The comprehensive expression of BCL2 family genes determines the prognosis of diffuse large B-cell lymphoma

Jin Roh  
Department of Pathology, Ajou University School of Medicine

Hyo-Kyung Pak  
Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine

Seongfeel Jeong  
Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine

Sewon Hwang  
Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine

Do Eon Kim  
Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine

Hwal-Seok Choi  
Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine

So-Jeong Kim  
Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine

Hyunji Kim  
Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine

Hyungwoo Cho  
Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine

Joon Seong Park  
Department of Hematology-Oncology, Ajou University School of Medicine

Seong Hyun Jeong  
Department of Hematology-Oncology, Ajou University School of Medicine

Yoon Seok Choi  
Department of Hematology-Oncology, Ajou University School of Medicine

Jae Ho Han  
Department of Pathology, Ajou University School of Medicine

Dok Hyun Yoon  
Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine
Chan-Sik Park (csikpark@amc.seoul.kr)
Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine

Research Article

Keywords: Diffuse large B-cell lymphoma, BCL2 family, Prognosis, BCL2 signature score, NanoString, Gene expression

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The comprehensive expression of BCL2 family genes determines the prognosis of diffuse large B-cell lymphoma

Jin Roh†, Hyo-Kyung Pak†, Seongfeel Jeong, Sewon Hwang, Do Eon Kim, Hwal-Seok Choi, So-Jeong Kim, Hyunji Kim, Hyungwoo Cho, Joon Seong Park, Seong Hyun Jeong, Yoon Seok Choi, Jae Ho Han, Dok Hyun Yoon, Chan-Sik Park

1Department of Pathology, Ajou University School of Medicine, Suwon, 16499, Republic of Korea.
2Department of Hematology-Oncology, Ajou University School of Medicine, Suwon, 16499, Republic of Korea.
3Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea.
4Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea.
5Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea.
6Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea.

†These authors contributed equally to this work.
Abstract

Diffuse large B-cell lymphoma (DLBCL) is a prevalent and aggressive non-Hodgkin’s lymphoma, and 40% of patients succumb to death. Despite numerous clinical trials aimed at developing treatment strategies beyond the conventional R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen, there have been no positive results thus far. Although the selective BCL2 inhibitor venetoclax has shown remarkable efficacy in chronic lymphocytic leukemia, its therapeutic effect in DLBCL was limited. We hypothesized that the limited therapeutic effect of venetoclax in DLBCL may be attributed to the complex expression and interactions of BCL2 family members, including BCL2. Therefore, we aimed to comprehensively analyze the expression patterns of BCL2 family members in DLBCL. We analyzed 157 patients with de novo DLBCL diagnosed at Asan Medical Center and Ajou University Hospital. The mRNA expression levels of BCL2 family members were quantified using the NanoString technology. BCL2 family members showed distinct heterogeneous expression patterns both intra- and inter-patient. Using unsupervised hierarchical cluster analysis, we were able to classify patients with similar BCL2 family expression pattern and select groups with clear prognostic features, C1 and C6. In the group with the best prognosis, C1, the expression of pro-apoptotic and pro-apoptotic BH3-only group gene expressions were increased, while anti-apoptotic group expression was significantly increased in both C1 and C6. Based on this, we generated the BCL2 signature score using the expression of pro-apoptotic genes BOK and BCL2L15, and anti-apoptotic gene BCL2. The BCL2 signature score 0 had the best prognosis, score 1/2 had intermediate prognosis, and score 3 had the worst prognosis (EFS, $p = 0.0054$; OS, $p = 0.0011$). Multivariate analysis, including COO and IPI, showed that increase in the BCL2 signature score was significantly associated with poor prognosis for EFS, independent of COO and IPI. The BCL2 signature score we proposed in this study provides information on BCL2 family deregulation based on the equilibrium of pro- versus anti-apoptotic BCL2 family, which can aid in the development of new treatment strategies for DLBCL in the future.

Keywords: Diffuse large B-cell lymphoma, BCL2 family, Prognosis, BCL2 signature score, NanoString, Gene expression

1 Introduction

Diffuse large B-cell lymphoma (DLBCL) is prevalent and aggressive type of non-Hodgkin’s lymphoma [12, 21]. The addition of rituximab (R) to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) has improved survival outcomes in patients with DLBCL. However, around 40% of patients are refractory to treatment or eventually experience a relapse [5, 6, 29, 34]. This treatment failure is partly due to the heterogeneity of DLBCL [37]. Recently, several clinical trials have been conducted to go beyond the conventional R-CHOP regimen by adding new targeted agents such as bortezomib [27], lenalidomide [26], and ibrutinib [36] to the R-CHOP in patients with non-germinal center B-cell (GCB) or activated B-cell...
Venetoclax is a highly selective BCL2 inhibitor and has been approved for the treatment of chronic lymphocytic leukemia (CLL) by US Food and Drug Administration and European Medicines Agency. Treatment with venetoclax demonstrated a significant therapeutic effect, with a 79% response rate and 20% complete response rate, in relapsed CLL patients [31]. Subsequent studies have confirmed that venetoclax induced rapid onset apoptosis in patient CLL cells both in vitro and in vivo [2]. Venetoclax treatment also achieved durable and significant therapeutic efficacy with manageable toxicity in 158 CLL patients, including those at high risk [35]. However in DLBCL, venetoclax treatment showed limited therapeutic response with 18% of overall response rate in patients with relapsed/refractory DLBCL [7]. Although venetoclax plus R-CHOP treatment in patients with BCL2-positive DLBCL demonstrated potential for improved efficacy compared with R-CHOP alone treatment [24], biomarker identification of likely responders would be required [20]. Recent reports suggest that resistance to venetoclax may be influenced by dysfunction of BCL2 family pro-apoptotic proteins, as well as the absence of BCL2 expression or a high dependence on MCL1 BCL2 family anti-apoptotic proteins in non-Hodgkin’s lymphoma [32]. These findings propose a hypothesis that the intricate interplay between BCL2 and other BCL2 family proteins may govern apoptosis regulation in DLBCL.

BCL2 family proteins comprise around 20 molecules and play a critical role in cellular apoptosis in combination of those molecules [4, 18]. They are typically divided into three groups: anti-apoptotic proteins, pro-apoptotic pore-formers, and pro-apoptotic BH3 domain [15]. Whether a B-cell undergoes apoptosis or survives is determined by the intricate and dynamic balance between the pro- and anti-apoptotic members of the BCL2 family [1, 28]. Therefore, an interpretation for the complex expression of BCL2 family members in DLBCL is necessary to understand its heterogeneity and identify appropriate targets. In this study, we performed a comprehensive functional assessment by evaluating the expression of pro- and anti-apoptotic members of BCL2 family, which allowed us to generate a BCL2 signature score. Using this assessment, we identified that decreased expression of pro-apoptotic members along with increased expression of anti-apoptotic member of BCL2 family are associated with poor prognosis.

2 Materials and methods

2.1 Patient cohorts

We retrospectively collected formalin-fixed paraffin-embedded (FFPE) diagnostic tissues from 157 patients with de novo DLBCL between February 2007 and December 2012 at Asan Medical Center (n = 122) and between February 2008 and December
2012 at Ajou University Hospital (n = 35), South Korea. All patients underwent the standard staging procedures including bone marrow aspiration and biopsy, computed tomography (CT) scans, and positron emission tomography-CT, and were treated with R-CHOP as first-line treatment. Patients with primary central nervous system lymphoma and patients who were initially treated with other treatment rather than R-CHOP regimen were excluded. Clinicopathological information was collected from medical records including sex, age, Ann Arbor stage, International Prognostic Index (IPI). Those without appropriate clinical information and/or the histologic materials were excluded. This study was approved by the institutional review boards of the participating institutions.

2.2 Isolation of RNA from FFPE tissues for NanoString analysis

Total RNAs were isolated from FFPE samples by using the MasterPure Complete DNA and RNA Purification Kit (Bioresearch Technologies, Hoddesdon, UK). The yield and purity of the isolated RNAs were evaluated using a DS 11 Spectrophotometer (Denovix Inc, DE, USA). To check the quality of the RNAs, we used a Fragment Analyzer from Advanced Analytical Technologies (IA, USA). The quality of the RNAs was assessed using the Fragment Analyzer (Advanced Analytical Technologies, IA, USA). At least 1 µg of total RNA was added to the sample preparation reaction in the available 5 µL volume for nanoString analysis.

2.3 Gene expression quantification by NanoString

The NanoString nCounter human mRNA expression assay (NanoString technologies, WA, USA) was performed using over 1 µg of total RNA isolated from FFPE tissue. RNA samples were combined with nCounter Reporter probes and nCounter Capture probes in hybridization buffer and incubated overnight at 65°C. Excess probes were removed using two-step magnetic bead-based purification on the nCounter Prep Station (NanoString technologies, WA, USA). The abundance of specific target molecules was quantified on the nCounter Digital Analyzer by counting the individual fluorescent barcodes. A high-density scan was performed, and data were collected using the nCounter Digital Analyzer (NanoString technologies, WA, USA) after taking images of the immobilized fluorescent reporters in the sample cartridge with a CCD camera.

Cell of origin (COO) subtypes and BCL2 family gene expression were assessed by the nCounter Analysis System (NanoString Technology). The COO of the cases was assigned into three groups: germinal center B-cell (GCB) group, activated B-cell (ABC) group, and unclassified group using the Lymph2Cx assay [33]. Expression of BCL2 family genes was measured by the NanoString nCounter Analysis System (NanoString Technology, WA, US) using customized probes (Supplementary Table S1). The customized probe set includes 24 known BCL2 family members to date [13, 25, 30]. Pro-apoptotic group includes BAX, BAK, BOK, BCL2L15, BCL2L13,
SPIKE. Anti-apoptotic group includes BCL2, BCL2L1, BCL2L2, MCL1, BCL2A1, and BNIP2. Pro-apoptotic BH3-only group includes BAD, PMAIP1, BCL2L11, BBC3, BID, BMF, HRK, BIK, and BCL2L14. The Other group includes BCAP31, MNT, and BECN1, which are reported to have close interactions with BCL2 family genes.

The raw NanoString counts for each target genes were normalized with the geometric mean of the housekeeping genes (ISY1, R3HDM1, TRIM56, UBXN4, and WDR55). The resulting data were preprocessed (batch-corrected, log2-transformed, z-score standardized) for further analyses.

2.4 Transcriptome datasets
To validate the association between the BCL2 signature score and the prognosis of DLBCL patients in other dataset, Affymetrix U133plus2 microarray data from the Dubois et al. study [10] were downloaded from Gene Expression Omnibus (GEO, GSE87371). Affymetrix U133plus2 microarray data from the Herishanu et al. study [14] were downloaded from GEO (GSE21029) to compare the gene expression pattern between chronic lymphocytic leukemia (CLL) and DLBCL. The data were normalized using Robust Multichip Average method. The expression of individual probesets was log2-transformed and z-score standardized.

2.5 Statistics
For continuous variables, Student’s t-test or Mann-Whitney test was used to compare between two groups, and one-way ANOVA test was used for comparisons among three groups. When the results of the one-way ANOVA test were statistically significant, Tukey’s multiple pairwise-comparisons were conducted to confirm significant differences between specific groups. Hierarchical cluster analyses were performed using Euclidean distance and ward.D2 linkage by multiClust[19] package in R/Bioconductor. The optimal number of clusters was determined using the gap statistic method. We compared patient survival rates among clusters using survival analysis.

Overall survival (OS) was defined as the time from diagnosis until death as a result of any cause. Event-free survival (EFS) was defined as the time from the date of diagnosis until death from any cause, tumor relapse or progression, or the initiation of subsequent anti-lymphoma therapy. OS and EFS time were measured in months. Survival curves were plotted using the Kaplan–Meier method and the log-rank test was used to analyze the statistical differences between survival curves. Multivariate analysis was performed using Cox proportional hazards regression model, and the results were presented as estimated hazard ratios (HRs) with 95% confidence intervals (CI). All statistical analyses were performed with the R programming environment (v4.2.2) (R Foundation for Statistical Computing, https://www.R-project.org/).
3 Results

3.1 Clinicopathologic characteristics of the enrolled patients

The baseline characteristics of the enrolled patients are presented in Table 2. The median age was 57.6 years (range, 20–81), 84 patients (53.5%) were male. The Lymph2Cx assay results showed that among all patients, 68 (43.3%) were classified as GCB, 42 (26.8%) as ABC, and 21 (13.4%) as unclassified. The classification of 26 patients (16.6%) was not possible due to poor material and/or RNA quality.

3.2 Expression of BCL2 family members in DLBCL

When comparing the mRNA expression levels of individual members of BCL2 family in DLBCL patients, a heterogeneous pattern was observed due to differences in expression levels among patients (Figure 1A). This heterogeneity was observed not only among individual patients, but also within the same functional group of BCL2 family in a single patient. Moreover, considerable expression level variations among patients were also observed when analyzing the average expression of BCL2 family members according to the functional groups (Figure S1). To support the hypothesis that BCL2 family members showed a heterogeneous expression pattern characteristically in DLBCL, we compared the expression profiles of the BCL2 family in CLL which is well-known for BCL2 dependency (Figure 1B). DLBCL showed more distinct inter-patient and intra-patient heterogeneity compared to CLL. The heterogeneity of BCL2 family expression patterns in DLBCL was also observed similarly in an independent DLBCL dataset (Figure S2). Through this, it can be inferred that the mechanism of tumor cell apoptosis in DLBCL is regulated by the complex actions of BCL2 family members or functional groups.

To explain the heterogeneous expression patterns of BCL2 members in DLBCL, additional analyses were performed. IPI and COO, which are classical prognostic features of DLBCL, were used to compare the expression patterns of BCL2 family members and to infer their relationship (Figure 1C–J, S3, and S4). The pro-apoptotic and Other groups showed significantly higher expression in the low IPI group (IPI 0–2). At the individual gene level, the expression of BAK, BOK, and BCL2L15 in the pro-apoptotic group was significantly higher in the low IPI group. Among the anti-apoptotic group, the expression of MCL1 was significantly lower in the low IPI group, while there were no significant expression differences on other genes. Among the pro-apoptotic BH3-only group, the expression of BAD, BID, and BCL2L14 was significantly higher in the low IPI group. Genes belonging to the Other group, such as MNT and BECN1, showed a significant increase in the low IPI group. Regarding COO, the pro-apoptotic group showed higher expression level in the GCB subtype compared to the ABC or Unclassified subtypes, while the anti-apoptotic group showed significantly higher expression level in the ABC subtype. At the individual gene level, BAK, BOK, and BCL2L13 from the pro-apoptotic group showed significantly higher expression levels in the GCB subtype. In the anti-apoptotic group, BCL2, MCL1, and BCL2A1 showed significant overexpression in the ABC subtype. The pro-apoptotic
BH3-only group showed heterogeneous expression patterns depending on COO, with BAD, BID, HRK, and BCL2L14 showing significant overexpression in the GCB subtype, while PMAIP1, BIM, and BMF showed significantly increased expression in the ABC subtype. BCAP31 expression was decreased in the GCB subtype, while MNT showed significant overexpression.

Overall, BCL2 family members showed diverse expression patterns depending on IPI and COO. However, pro-apoptotic genes tended to be upregulated in DLBCL with relatively favorable prognosis, such as low IPI or GCB subtype. On the other hand, anti-apoptotic genes tended to be upregulated in the ABC subtype.

3.3 Hierarchical cluster analysis

We performed unsupervised hierarchical cluster analysis (HCA) to identify common expression patterns of BCL2 family member. HCA grouped patients with similar BCL2 family expression pattern into seven clusters, determined by the largest gap statistic value (Figure 2A). The average expression of BCL2 family members for each of the seven clusters is shown in Figure 2B, and the comparison of survival rates for each cluster is shown in Figure S5. When comparing the survival rates among clusters, cluster 1 (C1) and cluster 6 (C6) showed the most distinct difference between survival curves. Comparing to the remaining clusters, C1 had the best prognosis while C6 had the worst prognosis ($p = 0.014$, Figure 2C). We focused on C1 and C6 for further analyses. To determine which genes contributed to the cluster classification, we analyzed the expression distribution of BCL2 family members in each cluster (Figure S6). In functional group, pro-apoptotic and pro-apoptotic BH3-only group expressions were significantly higher in C1. Anti-apoptotic group expression was significantly increased in both C1 and C6.

In detail, all pro-apoptotic group genes, except for BAX, were significantly upregulated in C1. In the anti-apoptotic group, the expression of MCL1 was significantly lower in C1, while the expression of BCL2, BCL2L2, and BNIP2 was significantly increased. Most of the genes belonging to the pro-apoptotic BH3-only group were also overexpressed in C1. Among the genes in the Other group, MNT and BECN1, except for BCAP31, were upregulated in C1. C6 generally showed an opposite expression pattern to C1, with the expression of genes belonging to the pro-apoptotic group, except for BAX, significantly decreased. For anti-apoptotic group genes, except for BCL2L2, all genes were overexpressed in C6. Among the genes in the pro-apoptotic BH3-only group, BAD, BBC3, BID, HRK, and BCL2L14 showed decreased expression in C6, while PMAIP1, BCL2L1, BMF, and BIK showed increased expression.

Using unsupervised HCA, we were able to classify groups showing similar expression patterns of BCL2 family members and select groups with clear prognostic features. The major differences in prognosis among clusters were primarily due to gene expression in the pro- and anti-apoptotic groups.
3.4 BCL2 signature score

Based on the results, it can be inferred that the integrated analysis of pro- and anti-apoptotic gene expression of the BCL2 family would be important for predicting the prognosis of DLBCL patients. Next, target genes were selected based on the expression pattern of BCL2 family members in C1 and C6, which showed clear prognostic impact in unsupervised HCA. Considering the average BCL2 family member expression in each cluster, genes with similar expression patterns were classified into seven groups. Subsequently, significant target genes with prognostic impact were selected based on their cluster context and univariate analysis results (Table 1). As a result, BOK and BCL2L15 were selected as target genes for the pro-apoptotic group, while BCL2 was selected for the anti-apoptotic group. For pro-apoptotic genes, a score 0 was given for high expression above the median and a score of 1 was given for low expression below the median. For anti-apoptotic genes, a score of 1 was given for high expression above the median and a score 0 was given for low expression below the median. Ultimately, a BCL2 signature score of 0–3 points was calculated based on the sum of each score.

Furthermore, the relationship between BCL2 signature score and patients’ prognosis was analyzed. As the BCL2 signature score increased from 0 to 3 points, patients showed poor prognosis for EFS ($p = 0.0088$) and OS ($p = 0.0015$) (Figure S7). When combining score 1 and 2, which showed similar prognostic outcomes, score 0 had the best prognosis, score 1/2 had intermediate prognosis, and score 3 had the worst prognosis (EFS, $p = 0.0054$; OS, $p = 0.0011$) (Figure 3 A and B). Multivariate analysis, including COO and IPI, showed that increase in the BCL2 signature score was significantly associated with poor prognosis for EFS, independent of COO and IPI. Although statistical significance was not achieved for OS, a similar trend was observed (Table 2). To verify the prognostic impact of the BCL2 signature score, the same scoring method was applied to an independent dataset (Figure 3 C and D). Although a clear trend was observed in which higher BCL2 signature scores were associated with poorer progression free survival (PFS) and OS, statistical significance was not achieved (PFS, $p = 0.071$; OS, $p = 0.089$) (Figure 3 C and D).

4 Discussion

There has been an ongoing unmet clinical need for treatment regimens beyond R-CHOP in DLBCL, but so far, there have been no promising results [3, 26, 27, 36]. The selective BCL2 inhibitor venetoclax was expected to be effective in DLBCL due to its mechanism of restoring apoptosis in malignant cells [16], but it has shown limited results in DLBCL [7]. The limited effectiveness of venetoclax in DLBCL, unlike in CLL, suggests that apoptosis in DLBCL may also be regulated through interactions of other BCL2 family proteins [20]. A recent report has shown that pro-apoptotic signaling defects are associated with poor response to venetoclax treatment in high-grade non-Hodgkin’s lymphoma [32]. Therefore, we hypothesized that the diverse interactions of BCL2 family members in DLBCL have caused the limited results so far. In this study, we identified specific expression patterns in the
heterogeneous expression of BCL2 family members in DLBCL that were associated with poor prognosis. Based on those expression patterns, BCL2 signature score was calculated. As summarized in Figure 4, apoptosis of tumor cells in DLBCL and resulting patient outcomes are determined by the composite interplay between the anti-apoptotic BCL2 and pro-apoptotic BOK and BCL2L15.

We analyzed the expression patterns of BCL2 family members using unsupervised HCA and there were some interesting findings in the expression patterns. C1, which had the best prognosis, and C6, which had the worst prognosis, generally showed opposite expression patterns. However, anti-apoptotic gene BCL2 was also upregulated in the C1. While these results may seem paradoxical when considering only BCL2, the tendency towards favorable prognosis in the C1 group in our study can be inferred by integrating the expression of other pro-apoptotic genes. Increased expression of pro-apoptotic BH3-only group genes, such as PMAIP1, in poor prognostic group is another observable result in this study. Similar findings have also been reported in prostate cancer [9]. The expression patterns and functions of genes belonging to the pro-apoptotic BH3-only group are complex, and additional research is needed to fully understand their molecular mode of action and their potential as tumor suppressor or tumorigenesis. Therefore, to predict the impact of BCL2 family on patient prognosis, it is necessary to perform an integrated analysis of the overall expression pattern and functional tendency, rather than focusing on individual genes.

In our study, BCL2 signature score 3 was found to be associated with generally poor prognosis in DLBCL. However, this association was more statistically significant in terms of EFS than OS. In multivariate analysis, it also showed statistical significance as an independent prognostic factor for EFS, regardless of IPI and COO. This trend was also repeated in an independent validation set. Because EFS provides a more direct assessment of treatment efficacy than OS, it can be considered a tumor-centered endpoint [8, 11]. These results suggest that the BCL2 signature score we derived may reflect tumor-centric nature, such as chemoresistance in DLBCL. The chemoresistance of DLBCL is known to be caused by blocking drug-induced apoptosis due to deregulation of BCL2 family members [17, 22]. Since the BCL2 signature score reflects this tumor-centric nature, it may show more positive results for tumor-based recurrence prediction. Further studies on the complex interplay of tumor and host factors associated with overall prognosis related to BCL2 family members should be conducted in the future.

To our knowledge, this study is the first comprehensive analysis of the expression patterns of all known BCL2 family members in DLBCL. Additionally, the use of the NanoString method, which is known to provide stable RNA quantification results in FFPE tissue, allowed for robust and reliable data on BCL2 family member expression to be obtained from a large number of archived samples [23]. Another advantage of this study is the detailed clinicopathologic information and long-term follow-up duration of enrolled cohorts. However, there are some limitations to this study. First, the relatively small number of subjects makes it necessary to verify some negative results.
in subgroup analysis with a larger number of subjects in future studies. Second, there is no standard method for determining cut-off values for BCL2 family member over-expression, so median values had to be used reflecting the use of routine statistical divisions in the absence of an underlying justification for division of expression levels.

5 Conclusion

In biologically heterogeneous disease such as DLBCL, targeting a single type of structure such as one BCL2 family member may not be sufficient to achieve therapeutic efficacy. In particular, for genes like BCL2 family members that exhibit diverse functions and interactions, a comprehensive approach is needed to design novel therapeutics. The BCL2 signature score we proposed in this study provides information on BCL2 family deregulation based on the equilibrium of pro-versus anti-apoptotic members, which can aid in the development of new treatment strategies for DLBCL in the future.

6 Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References


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Author information
Jin Roh and Hyo-Kyung Pak have contributed equally to this work.

Authors and Affiliations
Department of Pathology, Ajou University School of Medicine, Suwon, 16499, Republic of Korea
Jin Roh, Do Eon Kim & Jae Ho Han

Department of Hematology-Oncology, Ajou University School of Medicine, Suwon, 16499, Republic of Korea
Joon Seong Park, Seong Hyun Jeong & Yoon Seok Choi

Asan Institute for Life Science, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea
Hyo-Kyung Pak, So-Jeong Kim & Hyunji Kim
Department of Medical Science, AMIST, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea
Seongfeel Jeong, Sewon Hwang & Hwal-Seok Choi

Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea
Hyo-Kyung Pak, Seongfeel Jeong, Sewon Hwang, Hwal-Seok Choi, So-Jeong Kim, Hyunji Kim & Chan-Sik Park

Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, 05505, Republic of Korea
Hyungwoo Cho & Dok Hyung Yoon

Contributions
C-S.P. and D.H.Y. contributed to conceptualization, study design, and editing; J.R. and H-K.P. contributed to drafting the manuscript and coordinating the data collection and analysis; S-F.J. contributed to data analysis. D.E.K., H-S.C., S-J.K., S.H., and H.K. contributed to the interpretation of the statistical results and manuscript editing. H.C., D.H.Y., J.S.P., S.H.J., and Y.S.C. contributed to the patient care and collected the clinical data; J.R., C-S.P., and J.H.H. made the histopathological diagnoses. All authors had full access to the data, carefully reviewed the manuscript, and approved the final version.

Corresponding authors
Correspondence to Chan-Sik Park or Dok Hyung Yoon.

Ethics declarations

Conflict of interest
The authors declare that they have no competing interests.

Ethics approval and consent to participate
This study was approved by the Institutional Review Boards of Asan Medical Center and Ajou University Hospital.

Supplementary information. The Supplementary Information for this article can be found online.
Fig. 1  The expression pattern of BCL2 family members in DLBCL. (A) The overall expression pattern of BCL2 family members in DLBCL patients was visualized as a heatmap. The colors represent the mRNA expression levels of each gene. Blue represents low expression levels, while red represents high expression levels of each gene. On the right side of the heatmap, median expression values of each BCL2 family member are visualized. (B) The overall expression patterns of BCL2 family members in CLL were represented by a heatmap. On the right side of the heatmap, median expression values of each BCL2 family member are visualized. (C–J) Comparison of average expression levels of BCL2 family members by functional group according to IPI and COO. (C–F) Expression of (C) pro-apoptotic, (D) anti-apoptotic, (E) pro-apoptotic BH3-only, and (F) Other groups in DLBCL according to the IPI. (G–J) Expression of (G) pro-apoptotic, (H) anti-apoptotic, (I) pro-apoptotic BH3-only, and (J) Other groups in DLBCL according to the COO. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; DLBCL, diffuse large B-cell lymphoma; IPI, International Prognostic Index; COO, cell of origin.
Fig. 2 Unsupervised hierarchical cluster analysis according to the BCL2 family member expression. (A) A heatmap representing the expression of BCL2 family members in 7 distinct clusters identified by unsupervised hierarchical clustering. (B) A heatmap showing the average expression levels of BCL2 family members for each cluster. (C) Kaplan-Meier survival curves comparing the overall survival rates of C1 and C6, which show distinct prognostic impacts, with other clusters.
Fig. 3 Survival analysis according to the BCL2 signature score. Kaplan-Meier survival curves comparing the (A) event-free and (B) overall survival rates of enrolled DLBCL patients according to BCL2 signature score. Kaplan-Meier survival curves comparing the (C) progression-free and (D) overall survival rates of independent DLBCL dataset (GSE87371).

Fig. 4 Conceptual diagram of the impact of BCL2 signature score on prognosis in DLBCL patients. In DLBCL, pro- or anti-apoptotic BCL2 family members are expressed to varying degrees, and the overall expression levels of these members determine the survival or death of tumor cells. Eventually, the prognosis of DLBCL patients is influenced by the BCL2 family gene expression signature.
Table 1  Univariate analysis of the BCL2 family members expression for OS in DLBCL patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genes</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-apoptotic</td>
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<td>0.97</td>
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<td></td>
<td>BCL2L13</td>
<td>0.74 (0.44–1.26)</td>
<td>0.27</td>
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<tr>
<td></td>
<td>SPIKE</td>
<td>0.73 (0.43–1.23)</td>
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<tr>
<td>Anti-apoptotic</td>
<td>BCL2</td>
<td>1.70 (0.99–2.91)</td>
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</tr>
<tr>
<td></td>
<td>BCL2L1</td>
<td>1.10 (0.65–1.86)</td>
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<td>0.45 (0.26–0.78)</td>
<td>&lt; 0.01</td>
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<td></td>
<td>MCL1</td>
<td>1.20 (0.71–2.03)</td>
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<td></td>
<td>BCL2A1</td>
<td>0.94 (0.55–1.59)</td>
<td>0.81</td>
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<td>BNIP2</td>
<td>0.77 (0.46–1.31)</td>
<td>0.34</td>
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<tr>
<td>Pro-apoptotic(BH3-only)</td>
<td>BAD</td>
<td>0.67 (0.39–1.14)</td>
<td>0.14</td>
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<td></td>
<td>PMAIP1</td>
<td>1.68 (0.98–2.88)</td>
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<td></td>
<td>BCL2L11</td>
<td>0.81 (0.48–1.38)</td>
<td>0.44</td>
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<tr>
<td></td>
<td>BBC3</td>
<td>0.83 (0.49–1.40)</td>
<td>0.48</td>
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<tr>
<td></td>
<td>BID</td>
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<td>BMF</td>
<td>1.28 (0.76–2.18)</td>
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<td>BIK</td>
<td>0.84 (0.50–1.42)</td>
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<td>BCL2L14</td>
<td>1.03 (0.61–1.74)</td>
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<td>Other</td>
<td>BCAP31</td>
<td>1.00 (0.59–1.70)</td>
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<td>MNT</td>
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<td>BECN1</td>
<td>0.65 (0.38–1.12)</td>
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Table 2  Multivariate analysis of the EFS and OS in DLBCL patients.

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<th>Covariates</th>
<th>HR (95% CI)</th>
<th>p value</th>
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<td>Score 3</td>
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<td>EFS IPI (0-2)</td>
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<tr>
<td>IPI (3-5)</td>
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<td>GCB</td>
<td>reference</td>
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<tr>
<td>ABC</td>
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<td>reference</td>
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<tr>
<td>Score 1/2</td>
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<tr>
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<td>OS IPI (0-2)</td>
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<td>IPI (3-5)</td>
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<tr>
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<td>1.92 (0.91–4.07)</td>
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</tr>
</tbody>
</table>
**Supplementary Files**

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- SupplBCL2familyApoptosis.docx