Nucleotide Excision Repair Capacity Is Attenuated in Human Promyelocytic HL60 Cells That Overexpress BCL2

Yafei Liu, Louie Naumovski, and Philip Hanawalt

Abstract

We investigated the effect of the BCL2 overexpression on nucleotide excision repair (NER) and DNA replication in UV-irradiated HL60 cells. Forty-eight h after 10 J/m² irradiation, only 4% of the cyclobutane pyrimidine dimers were removed in the BCL2-overexpressing cells, in contrast to 38% removal in control cells. However, the repair of 6-4 pyrimidine pyrimidone photoproducts was not affected by BCL2 overexpression. Eight h after irradiation, DNA replication recovered to 60% of normal in the BCL2-overexpressing cells, whereas little DNA replication recovered in control cells. The antioxidant N-acetyl cysteine also attenuated cyclobutane pyrimidine dimer removal but did not enhance the recovery of DNA replication. Both BCL2-overexpressing and NAC-treated cells were more resistant to UV. Our data suggest that Bcl2 may promote mutagenesis and genomic instability in surviving cells.

Introduction

Bcl2 is a Mr 26,000 integral membrane protein that prevents apoptosis in multiple biological contexts (1). The activation of the interleukin 1β converting enzyme/ced-3 family cysteine proteases play a central role in the execution of apoptosis (2, 3). It is thought that the Bcl2 protein can inhibit apoptosis by preventing the activation of cysteine proteases (4). Overexpression of the BCL2 gene in transgenic mice results in lymphomagenesis, suggesting that the Bcl2 protein is oncogenic (5). Consistent with this notion, histopathological studies have revealed that the Bcl2 protein is frequently overexpressed in lung, breast, and skin cancers (6—8). BCL2 gene expression also becomes dysregulated by the t(14;18) translocation in over 15% of non-Hodgkin’s lymphomas (9, 10). The frequency of the t(14;18) translocation has been shown to correlate with lymphoma risk factors, such as aging and cigarette smoking in humans (11, 12). However, it is still not clear how Bcl2 might facilitate oncogenesis. We have investigated whether the Bcl2 protein might affect the efficiency of NER.

Complex NER Pathway Involves more than 30 Proteins (13) and Repairs a Variety of Mutagenic DNA Lesions (14). We found that NER is significantly attenuated in UV-irradiated, BCL2-overexpressing HL60 cells. Furthermore, DNA replication resumed in the BCL2-overexpressing cells, although the CPD lesions were not repaired. The BCL2 cells were also more resistant to UV toxicity than control cells. Similar to the Bcl2 protein, the antioxidant NAC is also shown to function as an antiapoptotic agent and to reduce apoptosis-associated oxidative damage (15). We found that NAC attenuated NER and enhanced cell survival but did not enhance post-UV DNA replication recovery. Because translesional DNA replication is usually mutagenic, our data suggest that Bcl2 may promote mutagenesis by attenuating DNA repair as well as by preventing apoptosis.

Materials and Methods

Cell Culture and Treatment. The establishment and maintenance of the BCL2-overexpressing and control HL60 lines have been described previously (16). Cells were irradiated in PBS solution at 0.33 J/m² incident dose rate with a Westinghouse IL 782–30 germicidal lamp (output primarily at 254 nm). NAC stock solution (200 mM) was freshly made and added into the medium to a 40 mM final concentration 2 h prior to irradiation. After UV, the NAC-treated cells were continuously incubated in medium containing 40 mM NAC.

Antibody Assay for CPDs and 6-4 Photoproducts. Cells were prelabeled with 0.1 μCi/ml [3H]thymidine for three generations before irradiation. After UV, cells were incubated in nonradioactive medium so that the newly synthesized DNA would not be labeled. DNA was quantified by fluorometry with Hoechst 33258 (17) and slot blotted (50 μg/slot for CPD; 150 μg/slot for 6-4 photoproduce) onto Hybond N+ membrane (Amersham Corp.) in triplicate. The membrane was probed with the TDM-2 (for CPD) or 64M-2 (for 6-4 photoproducts) mouse monoclonal antibodies (18, 19). Primary antibody binding was quantified using the ECL chemiluminescence procedure (Amersham), followed by a 2-h exposure on the CH chemiluminescent phosphor imaging screen (Bio-Rad). This procedure determined the relative level of DNA lesions in each slot. After the ECL exposure, the blot was covered with a thin tin foil screen guard (Bio-Rad) to block light emission and then re-exposed overnight on a CH weak energy isotope phosphor imaging screen (Bio-Rad) to quantify the amount of the 14C-labeled DNA on each slot. The level of DNA lesions on each blot was then normalized to the amount of the 14C-labeled DNA to quantify lesions per unit DNA.

DNA Replication Assay. Incorporation of [3H]thymidine into cellular DNA following UV irradiation could be the result of DNA replication and/or repair synthesis. To distinguish these two pathways, cellular DNA was incubated with 10 μM 5-bromodeoxyuridine, 1 μM fluorodeoxyuridine, and 3 μCi/ml [3H]thymidine immediately after 10 J/m² UV irradiation. Eight h after irradiation, 10 μg of DNA were subjected to isopycnic CsCl gradient ultracentrifugation (20) to separate the parental and the replicated DNAs. The gradients were eluted into 30 fractions, each fraction containing 200 μl of solution. Thirty μl from each fraction were spotted onto Whatman #1 filter paper and treated with 5% ice-cold trichloroacetic acid to precipitate the DNA. The filter papers were then washed with acetone, dried, and counted on a Beckman LS 5000 CE scintillation counter. The incorporation of [3H]thymidine due to genuine DNA replication can be analyzed by the counts in the density-labeled, replicated hybrid DNA peak.

Cytotoxicity Assay and Apoptotic DNA Laddering Assay. Log phase cells were irradiated with increasing UV doses. Three days after UV irradiation, triplicate cell samples were pelletted and resuspended in 0.4% trypan blue and 0.9% saline (1:1 v/v). The number of viable cells was then counted with a hemocytometer. For the DNA laddering analysis, cellular DNA was extracted 0, 8, and 24 h after UV irradiation. One μg of DNA samples were analyzed on 2% ethidium bromide agarose gels.

Results

Repair of CPDs Is Attenuated by BCL2 Overexpression or NAC Treatment in HL60 Cells. The antibody-based assay used in this study is a simple, rapid, and quantitative way to measure global...
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Genomic repair of photoproducts (18, 19). The assay requires blotting similar amounts of DNA onto membranes before the quantification of lesions. Previously, DNA lesions on each blot were normalized by the exact amount of DNA loaded onto the membrane (19). This procedure has been further refined in the present study. We prelabeled the cellular DNA with $^{14}$C-thymidine before the UV treatment and then normalized the quantity of DNA lesions to the $^{14}$C prelabel. Because the BCL2 cells continue replicating DNA rapidly after irradiation, the lesions cannot be normalized to the absolute DNA amount as described previously (19), because the post-UV DNA replication results in an increased quantity of lesion-free DNA during the repair incubation, thus leading to an overestimate of repair. The refined prelabel method quantifies removal of DNA lesions, regardless of DNA replication, and is thus a more accurate way to quantify repair, especially in cells undergoing replication.

As shown in Fig. 1, in the BCL2-overexpressing and in the NAC-treated HL60 cells, only 4% of the CPDs were removed from the genomic DNA within 48 h after UV exposure, whereas 38% of the CPDs were removed in the control cells during the same time period. Because CPDs are known to be repaired by NER enzymes, our data suggest that the Bcl2 protein or NAC can attenuate the NER capacity in HL60 cells.

Meanwhile, 6-4 photoproducts, another UV-induced DNA lesion that is repaired by NER in mammalian cells (21), were repaired at equal rates in all cases (Fig. 1). The repair of CPDs, but not 6-4 photoproduct, is relatively inefficient in HL60 cells as compared with that in human fibroblasts, in which 80% of CPDs are generally removed in 24 h (14, 22). Because HL60 cells are p53 null by gene deletion (23), our data are consistent with the previous report from our laboratory that global genomic NER is attenuated in cells without functional p53 tumor suppressor protein (22). CPDs and 6-4 photoproducts are chemically distinct di-pyrimidine photodadducts. The 6-4 lesion occurs at a lower frequency than does CPD but introduces a more severe distortion in the chromosome and is a better substrate for NER (14). The fact that BCL2 overexpression or NAC treatment can distinctly affect CPD but not 6-4 photoproduct removal suggests that these two types of lesions might be repaired by distinct biological pathways, or that the more distorting 6-4 photoproduct lesions are better recognized and repaired when repair enzyme levels are limiting.

DNA Replication Continues following UV Irradiation in BCL2-overexpressing Cells although the CPDs Are Not Repaired. We have measured the post-UV DNA replication in control, NAC-treated, and BCL2-overexpressing HL60 cells. Eight h after 10 J/m2 UV irradiation, the DNA replication is almost completely suppressed in the control or NAC-treated cells. However, in the BCL2-overexpressing cells, nearly 60% of DNA replication activity was retained compared to that in the unirradiated cells (Fig. 2). Thus, overexpression of BCL2 seems to prevent the UV-induced suppression of DNA replication, even when the CPD lesions are not removed.

Both BCL2 Overexpression and NAC Treatment Enhance Cellular Survival to UV Irradiation. The BCL2-overexpressing HL60 cells were about 100-fold more resistant to UV (at 40 J/m2) than the control HL60 cells. When the HL60 cells were treated with 40 mM NAC, they became much more resistant to UV (Fig. 3), consistent with previous reports (24).
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Discussion

Our global genome repair data are consistent with the results of a previous study on CPD repair in HL60 cells (25). Because of the extensive DNA degradation in HL60 cells, we were unable to obtain large-size DNA fragments to investigate the transcription-coupled repair pathway.

Possible Mechanisms for the Effect of Bcl2 Protein on NER.

Our findings suggest that the Bcl2 protein could attenuate the NER activity and thus delay CPD removal. However, because the Bcl2 protein is physically localized at the membranes of the nucleus, endoplasmic reticulum, mitochondria, and cytoplasm (26), it seems unlikely that the Bcl2 protein can affect the NER process by direct molecular interaction with repair proteins, although there is recent evidence that NER takes place at the nuclear matrix (27). Bcl2 protein might indirectly interfere with NER. Because NAC can inhibit NER to a similar extent as BCL2 overexpression, we sought explanations that might accommodate both observations. There are at least two possible mechanisms.

(a) The Bcl2 protein may block the nuclear trafficking of proteins that are needed for NER, resulting in reduced CPD removal. Both Bcl2 and NAC have been shown to block nuclear trafficking of proteins. For example, Bcl2 blocks the nuclear trafficking of the p53 tumor suppressor protein (28, 29), and NAC blocks the transport of the nuclear factor-κB transcriptional factor into the nucleus (30). The fact that 6-4 photoproduct repair is unaffected may be due to the fact that these lesions are much better substrates for NER than are the CPDs (31) and can be preferably repaired by the limited NER enzymes in the nucleus.

(b) The Bcl2 protein has been proposed to play an antioxidant role in the apoptotic process (26). The antioxidant NAC and Bcl2 are both known to increase the intracellular glutathione concentration (15, 26). It is possible that the antioxidant function of Bcl2 and NAC could attenuate NER activity. For example, UV irradiation can cause oxi-

Summary. We have compared NER, DNA synthesis, viability, and induction of apoptosis in control, NAC-treated, and BCL2 HL60 cells following UV irradiation. CPD removal is significantly reduced by BCL2 overexpression and by NAC treatment. DNA replication continues after UV irradiation in the BCL2 cells but does not significantly recover in the control or NAC-treated cells. Both BCL2-overexpressing and NAC-treated cells are significantly more resistant to UV.

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We have also studied the induction of apoptosis in these cells. DNA fragmentation is considered a hallmark for apoptosis. In the HL60 cells, DNA laddering appeared 4 h after UV exposure. The NAC treatment delayed DNA laddering until 24 h, whereas BCL2 overexpression further delayed the appearance of DNA laddering until after 24 h. These data suggest that: (a) the HL60 cells are subject to p53-independent apoptosis after UV irradiation; and (b) the NAC treatment and BCL2 overexpression delayed the onset of apoptosis (Fig. 3).

Fig. 3. Cytotoxicity assay by trypan blue exclusion. On day 3 after UV irradiation, cells were treated with trypan blue, and the fraction of surviving cells was determined. Bars, SD determined from three measurements. The lower panel shows that the onset of DNA fragmentation is delayed in NAC and BCL2 cells.

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Fig. 4. A model for the oncogenic action of Bcl2. When Bcl2 is overexpressed, both apoptosis and DNA repair are reduced, leading to enhanced mutagenesis. The dark nuclei represent the presence of DNA damage. The irregular cells represent apoptotic cells.
ative damage to the cell membrane, which is thought to induce a signal pathway that leads to the activation of an array of genes (32). It is possible that the Bcl2 protein and NAC could intervene in this signal pathway by precluding the UV-induced oxidative damage, thus affecting the NER reaction.

In the BCL2-overexpressing Cells, the Correlation between Repair and Survival Is Reversed. Traditionally, cellular survival against UV irradiation is well correlated with the ability of the cell to repair DNA lesions (14). However, in the BCL2-overexpressing cells, although their NER activity was repressed, their survival against UV toxicity was enhanced. This finding suggests that Bcl2 protein can block apoptosis, regardless of reduced DNA repair capacity. Our data are similar to the recent report (22) that p53-defective cells are more resistant to UV cytotoxicity, although their global NER capacity is attenuated.

Our Data Suggest That BCL2 Plays an Active Role in Carcinogenesis. When comparing the time course of DNA repair and DNA replication after UV treatment, we observe, in the BCL2 cells, that the majority of CPD lesions remain unrepaired 48 h after UV irradiation and that DNA has been replicated although the CPD lesions have not been repaired. This finding is consistent with the report that efficient translesional DNA replication can occur in UV-irradiated mammalian cells (33).

DNA repair is an important biological pathway that promotes survival and minimizes mutagenesis (Fig. 4). Severely damaged cells cannot give rise to mutants because they die through apoptosis. However, in the BCL2-overexpressing cells, both apoptosis and DNA repair processes are attenuated, leading to DNA synthesis that may potentially fix unrepaired DNA into mutations (Fig. 4). Our data suggest that the Bcl2 protein may actively enhance mutagenesis and carcinogenesis by both attenuating DNA repair and promoting replication in addition to inhibiting apoptosis. In fact, it has been shown recently that overexpression of BCL2 can promote radiation-induced mutagenesis in human cells (34).

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References

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