural cells induced by GSH depletion (30). We assayed the survival of ABT-737-resistant clones treated with MNNG, ABT-737, ABT-888, or combinations (Fig. 4A). As expected, very little cell death was observed with single-agent ABT-737 (Fig. 4A). However, ABT-737 significantly increased death induced by MNNG in ABT-737-resistant
OCI-LY1 and SU-DHL4 clones as well as Toledo cells (Fig. 4A and B; \( P < 0.05 \) compared with MNNG plus vehicle in all lines). Importantly, MNNG \( + \) ABT-737–induced death was partially reversed by ABT-888, supporting a specific role for PARP1 (Fig. 4A and B).

Transmission electron microscopy of OCI-LY1-10R and SU-DHL4-2R cells treated with MNNG \( + \) ABT-737 showed ultrastructural features of nonapoptotic cell death, including loss of membrane integrity with preservation of nuclear architecture (Fig. 4C). These findings are most consistent with necrotic cell death. Double-membrane structures characteristic of autophagy were not present. In contrast, treatment with ABT-737 plus the topoisomerase II poison etoposide resulted in features characteristic of apoptosis, including chromatin condensation and preservation of membrane integrity (Fig. 4C). Thus, ABT-737 can promote PARP1-dependent nonapoptotic death in DLBCL cells.

We treated Bax\(^{-/-}\)/Bak\(^{-/-}\) MEFs (referred to as "DKO cells") with MNNG and confirmed that nonapoptotic cell death is independent of BAX and BAK (Fig. 5A and B). DKO cells that stably overexpress BCL2 ("BCL2-DKO cells") were less sensitive to MNNG \( (P < 0.05 \) compared with DKO cells; Fig. 5A), consistent with the inhibition of PARP1-dependent cell death by BCL2. In both lines, MNNG-induced death was completely blocked by ABT-888 (Fig. 5A). Thus, BCL2 inhibition can promote at least 2 forms of cell death. The first is BAX/BAK-dependent intrinsic apoptosis, whereas the second involves PARP1-dependent nonapoptotic death that is independent of BAX and BAK.

PARP1-mediated cell death induced by MNNG is characterized by reductions in cellular NAD\(^+\) and ATP (24, 28). We confirmed that both NAD\(^+\) and ATP concentrations are significantly reduced in DKO cells after 30-minute treatment with MNNG (Fig. 5C). In DKO cells, reductions in NAD\(^+\) and
ABT-737 promotes nonapoptotic death in primary CLl cells independent of stromal protection

We hypothesized that nonapoptotic cell death induced by MNNG + ABT-373 could be used to overcome stroma-induced resistance to apoptosis. CLl cells are known to be dependent on BCL2 and sensitive to treatment with ABT-737 (31). Cocculture of primary CLl cells with murine or human marrow stromal cells (HMSC) reduces apoptosis of CLl cells upon treatment with antineoplastic agents or ABT-373 through upregulation of antiapoptotic BCL2 family members (12, 13).

We cultured primary CLl cells from 14 patients (Supplementary Table S2) with or without HMSCs for 48 hours (Fig. 6). As expected, cells cultured in the presence of human HMSCs downregulated CXCR4 (Fig. 6A), the CLl cell surface homing receptor for SDF-1/CXCR12 (12, 32). ABT-737 potently induced mitochondrial depolarization in primary CLl cells cultured in the absence of HMSCs (Fig. 6B), whereas cells cocultured with HMSCs had significant resistance to 100 nmol/L ABT-737 (P < 0.01; Fig. 6C and D). In contrast, coculture with HMSCs did not block the additive killing from the combination of 50 μmol/L MNNG plus 100 nmol/L ABT-737 but killing was reduced by ABT-888 (P < 0.05; Fig. 6C and D), showing partial dependence on PARP1. Of note, 100 nmol/L ABT-737 plus 50 μmol/L MNNG resulted in only 20% loss of viability among the HMSC cells (data not shown).

BCL2 expression induces death in PARP inhibitor–sensitive cells

A logical extension of our findings that BCL2 overexpression phenocopies PARP1 inhibition is that cells sensitive to therapeutic PARP inhibitors could be killed by overexpression of BCL2. As a proof-of-principle, we retrovirally transduced BCL2 into the BRCA1-mutated breast cancer cell line MDA-MB-436 (33). Strikingly, ectopic expression of BCL2 completely blocked colony formation, which was partially reversed by co-administration of ABT-737 (Fig. 7A).

To expand this finding, we screened approximately 30 lung cancer cell lines for sensitivity to the PARP1 inhibitor AG014699. The lung cancer cell lines H1520 (GI50, 30 nmol/L) and HCC827 (GI50, 65 nmol/L) were highly sensitive to AG014699, compared with insensitive lines such as H1299 (GI50 >1 μmol/L; Fig. 7B; Supplementary Fig. S3). Similar to
MDA-MB-436 cells, ectopic expression of BCL2 in the PARP inhibitor–sensitive cell lines reduced colony formation by 90% to 100% but did not affect colony formation in H1290 cells (Fig. 7C). The effect from BCL2 was reversed by ABT-737 at therapeutic concentrations (Fig. 7C).

Discussion

BCL2 suppresses the intrinsic pathway of apoptosis by binding proapoptotic BCL2 family members (8). We have identified 2 additional pathways that can modulate survival through BCL2. First, inhibition of PARP1 activity by BCL2 delays the repair of DNA damage as well as nonapoptotic cell death. The pharmacologic BH3 mimetic ABT-737, which was designed to induce BAX/BAK-dependent apoptosis in cells dependent on BCL2 (5), can also promote PARP1-dependent nonapoptotic death by dissociating PARP1 from BCL2 and thereby restoring PARP1 enzymatic activity.

The second new pathway of survival modulation involving BCL2 results from the ability of BCL2 overexpression to phenocopy PARP deficiency. More specifically, BCL2 overexpression in PARP inhibitor–sensitive cells can promote cell death, and this death can be reversed by interrupting the interaction between BCL2 and PARP1. The "pro-death" effect from BCL2 and the "pro-survival" effect from ABT-737 are specific to cells that lack the ability to repair damage that results from PARP deficiency. In this model (Fig. 7D), basal levels of damage are repaired through PARP1 without drastically affecting cellular energy stores. PARP1 deficiency, either through interaction with BCL2 or pharmacologic inhibition, results in the conversion of single-strand lesions to double-strand breaks, which can be repaired by homology-directed repair (HDR; refs. 34, 35). Extensive DNA damage resulting from exogenous clastogens can drive both apoptosis and PARP1-mediated nonapoptotic cell death, which are both suppressed by BCL2.

The extent of damage necessary to induce PARP1-mediated nonapoptotic death within any particular cell is likely to depend on several factors, including energy metabolism, repair efficiency, threshold for undergoing apoptosis (36), and the downstream effectors of PARP1-dependent death. One testable prediction based on our findings is that tumors with BCL2 overexpression must maintain some extent of HDR, as concurrent deficiencies in HDR and PARP1 result in synthetic lethality (34, 35). Challenging our model, overexpression of BCL2 or BCL-xL can suppress HDR in rodent (37) and human (38, 39) cells. In addition, BCL2 inhibits the formation of HDR.
radiation-induced BRCA1 foci in human lymphoma cells (39), further suggesting that BCL2 may negatively modulate HDR. However, another study in human lymphoblastoid cells noted the opposite effect, with a 3-fold increase in HDR upon BCL-xL overexpression (40).

A series of studies have reported additional effects of BCL2 in various cell types on other DNA pathways, including nucleotide excision repair, base excision repair, mismatch repair, and homologous recombination (16–19, 37, 38, 41–43). The described effects from BCL2 include both direct binding and decreased transcription of DNA repair factors. The extent to which these effects are present within lymphoid tumor cells and their contribution to sustaining DNA damage after treatment with clastogens have not been addressed.

Several aspects of the BCL2-mediated suppression of PARP1 activity remain unclear. First, the precise function of the PARP1 BRCT domain is poorly understood. In chicken DT40 cells, the PARP1 BRCT domain appears to be involved in mutagenic repair at immunoglobulin loci, possibly through an interaction with nonhomologous end-joining factors (44, 45). Aberrant somatic hypermutation at oncogene loci is a common feature of lymphomas with BCL2 overexpression (46). Thus, it is intriguing to speculate that BCL2-mediated suppression of PARP1 function could promote these events by inhibiting more fidelitous repair.

It also remains unclear whether targeting nonapoptotic death would offer an adequate therapeutic index, as some populations of nonneoplastic cells may be similarly sensitive to PARP1-mediated death. Zong and colleagues identified greater sensitivity to MNNG-induced death in proliferating versus vegetative cells, despite equivalent amounts of DNA damage and PARP activity (47). The greater sensitivity among proliferating cells resulted from dependence on glycolysis for the generation of ATP (i.e., the Warburg effect).

In conclusion, we have identified a novel interaction between BCL2 and PARP1 that blocks PARP1 enzymatic activity and suppresses PARP1-dependent repair. Targeting of the BCL2–PARP1 interaction with BH3 mimetics is a potential approach for killing BCL2-expressing tumors that are resistant to apoptosis, either through upregulation of other antiapoptotic BCL2 family members or from defects in the intrinsic pathway (11, 13).
Disclosure of Potential Conflicts of Interest
No potential conflicts of interests were disclosed.

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Acknowledgments
The authors thank Margaret Shipp and Bjorn Chapuy for providing reagents and helpful discussion and Maria Ericsson, Simon Dillon, and John Daley for assistance with electron microscopy, mass spectrometry, and flow cytometry, respectively.

Grant Support
This work was supported by a Burroughs-Wellcome Fund Career Award in the Biomedical Sciences (6150801). The Claudia Adams Barry Program in Cancer Research, and the John Stellato Fund.

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Received December 27, 2011; revised May 15, 2012; accepted May 31, 2012; published OnlineFirst June 11, 2012.

Dutta et al.


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