Identification of B-cell translocation gene 1-controlled gene networks in diffuse large B-cell lymphoma: A study based on bioinformatics analysis

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Abstract. B-cell translocation gene 1 (BTG1) is a member of the BTG/transducer of Erb family. The present study evaluated the impact of BTG1 gene expression on the clinical outcome of diffuse large B-cell lymphoma (DLBCL) and investigated potential mechanisms using the Gene Expression Omnibus (GEO) database. The gene expression profile datasets GSE31312, GSE10846, GSE65420 and GSE87371 were downloaded from the GEO database. BTG1 expression and clinicopathological data were obtained from the GSE31312 dataset. In 498 cases, the expression of BTG1 in DLBCL was associated with treatment response (χ^2=19.020; P<0.001) and International Prognostic Index score (χ^2=5.320; P=0.025). Using the Kaplan-Meier method, it was identified that the expression of BTG1 was associated with overall survival (OS) and progression-free survival (PFS) times. Univariate and multivariate Cox regression analysis demonstrated that BTG1 was an independent predictive factor for OS and PFS. From the overlapping analysis of 407 BTG1-associated genes and 22,187 DLBCL-associated genes, 401 genes were identified as BTG1-associated DLBCL genes. Pathway analysis revealed that BTG1-associated DLBCL genes were associated with cancer progression and DLBCL signaling pathways. Subsequently, a protein-protein interaction network was constructed of the BTG1-associated genes, which consisted of 235 genes and 601 interactions. Additionally, 24 genes with high degrees in the network were identified as hub genes, which included genes associated with 'ribosome' [ribosomal protein (RP) L11, RPL3, RPS29, RPL19, RPL15 and RPL12], 'cell cycle' (ubiquitin carboxyl extension protein 52, ATM and Ras homolog family member H), 'mitogen-activated protein kinase pathway' (mitogen-activated protein kinase 1), 'histone modification' (ASH1-like protein) and 'transcription/translation' (eukaryotic translation initiation factor 3 subunit E, eukaryotic translation elongation factor 1 δ, transcription termination factor 1, cAMP responsive element binding protein 1 and RNA polymerase II subunit F). In conclusion, BTG1 may serve as a predictive biomarker for DLBCL prognosis. Additionally, bioinformatics analysis indicated that BTG1 may exhibit key functions in the progression and development of DLBCL.

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Abbreviations: BTG1, B-cell translocation gene 1; DLBCL, diffuse large B-cell lymphoma; GEO, Gene Expression Omnibus; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; OS, overall survival; PFS, progression-free survival; IPI, International Prognostic Index; GCB, germinal center B-like; BCR, B-cell receptor; HR, hazard ratio; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; MCODE, Molecular Complex Detection; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease; DEG, differentially-expressed gene; RP, ribosomal protein; mTOR, mammalian target of rapamycin

Key words: diffuse large B-cell lymphoma, B-cell translocation gene 1-associated genes, prognosis, enrichment analysis, interaction network

Introduction

Despite improvements in diagnostic techniques, the incidence rate of lymphoma has been increased by 75% in the past 20 years (1). Less than half of the patients who are diagnosed with diffuse large B-cell lymphoma (DLBCL) achieve complete remission (2). Patients with DLBCL exhibit various clinical outcomes due to tumors possessing different histology, morphology and clinical features (3). Treatment for patients with DLBCL includes combinations of radiation therapy, chemotherapy and targeted therapy. The long-term remission rate of the disease has improved with the introduction of rituximab; however, this treatment has poor efficacy in certain patients (4). Therefore, there is an increasing requirement to further understand the molecular mechanisms underlying the disease. This would assist with survival prediction and enable the design of improved targeted therapeutic strategies.

DLBCL is one of the most studied diseases for prognostic markers. Since its publication, the International Prognostic Index (IPI) has been used to predict the prognosis of patients

with DLBCL. Immunohistochemistry has been used to classify DLBCL into germinal center B-like (GCB) and non-GCB subgroups, with various positive staining combinations of cluster of differentiation 10, mutated melanoma-associated antigen 1, B-cell lymphoma 6 and CD138 (5). Numerous studies have confirmed that GCB subgroups improve prognosis estimations of DLBCL (6-8). According to the 2016 World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissue, Epstein-Barr virus-positive DLBCL is frequently diagnosed in immunocompromised patients and demonstrates a poor response to treatment (9). Previously, a number of studies investigated MYC. Multivariate analysis illustrated that extra copies of MYC and MYC rearrangement in DLBCL are independent poor prognostic factors (10). Numerous studies confirmed that the development and progression of DLBCL is associated with multiple signaling pathways, including the Wnt, nuclear factor-κB (NF-κB), mammalian target of rapamycin (mTOR) and B-cell receptor (BCR) signaling pathways (11,12).

B-cell translocation gene 1 (BTG1) is a member of the BTG/transducer of Erb (TOB) family. This family consists of six members, BTG1, BTG2/PC3/TIS21, BTG3, BTG4/PC3B, TOB1 and TOB2, which regulate cell cycle progression and differentiation, and inhibit proliferation (13). The BTG/TOB family consists of two characteristic and conserved domains, Box A and Box B (14). Additionally, BTG/TOB proteins are nuclear proteins that are transported into the nucleus by nuclear localization signaling (15). Human BTG1 is located on chromosome 12q22 and consists of 4,704 nucleotides that encode 171 amino acids and a 19 kDa protein (16). BTG1 promotes apoptosis, stimulates cellular differentiation, maintains cell cycle progression and inhibits proliferation, and therefore functions as a tumor suppressor gene (17). A previous study identified that BTG1 expression is increased in the G0/G1 phase and decreased in the G1 phase of the cell cycle (18). Therefore, BTG1 is considered to be a potential suppressor gene due to its effects on cell cycle progression and proliferation (19). BTG1 interacts with arginine N-methyltransferase 1 *in vitro,* which regulates transcription and affects cytokine signaling pathways (20). BTG1 enhances the inhibitory function of homeobox B9-mediated transcription (21). Additionally, overexpression of BTG1 enhances apoptosis of NIH/3T3 cells (22). A recent study revealed that BTG1 serves as a tumor suppressor in B-cell precursor acute lymphoblastic leukemia (23). Similarly, another study demonstrated that BTG1 acts as a regulator of B-cell differentiation, which supports a role of BTG1 as a tumor suppressor in B-cell malignancies (24). However, a limited number of studies have performed global network analysis for BTG1, which limits the investigation of BTG1's role in DLBCL.

The present study investigated the association between BTG1 expression and clinicopathological parameters in patients with DLBCL. Subsequently, the prognostic value and functional mechanism of BTG1 in DLBCL were further analyzed by utilizing certain bioinformatics methods. Additionally, Oncomine analysis was performed, which revealed that BTG1 was downregulated in DLBCL. Furthermore, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and Cytoscape analysis demonstrated that hub genes of BTG1-associated DLBCL interaction networks were enriched in 'Ribosome', 'Cell cycle' and 'B cell receptor signaling pathway'. In conclusion, BTG1 may serve as an independent predictor for DLBCL prognosis and as a potential therapeutic target.

Materials and methods

Patient characteristics from the Gene Expression Omnibus (GEO) database and statistical analysis. A gene expression profile, GSE31312 (25), was downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo). GSE31312 is a human DLBCL expression profile that contains BTG1 expression data, which were sequenced using the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array). GSE31312 contains 498 samples of DLBCL, of which 470 samples have clinical data. According to GSE31312 data, the median value of BTG1 expression was calculated and the 470 patients with BTG1 expression ≥4.34 were placed in the high expression group and patients with BTG1 expression <4.34 were placed in the low expression group. The association between BTG1 expression level and numerous factors, including sex, age, Ann Arbor stage (26), Eastern Cooperative Oncology Group (ECOG) score, subtype, IPI score, B symptoms, bulky disease, lactate dehydrogenase (LDH) level, treatment response and survival data, were analyzed by extraction of clinical data from GSE31312. All analysis was performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Associations between BTG1 expression and clinical parameters were examined with the χ^2 test. Survival analysis was performed using the Kaplan-Meier method and differences were analyzed by log-rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to identify independent predictors. The hazard ratios (HRs) and 95% confidence interval (CIs) of the prognostic factors were calculated. $P<0.05$ was considered to indicate a statistically significant difference.

Microarray data and data processing. Expression levels of BTG1 in DLBCL were obtained from the Oncomine database (http://www.oncomine.com/resource/main.html). The GSE31312 (25), GSE10846 (27) and GSE87371 (28) datasets were downloaded from the GEO database and the R2 platform (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi) was applied to identify the BTG1-associated genes. The cut-off point was defined as: $P<0.01$ and PresCalls ≥1. Only BTG1-associated genes identified in all three independent datasets were selected. Furthermore, GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) was applied to reveal differentially-expressed genes (DEGs) in DLBCL, compared with normal lymphocytes. The following cut-off criteria was applied: $P<0.05$ and |log (fold-change) |>1. A Venn diagram was generated to visualize the overlapping BTG1-associated genes and DLBCL-associated DEGs. The resulting overlapping genes were defined as BTG1-associated DLBCL genes.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. GO and pathway analysis were performed using the Database for Annotation,

Table I. Association between BTG1 expression level and clinical characteristics obtained from the GSE31312 dataset.

Characteristic	Case, n (%)	Low BTG1 expression, n	High BTG1 expression, n	χ^2 value	P-value
Sex				0.218	0.709
Male	199 (42.3)	138	133		
Female	271 (57.7)	97	102		
Age, years				0.690	0.460
<63	229 (48.7)	110	119		
≥63	241 (51.3)	125	116		
Stage				0.000	1.000
I/II	220 (46.8)	110	110		
III/IV	250 (53.2)	125	125		
ECOG score				0.209	0.732
Low	374 (79.6)	185	189		
High	96 (20.4)	50	46		
Subtype				0.034	0.926
Non-GCB	222 (47.2)	112	110		
GCB	248 (52.8)	123	125		
IPI score				5.320	0.025
Low	274 (64.6)	135	139		
High	150 (35.4)	79	71		
B symptom				0.513	0.526
No	276 (67.6)	138	138		
Yes	132 (32.4)	61	71		
Bulky disease				0.141	0.724
No	268 (73.2)	129	139		
Yes	98 (26.8)	45	53		
LDH level				0.071	0.839
Normal	148 (34.7)	76	72		
High	278 (65.3)	139	139		
Treatment response				19.020	<0.001
CR	354 (75.3)	157	197		
PR	72 (15.3)	48	24		
PD	24 (5.1)	15	9		
SD	20 (4.3)	15	5		

BTG1, B-cell translocation gene 1; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-like; IPI, International Prognostic Index; LDH, lactate dehydrogenase; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov/), and the KEGG database (29). P<0.05 indicated a statistically significant enriched GO and pathway term for the BTG1-associated DLBCL genes.

Establishment of a protein-protein interaction (PPI) network and cluster selection. The STRING database (http://string-db.org) was used to predict interaction networks of the protein products of BTG1-associated DLBCL genes. A confidence score of ≥0.4 was set as the cut-off point. Cytoscape 3.5.1 software (Institute for Systems Biology, Seattle, WA, USA) was used to construct the PPI networks for BTG1-associated DLBCL genes. The hub genes were identified using the cytohubba plugin in Cytoscape software and a degree ≥17 was set as the cut-off criterion. Molecular Complex Detection (MCODE) v1.5 (30) was subsequently used to reveal clusters of genes in the PPI network.

Results

Patient characteristics from the GSE31312 dataset. Patient data downloaded from GEO database are presented in Table I. The patients included 199 males and 271 females, and the median age at diagnosis was 63 years (range, 18-92 years). All patients were assessed according to the Ann Arbor staging system (26) and patients were divided into a low stage group (I and II; 220 patients) or high stage group (III and IV; 250 patients). A total of 374 patients had a low ECOG score (≤1) and 96 had a

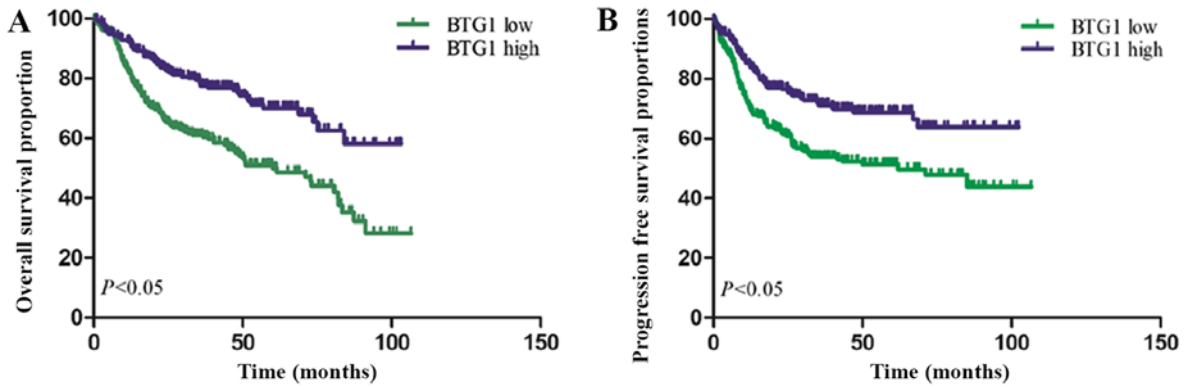

Figure 1. Kaplan-Meier survival curves for OS and PFS time of patients with diffuse large B-cell lymphoma stratified by median BTG1 expression level. (A) OS curve. (B) PFS curve. OS, overall survival; PFS, progression-free survival; BTG1, B-cell translocation gene 1.

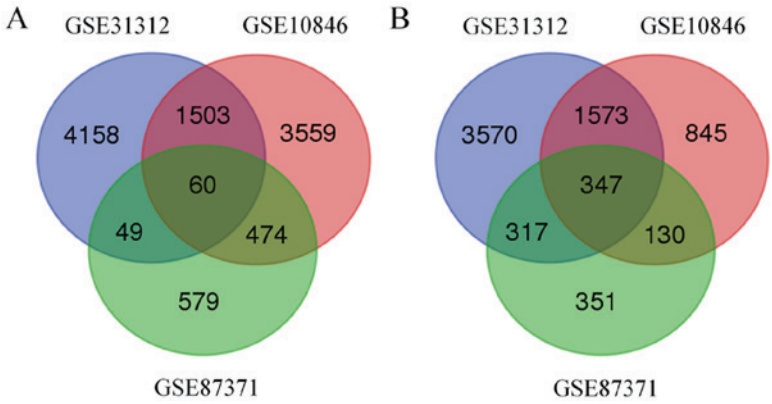

Figure 2. Identification of B-cell translocation gene 1-associated genes. (A) Downregulated and (B) upregulated genes in mRNA expression profiling datasets GSE31312, GSE10846 and GSE87371.

high ECOG score (>1). Information regarding IPI score was available for 424 cases. A total of 274 patients had a low IPI score (≤2) and 150 had a high IPI score (>3). Only 408 of the 470 cases had B symptom data, 366 cases had bulky disease data and 426 cases had LDH level data. The 470 cases were divided into complete response (CR), partial response (PR), progressive disease (PD) and stable disease (SD) groups, according to their treatment response.

Associations between BTG1 expression level and the clinical characteristics of patients with DLBCL. A total of 470 samples in the GSE31312 dataset contained BTG1 expression data. The associations between BTG1 expression level and clinical features of patients with DLBCL were investigated (Table I). It was identified that the BTG1 expression level was significantly different in treatment response (P<0.001) and IPI score (P=0.025) groups. However, no significant difference in BTG1 expression level was observed for age, sex, stage, subtype, ECOG score, B symptom, bulky disease or LDH level (P>0.05).

Prognostic performance of BTG1 for DLBCL. Based on the median expression level of BTG1, Kaplan-Meier analysis was preformed to estimate overall survival (OS_ and progression-free survival (PFS) times. As demonstrated in Fig. 1, Kaplan-Meier survival curves revealed that patients with low

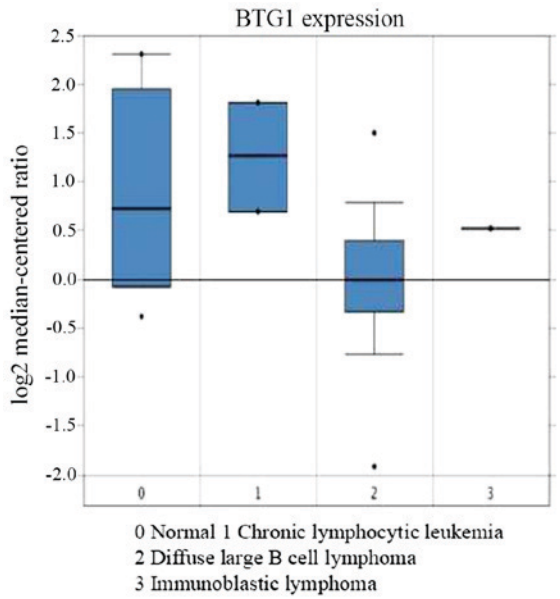

Figure 3. The mRNA expression level of BTG1 in diffuse large B-cell lymphoma, obtained from the Oncomine database. Data are present as the mean ± standard deviation. BTG1, B-cell translocation gene 1.

BTG1 expression exhibited a reduced OS time, compared with patients with high BTG1 expression (P<0.001). Furthermore,

Table II. Univariate and multivariate Cox regression analysis for patients with diffuse large B-cell lymphoma.

A, Overall survival

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Sex (males vs. females)	1.050 (0.775-1.423)	0.752		
Age (≥63 vs. <63), years	1.697 (1.246-2.312)	0.752	1.742 (1.244-2.441)	0.001
Stage (low vs. high)	2.307 (1.668-3.189)	<0.001	1.594 (1.103-2.302)	0.013
ECOG score (low vs. high)	2.021 (1.450-2.818)	<0.001	1.978 (1.376-2.844)	<0.001
Subtype (non-GCB vs. GCB)	0.668 (0.494-0.904)	0.009	1.978 (1.376-2.844)	<0.001
IPI score (low vs. high)	1.411 (1.026-1.940)	0.034		
B symptom (no vs. yes)	1.105 (1.787-1.551)	0.565		
LDH (normal vs. high)	1.120 (0.803-1.563)	0.503		
Bulky disease (no vs. yes)	1.051 (0.732-1.509)	0.787		
Treatment response (CR+PR vs. PD+SD)	2.605 (2.828-2.990)	<0.001	2.612 (2.214-3.081)	<0.001
BTG1 expression (low vs. high)	2.066 (1.508-2.829)	<0.001	1.692 (1.193-2.401)	0.003

B, Progression free survival

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Sex (males vs. females)	0.864 (0.639-1.169)	0.343		
Age (≥63 vs. <63), years	1.156 (0.855-1.563)	0.346		
Stage (low vs. high)	2.469 (1.781-3.422)	<0.001	1.538 (1.063-2.226)	0.022
ECOG score (low vs. high)	1.678 (1.191-2.366)	0.003	1.187 (0.815-1.729)	0.371
Subtype (non-GCB vs. GCB)	0.624 (0.461-0.846)	0.002	0.563 (0.406-0.782)	0.001
IPI score (low vs. high)	1.545 (1.124-2.124)	0.007		
B symptom (no vs. yes)	1.261 (0.902-1.763)	0.125		
LDH (normal vs. high)	1.078 (0.774-1.501)	0.659		
Bulky disease (no vs. yes)	1.175 (0.820-1.682)	0.379		
Treatment response (CR+PR vs. PD+SD)	2.401 (2.095-2.751)	<0.001	2.220 (1.889-2.607)	<0.001
BTG1 expression (low vs. high)	1.801 (1.324-2.449)	<0.001	1.403 (1.004-1.960)	0.047

HR, hazard ratio; CI, confidence interval; BTG1, B-cell translocation gene 1; ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-like; IPI, International Prognostic Index; LDH, lactate dehydrogenase; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

low BTG1 expression was identified to be associated with a reduced PFS time in patients with DLBCL (P<0.001).

To assess whether BTG1 is an independent prognostic factor for DLBCL, univariate and multivariate Cox regression analysis was performed. The results revealed that age (HR, 1.742; 95% CI, 1.244-2.441; P=0.001), stage (HR, 1.594; 95% CI, 1.103-2.302; P=0.013), ECOG score (HR, 1.978; 95% CI, 1.376-2.844; P<0.001), subtype (HR, 1.978; 95% CI, 1.376-2.844; P<0.001), treatment response (HR, 2.612; 95% CI, 2.214-3.081; P<0.001) and BTG1 expression (HR, 1.692; 95% CI, 1.193-2.401; P=0.003) were independent prognostic factors for OS time. Subsequently, multivariate Cox regression analysis was performed to determine the independence of the prognostic power of BTG1 for PFS time. The results demonstrated that stage (HR, 1.538; 95% CI, 1.063-2.226; P=0.022), subtype (HR, 0.563; 95% CI, 0.406-0.782; P=0.001), treatment response (HR, 2.220; 95% CI, 1.889-2.607; P<0.001) and BTG1 expression (HR, 1.403; 95% CI, 1.004-1.960; P=0.047) could predict a reduced PFS time for patients with DLBCL (Table II).

Analysis of BTG1-associated genes. BTG1-associated genes from DLBCL gene expression profiling datasets were identified using the R2 platform and the following criteria: P<0.01 and PresCalls ≥1. A total of 11,577, 8,491 and 2,307 genes were identified to be associated with BTG1 in the GSE1312, GSE10846 and GSE87371 datasets, respectively. Additionally, 407 BTG1-associated genes were identified in all three

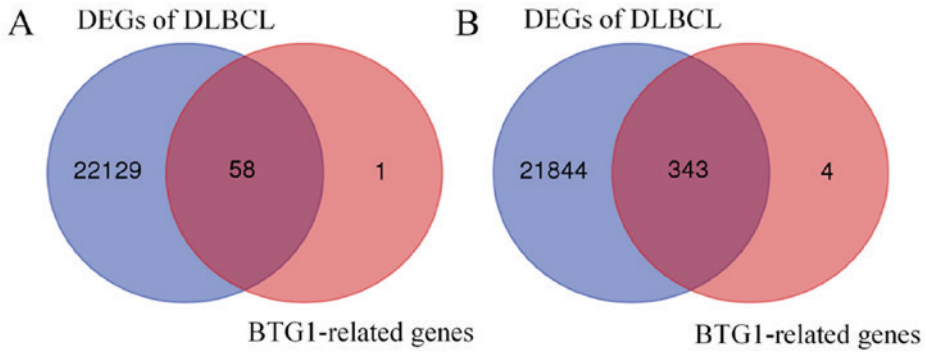

Figure 4. Venn diagram of BTG1-associated genes and DLBCL-associated genes. (A) Downregulated and (B) upregulated genes. BTG1, B-cell translocation gene 1; DLBCL, diffuse large B-cell lymphoma; DEG, differentially-expressed gene.

datasets (Fig. 2). Of the 407 BTG1-associated genes, 347 were upregulated and 60 were downregulated.

BTG1 serves a role in DLBCL progression. The association between BTG1 expression and DLBCL was then analyzed. Using Oncomine analysis, the expression of BTG1 was identified to be downregulated in DLBCL (31) (Fig. 3). Using the GSE65720 dataset and GEO2R analysis, a total of 22,187 DEGs were identified in DLBCL compared with normal lymphocytes. Overlapping analysis of the 407 BTG1-associated genes and the 22,187 DEGs revealed that 401 genes were BTG1-associated DLBCL genes (Fig. 4). Subsequently, GO and KEGG pathway analysis was performed to classify the 401 overlapping genes. The most significantly enriched GO terms were 'transcription' (GO: Biological process), 'nucleus' (GO: Cellular component) and 'protein binding' (GO: Molecular function) (Fig. 5). Additionally, KEGG pathway analysis revealed that the BTG1-associated DLBCL genes were involved in seven pathways, including 'Ribosome', 'Cell cycle' and 'B cell receptor signaling pathway' (Fig. 6). In summary, BTG1 may be involved in DLBCL progression.

Establishment of a PPI network and identification of hub genes. The STRING database and Cytoscape analysis were used to predict a potential interaction network for the BTG1-associated DLBCL genes. The PPI network was composed of 235 nodes and 601 edges, including 343 upregulated genes and 58 downregulated genes (Fig. 7). Additionally, when a degree ≥17 was set as the cut-off point, 24 genes in the PPI network were identified as hub genes, including ubiquitin carboxyl extension protein 52 (UBA52), ribosomal protein (RP) L11, mitogen-activated protein kinase 1 (MAPK1) and exosome component 4.

Furthermore, 11 clusters were selected from a PPI network using MCODE, which revealed that the most significant cluster consisted of 21 nodes and 203 edges. Additionally, MCODE analysis demonstrated that each cluster contained one 'seed' gene (32), including RPL31, hect domain and RLD 4 and heterogeneous nuclear ribonucleoprotein A3 (Fig. 8).

Discussion

DLBCL is the most common lymphoid malignancy, with the incidence rate of lymphoma in China reported as 643/100,000 in 2012, and is part of a heterogeneous group of fast growing neoplasms, which exhibit an aggressive clinical course (33). Multi-agent chemotherapy has the potential to cure ~40% of patients and combination with an anti-CD20 monoclonal antibody has further improved the treatment response for an additional 10-25% of patients (34). Despite improvements in therapy for DLBCL, 30% of patients do not respond to treatment attempts (35). The variation in prognosis for patients with DLBCL supports investigations of prognostic factors that can predict treatment response and the clinical course.

The BTG family serves a role in cancer, as BTG proteins can regulate the cell cycle (36). As a member of the BTG family, BTG1 has been identified to possess a t (q24;q22) translocation in B-cell chronic lymphocytic leukemia and serve as a biomarker for complete remission of acute lymphoblastic leukemia (37). Additionally, BTG1 is considered to be a tumor suppressor gene that is typically downregulated in various types of cancer, including colorectal, ovarian and renal cancer (13,20,38). However, to the best of our knowledge, the role of BTG1 in DLBCL remains unclear. The present study performed systemic bioinformatics analysis to investigate the mechanism and gene network of BTG1 in DLBCL.

The present study investigated the association between BTG1 and clinical characteristics, as well as the diagnostic value of BTG1 for DLBCL. According to 470 samples obtained from the GSE31312 dataset, the expression level of BTG1 was associated with treatment response and IPI score. Furthermore, univariate and multivariate Cox regression analysis indicated that BTG1 expression level was a prognostic factor for overall survival and progression-free survival times. Although clinical data is missing for 28 patients, which may have certain effects on the results, it can be indicated that BTG1 is a protective factor in DLBCL.

A total of 401 BTG1-associated DLBCL genes were identified from the GSE31312, GSE10846 and GSE87371 datasets, consisting of 343 upregulated genes and 58 downregulated genes. These genes were enriched in seven pathways, including 'Ribosome', 'Cell cycle' and 'B cell receptor signaling pathway'. According to their degree in the PPI network, 24 genes were recognized as hub genes. The hub genes were associated with 'Ribosome' (RPL11, RPL5, RPS15, RPS14, RPL22 and RPL37), 'Cell cycle' (UBA52, ATM and Ras homolog family member H), 'MAPK pathway' (MAPK1), 'histone modification' (ASH1-like protein) and 'transcription/translation' (eukaryotic translation

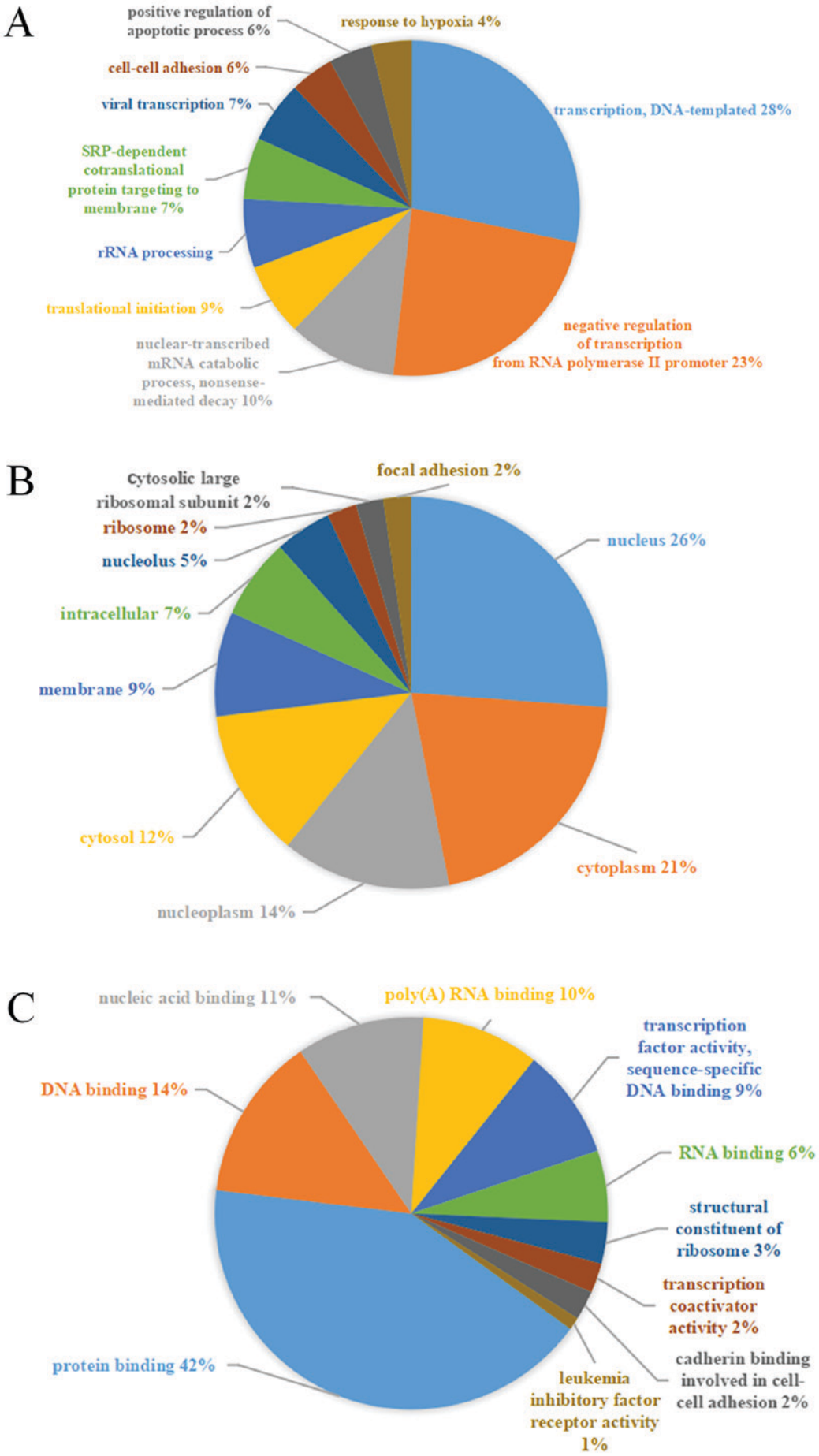

Figure 5. Enriched Gene Ontology terms for B-cell translocation gene 1-associated diffuse large B-cell lymphoma genes. (A) Biological process terms. (B) Cellular component terms. (C) Molecular function terms.

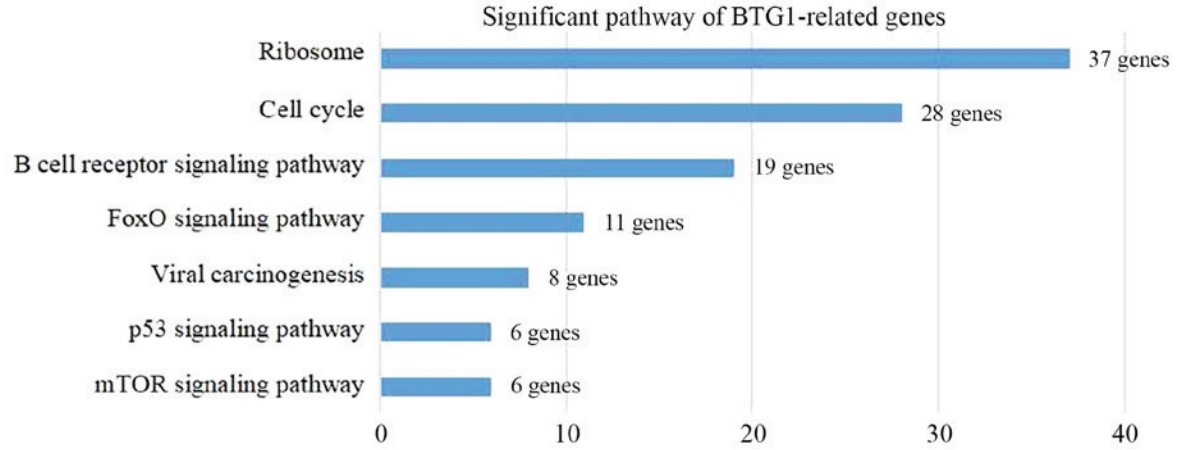

Figure 6. Enriched pathways for BTG1-associated diffuse large B-cell lymphoma genes. BTG1, B-cell translocation gene 1; FoxO, forkhead box O; mTOR, mammalian target of rapamycin.

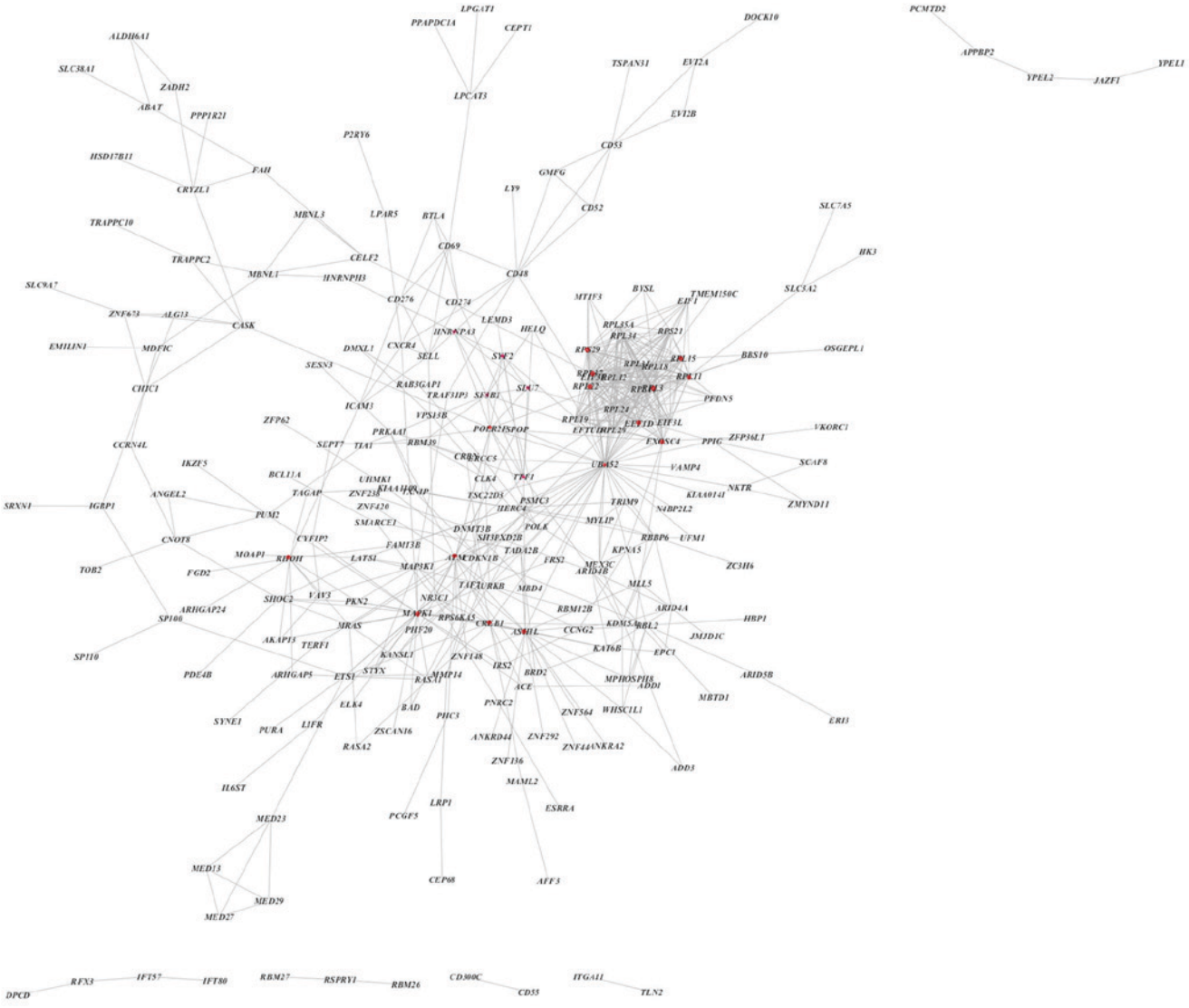

Figure 7. Protein-protein interaction network of B-cell translocation gene 1-associated genes in diffuse large B-cell lymphoma. Red nodes indicate hub genes.

initiation factor 3 subunit E, eukaryotic translation elongation factor 1 δ, transcription termination factor 1, cAMP responsive element binding protein 1 and RNA polymerase II subunit F).

Notably, a panel of genes that encode RPs, including RPL11, RPL3, RPS29, RPL19, RPL15 and RPL12, were identified to be highly associated with the expression level of BTG1. Cancer

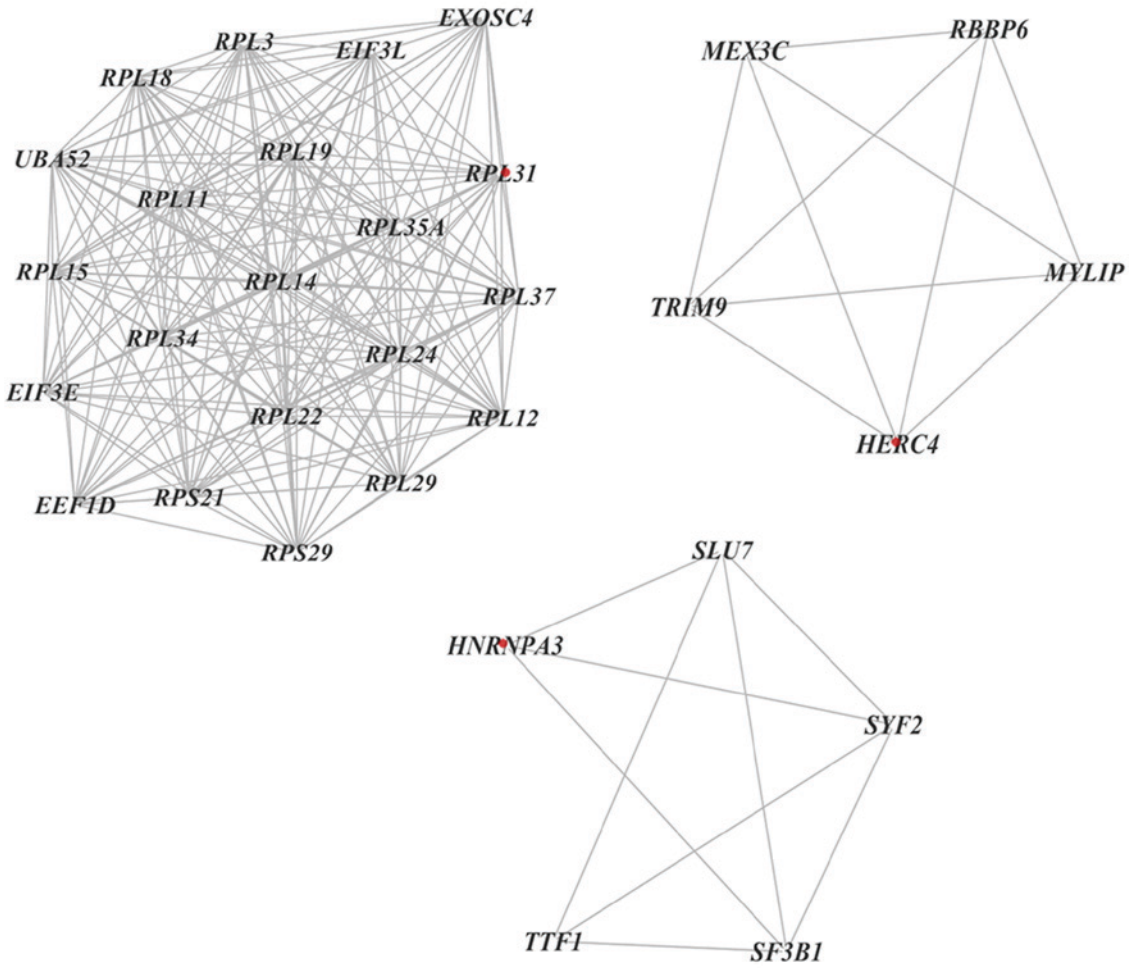

Figure 8. Three most significant clusters selected from the protein-protein interaction network. Red nodes indicate seed genes.

cells require large amounts of protein and increased protein synthesis, and consequently require efficient ribosome translational machinery (39). Therefore, a number of carcinogens and tumor suppressors, including p53, p21 and mMRPS36, frequently affect the growth of cancer cells by regulating ribosome biogenesis and protein synthesis (40). Numerous RPs, including RPL11, RPL5, RPL37, RPS15 and RPS14, have been identified to suppress tumor cell proliferation by regulating the mouse double minute (MDM) 2 homolog/MDMX-p53 cascade (41-44). RPL11 has also been revealed to suppress c-Myc activity and promote microRNA (miR)-24/miR-induced silencing complex-mediated c-Myc mRNA degradation (45). Additionally, mutations in certain RP-encoding genes, including RPL5 and RPL22, in tumors further indicates that RPs can be regarded as tumor suppressors (46).

A high degree of interaction was also observed between the hub genes UBA52, MAPK1 and BTG1. Ubiquitination is an important post-translational modification. UBA52 encodes a fusion protein, which consists of ubiquitin at the N-terminus and RPL40 at the C-terminus. UBA52 deficient cells exhibit inhibited protein synthesis and cell cycle arrest (47). As an ubiquitin-coding gene, UBA52 also serves a role in the regulation of the ribosomal protein complex (48). The MAPK signaling cascade is a pathway that mediates the proliferation and differentiation of hematopoietic cells. Among the MAPKs, MAPK1 serves a role in various mitogenic signaling pathways and participates in a diversity of cellular programs, including cell cycle progression and differentiation (49).

The majority of hub genes associated with BTG1 were identified to be involved in the ribosomal, cell cycle and p53 pathways. These results were consistent with GO and KEGG analysis of the BTG1-associated DLBCL genes. Other pathways identified by KEGG analysis included the BCR signaling pathway, the forkhead box O (FoxO) signaling pathway and the mTOR signaling pathway. DLBCL activates BCR signaling to maintain malignant growth and survival, which is mediated by NF-κB and other signals (12). The FoxO proteins are a subfamily of the fork head transcription factor family, which exhibit important roles in cell fate and tumor suppression (50). mTOR has been investigated for a number of years as a central regulator of cell growth, proliferation, survival and differentiation (51). The mechanism of BTG1 in DLBCL may involve these aforementioned pathways. However, potential mechanisms have not been investigated in DLBCL *in vivo* or *in vitro*. Therefore, further studies are required to support the results of the present study.

In conclusion, the present study indicated that BTG1 may be an independent prognostic factor for DLBCL and may serve a role in the progression and development of the disease. The aim of the present study was to predict the mechanism of BTG1 in DLBCL using bioinformatics analysis. It was identified that BTG1 may interact with RPs, UBA52, MAPK1

and other genes to participate in the development of DLBCL, which would involve numerous tumor-associated signaling pathways. Future studies are required to verify the potential regulatory network proposed in the present study.

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Availability of data and material

The datasets analyzed during the current study are available from the GEO database (http://www.ncbi.nlm.nih.gov/geo). All clinicopathological data analyzed during this study are included in the published GEO dataset, GSE31312 (25).

Authors' contributions

WYan and WYang designed the study and conducted bioinformatics analysis. WYan, SXL and HG performed statistical analysis, participated in data collection and drafted the manuscript. WYang supervised the scientific work and revising it critically amended the manuscript. She also gave final approval of the version to be published. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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