Identification of key candidate genes and miRNA-mRNA target pairs in chronic lymphocytic leukemia by integrated bioinformatics analysis

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Abstract. Chronic lymphocytic leukemia (CLL) is a malignant clonal proliferative disorder of B cells. Inhibition of cell apoptosis and cell cycle arrest are the main pathological causes of this disease, but its molecular mechanism requires further investigation. The purpose of the present study was to identify biomarkers for the early diagnosis and treatment of CLL, and to explore the molecular mechanisms of CLL progression. A total of 488 differentially expressed genes (DEGs) and 32 differentially expressed microRNAs (miRNAs; DEMs) for CLL were identified by analyzing the gene chips GSE22529, GSE39411 and GSE62137. Functional and pathway enrichment analyses of DEGs demonstrated that DEGs were mainly involved in transcriptional dysregulation and multiple signaling pathways, such as the nuclear factor-κB and mitogen-activated protein kinase signaling pathways. In addition, Cytoscape software was used to visualize the protein-protein interactions of these DEGs in order to identify hub genes, which could be used as biomarkers for the early diagnosis and treatment of CLL. Cytoscape software was also used to analyze the association between the predicted target mRNAs of DEMs and DEGs and increase knowledge about the miRNA-mRNA regulatory network associated with the progression of CLL. Taken together, the present study provided a bioinformatics basis for advancing our understanding of the pathogenesis of CLL by identifying differentially expressed hub genes, miRNA-mRNA target pairs and molecular pathways. In addition, hub genes may be used as novel biomarkers for the diagnosis of CLL and to guide the selection of CLL drug combinations.

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Introduction

Chronic lymphocytic leukemia (CLL) is a clonal proliferative tumor of mature B lymphocytes characterized by the accumulation of lymphocytes in the peripheral blood, bone marrow, lymph nodes and spleen (1). It is the most common type of leukemia in western populations, causing ~5,000 mortalities in the United States of America annually (2). Unfortunately, the exact molecular mechanism underlying the development of CLL remains unclear, which limits early diagnosis and timely treatment. Therefore, exploring the molecular mechanism of CLL progression and its biomarkers to improve the likelihood of early diagnosis and treatment have become the focus in CLL research. Increasing evidence suggests that numerous genes, miRNAs and cellular pathways are involved in the occurrence and progression of CLL (3,4).

In recent years, the rapid development of computer-based technologies, such as molecular dynamics simulation, has gradually revealed novel molecular mechanisms of the disease (5,6). Through the combination of Gene Expression Omnibus (GEO) chip, The Cancer Genome Atlas data mining (cancergenome.nih.gov/) and computer-based analysis techniques, a series of highly specific and sensitive markers have been reported (7-9). Gene chips are widely used as a genetic testing tool as they can be used to detect rapid gene expression of a sample at a certain time point and are particularly suitable for the screening of differentially expressed genes (10). MicroRNAs (miRNAs/miRs) are endogenous non-coding small RNAs which have several important regulatory roles in cells (11). Computer-based analysis techniques have demonstrated a mutation of a miRNA binding site that is associated with cancer and revealed its mechanism of gene regulation (12).

In the present study, two original gene microarray datasets (GSE22529 and GSE39411) and one original miRNA microarray dataset (GSE622137) were downloaded from the National Center for Biotechnology Information-Gene Expression Omnibus database (NCBI-GEO). The raw gene microarray datasets were initially processed online using the Gene-Cloud of Biotechnology Information (GCBI) software to screen for differentially expressed genes (DEGs) in CLL and normal samples. In addition, the Gene Ontology (GO) Consortium and the Kyoto Encyclopedia of Genes and Genomes (KEGG), in conjunction with the Database for Annotation, Visualization, and Integrated Discovery (DAVID) software program were used to analyze the pathway enrichment of DEGs. The STRING online database and Cytoscape software were then used to develop the protein-protein interaction (PPI) network of the DEGs and for modular analysis to identify CLL central genes. In addition, GEO2R analysis of the miRNA dataset was employed to obtain the differentially expressed miRNAs (DEMs). Cytoscape software was used to analyze the association between the predicted target mRNAs of DEMs and DEGs, which helped to define the regulatory network of miRNA-mRNA in relation to the development of CLL. Our findings suggest that data mining and integration may be useful methods for predicting and understanding the mechanisms of CLL disease development and progression. Ultimately, the early diagnosis and personalized treatment of CLL may be facilitated by identifying new biomarkers (3).

Materials and methods

Microarray data. Data of gene chips GSE22529 (13), GSE39411 (14) and GSE62137 (15) were obtained from the GEO database (www.ncbi.nlm.nih.gov/geo). GSE22529 was based on Agilent GPL96 (Agilent Technologies, Inc., Santa Clara, CA, USA; [HG-U133A] Affymetrix Human Genome U133A Array, Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and GPL97 [(HG-U133B) Affymetrix Human Genome U133B Array, Affymetrix; Thermo Fisher Scientific, Inc.)]. To reduce the offset, based on the GCBI online laboratory (www.gcbi.com.cn/gclib/html/index), the GPL96 annotation data set as the research object, was screened. The GSE22529 dataset included 52 samples, containing 41 CLL samples and 11 normal samples. The GSE39411 data were based on GPL570 [(HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array; Affymetrix, Thermo Fisher Scientific, Inc.], consisting of 41 CLL samples and 11 normal samples. The GSE62137 data were based on GPL14767 [(Agilent-021827 Human miRNA Microarray G4470C)], consisting of 15 CLL samples and 3 normal samples.

Identification of DEGs and DEMs. To identify DEGs in the CLL and normal samples, a web-based online tool, GCBI, was utilized. GCBI is an interactive online tool that allows users to compare two or more groups of samples in a GEO series and analyzes the majority of GEO series with gene symbols. DEGs were screened according to $P<0.05$ and |log fold change (FC)|>1.5. DEMs were obtained by analyzing miRNA expression profiles using the GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) and screening according to the |log FC|>2 and $P<0.05$. In addition, DEG heat maps from datasets GSE22529 and GSE39411, were created using Morpheus online software (software.broadinstitute.org/morpheus).

GO and pathway enrichment analysis of DEGs. DAVID version 6.8 (david.abcc.ncifcrf.gov/), an open source platform, was utilized to determine the association among target molecules (16). To gain insight into the biological functions of these misaligned genes, GO and (KEGG) pathway enrichment analyses were performed on the DEGs based on DAVID, and $P<0.05$ was set as the cut-off criterion. The molecular function, biological processes, cell component and KEGG pathways of these DEGs and target genes were investigated using DAVID.

Protein-protein interaction (PPI) network and module analysis. As a search tool for the retrieval of interacting genes, STRING version 10.5 (string-db.org) was used to assess PPI information (17). To further examine the potential association between these DEGs, STRING was used to characterize the PPI network, and a confidence score >0.4 was set as the cut-off criterion. Cytoscape_v3.5.1 software was used to visualize the resulting PPI network (18). The Molecular Complex Detection (MCODE version 1.4.2) application (http://apps.cytoscape.org/apps/mcode) was used to select significant modules from the PPI network in Cytoscape with MCODE scores >5 and number of nodes >10 as the standard. Furthermore, a topological analysis of the PPI network was performed and the key genes were screened for further biological analysis of the node degree of these DEGs.

Target mRNA prediction of DEMs and construction of miRNA-mRNA regulatory networks. The target mRNAs of DEMs were predicted using TargetScan (www.targetscan.org/), miRDB (www.mirdb.org/miRDB/) (19), and miRTarBase version 7 (mirtarbase.mbc.nctu.edu.tw/) (20) online analysis tools. To further improve the reliability of bioinformatics analysis, overlapping target genes between DEGs and predicted target mRNAs were identified using a Venn diagram. The miRNA-mRNA networks were visualized using the Cytoscape platform software.

Results

Identification of DEGs and DEMs. NCBI-GEO is a free database containing microarray/gene profiles and next-generation sequences. Data for gene expression in CLL patients and normal samples was obtained from GSE22529 and GSE39411 along with miRNA expression data from GSE62137. The original gene data files were uploaded to the GCBI online laboratory to screen for the DEGs in CLL and normal samples. Using $P<0.05$ and |log FC|>1.5 as cut-off criteria, 781 and 1,607 DEGs were extracted from the expression profile datasets GSE22529 and GSE39411, respectively. Following integrated bioinformatics analysis, a total of 488 consistently expressed genes were identified from the two profile datasets, including 204 upregulated and 284 downregulated genes in the CLL samples compared with the normal population. Using a novel web-based tool, Morpheus, heat maps (illustrating the top 100 upregulated and top 100 downregulated genes) of DEGs were constructed from the GSE22529 and GSE39411 datasets

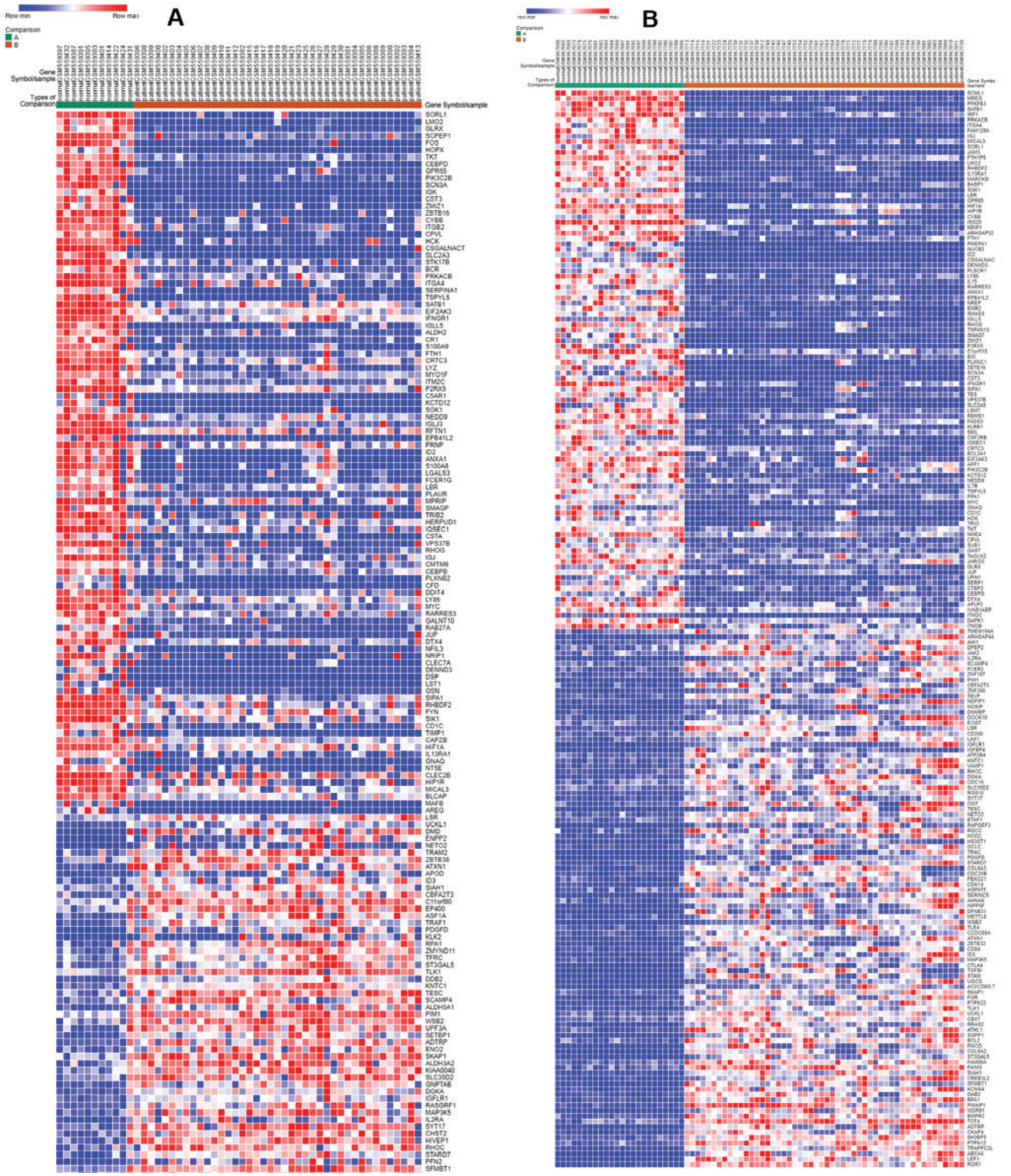

Figure 1. Heat map of Gene Expression Omnibus series. Top 100 upregulated and 100 downregulated genes from (A) GSE22529 and (B) GSE39411 datasets. Red corresponds to gene upregulation and blue to gene downregulation.

(Fig. 1A and B). In addition, the miRNA profile dataset, GSE62137, was analyzed to screen for DEMs in CLL using the GEO2R tool. According to the cut-off criteria ($P<0.05$ and $|logFC|>2.0$), a total of 34 differentially expressed miRNAs were identified from this microarray dataset, including 29 upregulated and 5 downregulated miRNAs (Table I). Among them, miR-144 and miR-181a were the most downregulated miRNAs in the GSE62137 dataset, whereas miR-582 was the most upregulated. The 488 consistently expressed genes and 34 differentially expressed miRNAs were used for further functional analysis.

GO function and KEGG pathway enrichment analysis of the DEGs. To gain a deeper understanding of the selected DEGs, GO and KEGG pathway analyses were performed using DAVID. GO analysis demonstrated that upregulated

Table I. Main dysregulated miRNAs in chronic lymphocytic leukemia.

miRNA	LogFC	P-value
hsa-miR-582-5p	8.23	2.78×10^{-21}
hsa-miR-451	-6.25	1.91×10^{-5}
hsa-miR-548c-3p	3.65	9.27×10^{-5}
hsa-miR-181c-3p	3.22	1.03×10^{-4}
hsa-miR-181a	-8.66	1.12×10^{-4}
hsa-miR-95	4.34	1.16×10^{-4}
hsa-miR-132	6.46	2.84×10^{-4}
hsa-miR-144	-8.52	3.25×10^{-4}
hsa-miR-486-5p	-5.57	3.88×10^{-4}
hsa-miR-885-3p	3.23	5.86×10^{-3}
hsa-miR-199a-3p	5.76	7.00×10^{-3}
hsa-miR-126	6.45	9.25×10^{-3}
hsa-miR-145	2.59	1.98×10^{-2}
hsa-miR-136	2.78	1.98×10^{-2}
hsa-miR-326	2.02	1.98×10^{-2}
hsa-miR-346	2.03	1.98×10^{-2}
hsa-miR-376c	2.96	1.98×10^{-2}
hsa-miR-139-5p	2.14	1.98×10^{-2}
hsa-miR-410	2.33	1.98×10^{-2}
hsa-miR-339-5p	2.05	1.98×10^{-2}
hsa-miR-584	2.44	1.98×10^{-2}
hsa-miR-1	2.24	1.98×10^{-2}
hsa-miR-495	2.11	1.98×10^{-2}
hsa-miR-376a	2.89	1.98×10^{-2}
hsa-miR-377	2.76	1.98×10^{-2}
hsa-miR-26b-3p	2.1	1.98×10^{-2}
hsa-miR-181c	2.09	1.98×10^{-2}
hsa-miR-381	2.37	1.98×10^{-2}
hsa-miR-296-3p	2.16	1.98×10^{-2}
hsa-miR-409-3p	2.36	1.98×10^{-2}
hsa-miR-199a-5p	3.42	1.98×10^{-2}
hsa-miR-126-5p	3.11	1.98×10^{-2}
hsa-miR-664a-5p	-5.32	1.98×10^{-2}
hsa-miR-501-5p	4.2	1.98×10^{-2}

FC, fold-change; miR, microRNA.

and downregulated DEGs were particularly abundant in the 'molecular function', 'biological processes' and 'cell component classification' (Table II). As demonstrated in this Table, in the 'molecular function group', upregulated genes were mainly enriched in 'protein binding', 'protein kinase binding' and 'non-membrane spanning protein tyrosine kinase activity', while the downregulated DEGs were enriched in various combinations, such as 'antigen binding', 'immunoglobulin receptor binding' and 'cell adhesion molecule binding'. In the 'biological processes' group, upregulated DEGs were mainly associated with 'intracellular signaling transduction', the 'transmembrane receptor protein tyrosine kinase signaling pathway' and 'positive regulation of granulocyte differentiation', and downregulated DEGs were significantly enriched in 'immune response' and the 'B cell receptor signaling pathway'. In addition, GO cell component analysis demonstrated that the upregulated DEGs were enriched in 'cytosol and plasma membrane', and downregulated DEGs were enriched in 'cell surface', 'extracellular exosome' and 'cell-cell adherent junction'. These results demonstrated that most DEGs are significantly enriched in 'binding', 'adhesion' and 'cell cycle'.

The results of the enrichment of DEG function and signaling pathways (Table III) demonstrated that the downregulated genes were mainly associated with the complex biological behavior of CLL. Using $P<0.05$ as a cut-off criterion, 17 KEGG pathways were over-represented in downregulated DEGs, including 'hematopoietic cell lineage', 'transcriptional dysregulation in cancer' and 'pathways in cancer', while the four KEGG pathways involved in the upregulated DEGs were enriched in multiple signaling pathways, the 'cyclic adenosine monophosphate (cAMP) signaling pathway' and the 'mitogen-activated protein kinase (MAPK) signaling pathway'. These most significantly enriched GO terms and KEGG pathways demonstrated the interactions of DEGs at the functional level.

PPI network construction and module selection. Using the STRING online database and Cytoscape software, a total of 354 DEGs were filtered into the complex DEG PPI network, containing 354 nodes and 1,392 edges (Fig. 2); 134 of the 488 DEGs did not fall into the DEG PPI network. Among the 354 nodes, 12 differentially expressed genes with a high degree of node were screened from the PPI network, and the most significant 12 node degree genes were *MYC*, interleukin *(IL)8*, B-cell lymphoma 2 *(BCL2)*, integrin subunit alpha M *(ITGAM)*, transforming growth factor *(TGF)B1*, cluster of differentiation *(CD)44*, *LCK*, *FYN*, Janus kinase *(JAK)2*, *FOS*, *CD86 and* z chain of T cell receptor associated protein kinase *(ZAP)-70* (Fig. 3). According to their degree of importance, 2 important modules from the PPI network complex were selected for further analysis based on Cytoscape MCODE. Functional and pathway enrichment analysis of the genes in these two modules were performed using DAVID. The results revealed that Module 1 consisted of 14 nodes and 82 edges (Fig. 4A and B), which were mainly associated with cytokine-cytokine receptor interaction, the JAK/signal transducer and activator of transcription (Jak-STAT) signaling pathway, and that Module 2 consisted of 28 nodes and 101 edges (Fig. 4C and D), which were enriched in the B cell receptor signaling pathway and the chemokine signaling pathway. The results of the module analysis revealed that eight of the 12 DEGs with a high degree were clustered in Module 1, and the other four genes were in Module 2. In order to further understand the interaction of these 12 key genes, the PPI network of these genes was constructed by STRING (Fig. 5). These results suggested that these central genes are closely associated with CLL and interact to promote the development of disease, which may suggest novel therapeutic approaches against CLL.

Construction of miRNA-mRNA regulatory network in the pathogenesis of CLL. The target mRNAs of DEMs were

Table II. Gene ontology analysis of differentially expressed genes associated with chronic lymphocytic leukemia[a].

Category	Term	Count	P-value
Upregulated			
GOTERM_BP_DIRECT	GO:0035556~intracellular signal transduction	16	2.64×10^{-5}
	GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	7	5.49×10^{-4}
	GO:0008283~cell proliferation	12	1.89×10^{-3}
	GO:0030854~positive regulation of granulocyte differentiation	3	2.27×10^{-3}
	GO:1901385~regulation of voltage-gated calcium channel activity	3	2.27×10^{-3}
GOTERM_MF_DIRECT	GO:0005515~protein binding	122	6.86×10^{-6}
	GO:0019901~protein kinase binding	14	1.72×10^{-4}
	GO:0005524~ATP binding	31	4.04×10^{-4}
	GO:0004715~non-membrane spanning protein tyrosine kinase activity	5	1.35×10^{-3}
	GO:0042169~SH2 domain binding	4	3.39×10^{-3}
GOTERM_CC_DIRECT	GO:0005886~plasma membrane	65	1.81×10^{-4}
	GO:0005829~cytosol	53	7.60×10^{-4}
	GO:0009897~external side of plasma membrane	9	1.63×10^{-3}
	GO:0045121~membrane raft	8	5.46×10^{-3}
	GO:0030175~filopodium	5	6.11×10^{-3}
Downregulated			
GOTERM_BP_DIRECT	GO:0006955~immune response	31	1.58×10^{-12}
	GO:0050776~regulation of immune response	21	2.84×10^{-12}
	GO:0006958~complement activation, classical pathway	14	2.97×10^{-9}
	GO:0006911~phagocytosis, engulfment	8	7.61×10^{-7}
	GO:0050853~B cell receptor signaling pathway	9	1.36×10^{-6}
GOTERM_MF_DIRECT	GO:0003823~antigen binding	15	3.62×10^{-10}
	GO:0042803~protein homodimerization activity	29	4.07×10^{-6}
	GO:0034987~immunoglobulin receptor binding	5	5.43×10^{-4}
	GO:0004872~receptor activity	11	1.51×10^{-3}
	GO:0005515~protein binding	154	2.01×10^{-3}
GOTERM_CC_DIRECT	GO:0070062~extracellular exosome	97	8.36×10^{-18}
	GO:0005886~plasma membrane	113	1.27×10^{-13}
	GO:0009986~cell surface	24	3.43×10^{-6}
	GO:0072562~blood microparticle	12	1.19×10^{-5}
	GO:0005887~integral component of plasma membrane	37	5.02×10^{-4}

[a]If there were more than five terms in this category, the first five terms were only selected based on the P-value. 'Count' corresponds to the number of enriched genes in each term. BP, biological processes; CC, component classification; MF, molecular function.

Table III. Kyoto Encyclopedia of Genes and Genomes pathway analysis of differentially expressed genes associated with chronic lymphocytic leukemia[a].

Category	Term	Count	P-value	Genes
Upregulated DEGs	hsa04261:Adrenergic signaling in cardiomyocytes	7	$9.44x10^{-3}$	ADRB2, ATP2B4, BCL2, PPP2R5C, CREB3L2, CACNB2, RAPGEF3
	hsa00561:Glycerolipid metabolism	4	$3.52x10^{-2}$	DGKA, LPL, AGPAT5, ALDH3A2
	hsa04024:cAMP signaling pathway	7	$3.63x10^{-2}$	ADRB2, ATP2B4, PDE4A, RRAS2, CREB3L2, RAPGEF3, MYL9
	hsa04010:MAPK signaling pathway	7	$3.89x10^{-2}$	PTPN7, MAP3K5, RASGRF1, RRAS2, CACNB2, MYC, RASA1, CDC25B
Downregulated DEGs	hsa05144:Malaria	8	$3.32x10^{-5}$	KLRB1, GYPC, CR1, KLRC4-KLRK1, ITGB2, HBA1, HBB, TGFB
	hsa04640:Hematopoietic cell lineage	9	$1.90x10^{-4}$	CR1, CR2, CD44, CD1C, CD22, ITGA4, IL7R, ITGAM, CD1D
	hsa05202:Transcriptional dysregulation in cancer	12	$3.22x10^{-4}$	JUP, CD86, CEBPB, ID2, REL, LMO2, BCL2A1, AFF1, ZBTB16, MYC, ITGAM, KLF3
	hsa05140:Leishmaniasis	8	$3.64x10^{-4}$	FOS, CR1, NFKBIA, ITGB2, ITGA4, ITGAM, TGFB1, IFNGR1
	hsa05323:Rheumatoid arthritis	7	$6.30x10^{-3}$	FOS, CD86, CCL3, ITGB2, IL15, LTB, TGFB1

[a]If there were more than five terms in this category, the first five terms were only selected based on the P-value. 'Count' corresponds to the number of enriched genes in each term.

predicted using the TargetScan, miRDB and miRTarBase online analysis tools. A total of 621 target mRNAs were predicted, and 32 target mRNAs associated with CLL were screened further by analyzing the association between them and the corresponding DEGs. In the present study, miR-582 was the most significantly upregulated miRNA and was predicted to target the *ETS2* and ArfGAP with RhoGAP domain, ankyrin repeat and PH domain *(ARAP)2* genes. MiR-144 and miR-181a were the most significantly downregulated miRNAs. MiR-144 was predicted to target the titin *(TTN)* gene, and miR-188a was predicted to target the zinc finger (*ZNF)266*, *NOTCH2*, FK506 binding protein *(FKBP)1A*, DNA damage inducible transcript *(DDIT)4*, oxysterol binding protein like *(OSBPL)3* and heat shock protein 90 β family member *(HSP90B)1* genes. The results also demonstrated that miR-181c and miR-145 targeted six different differentially expressed genes, while miR-584, miR-132 and miR-548c-3p targeted 3 different genes. In addition, *DDIT4* could be regulated by hsa-miR-181a, miR-181c, miR-199a-3p and miR-495, while *ZNF266*, *influenza virus NS1A binding protein (IVNS1ABP)*, *OSBPL3*, *HSP90B1*, *HSPA5* and *NOTCH2* could be regulated by two different miRNAs. A total of 32 target mRNAs corresponded to 17 differentially expressed miRNAs. To better understand the pathogenesis of CLL, miRNA-mRNA regulatory networks (Fig. 6) were constructed and analyzed, and a total of 42 miRNA-mRNA pairs were identified, including 16 positively associated target pairs and 26 negatively associated target pairs (Table IV).

Discussion

As an incurable B-cell malignancy, CLL is the most common leukemia in the western hemisphere (21). Recently, methods such as chemical compounds, stem cell transplantation and monoclonal antibody treatment have demonstrated high remission rates in the treatment of CLL (22,23); however, these treatments have a weak effect on the overall survival of CLL (24). Therefore, there is an urgent need to identify

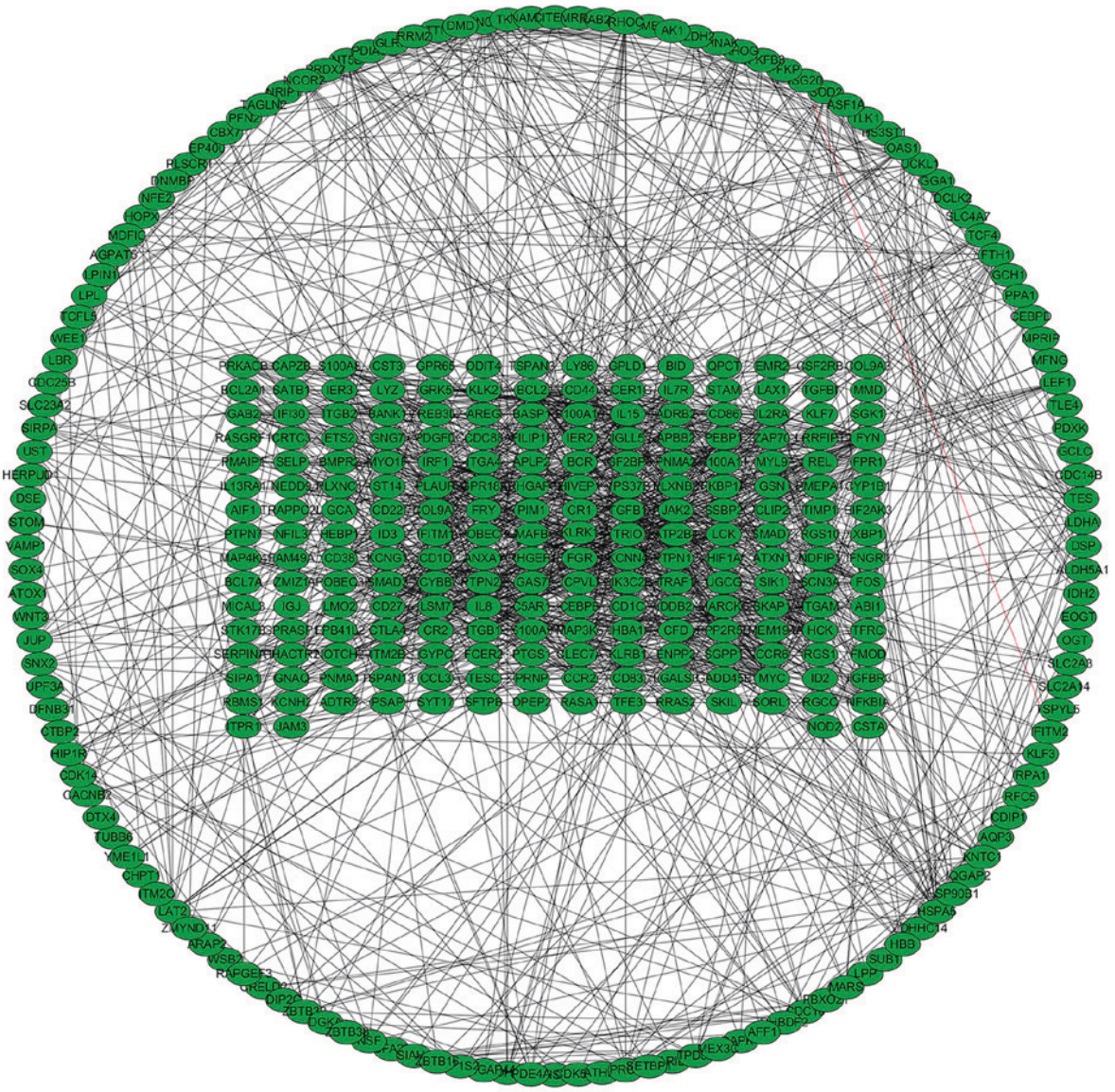

Figure 2. Protein-protein interaction network of differentially expressed genes in chronic lymphocytic leukemia.

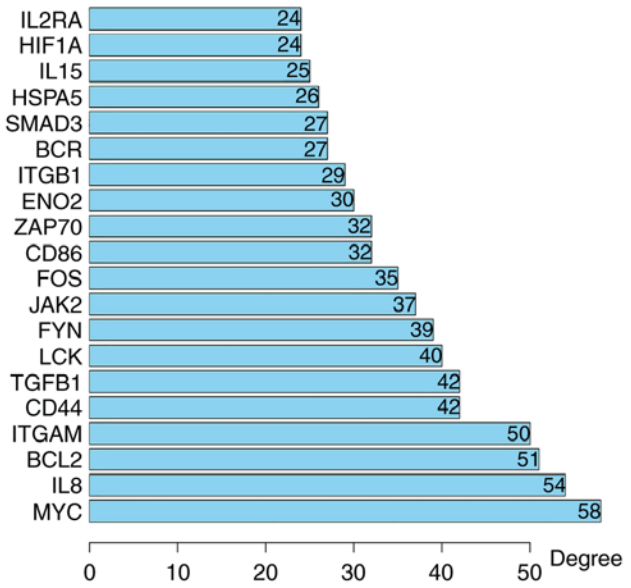

Figure 3. Top 20 differential hub genes in chronic lymphocytic leukemia.

sensitive and specific biomarkers of CLL and to find novel therapeutic targets.

Recently, the rapid development of microarray technology has provided a research platform for further investigation of disease progression and identification of tumor biomarkers, which may facilitate the identification of targets for diagnosis, therapeutics, and prognosis of tumors (25,26). In the present study, genes whose expression was significantly different between CLL and normal samples were identified, and a series of bioinformatics analyses were conducted. A total of 42 miRNA-mRNA pairs, including 17 differential miRNAs and 32 differential mRNAs were identified, and a miRNA-mRNA network analysis was performed to explore the pathogenesis of CLL.

GO and KEGG analysis demonstrated that the DEGs enrichment pathway reflected the complex biological behavior of CLL, including 'transcriptional dysregulation in cancer' and 'hematopoietic cell lineage'. Upregulated DEGs were associated with multiple signaling pathways, such as cAMP and MAPK. MAPK signaling pathway is fundamental for the

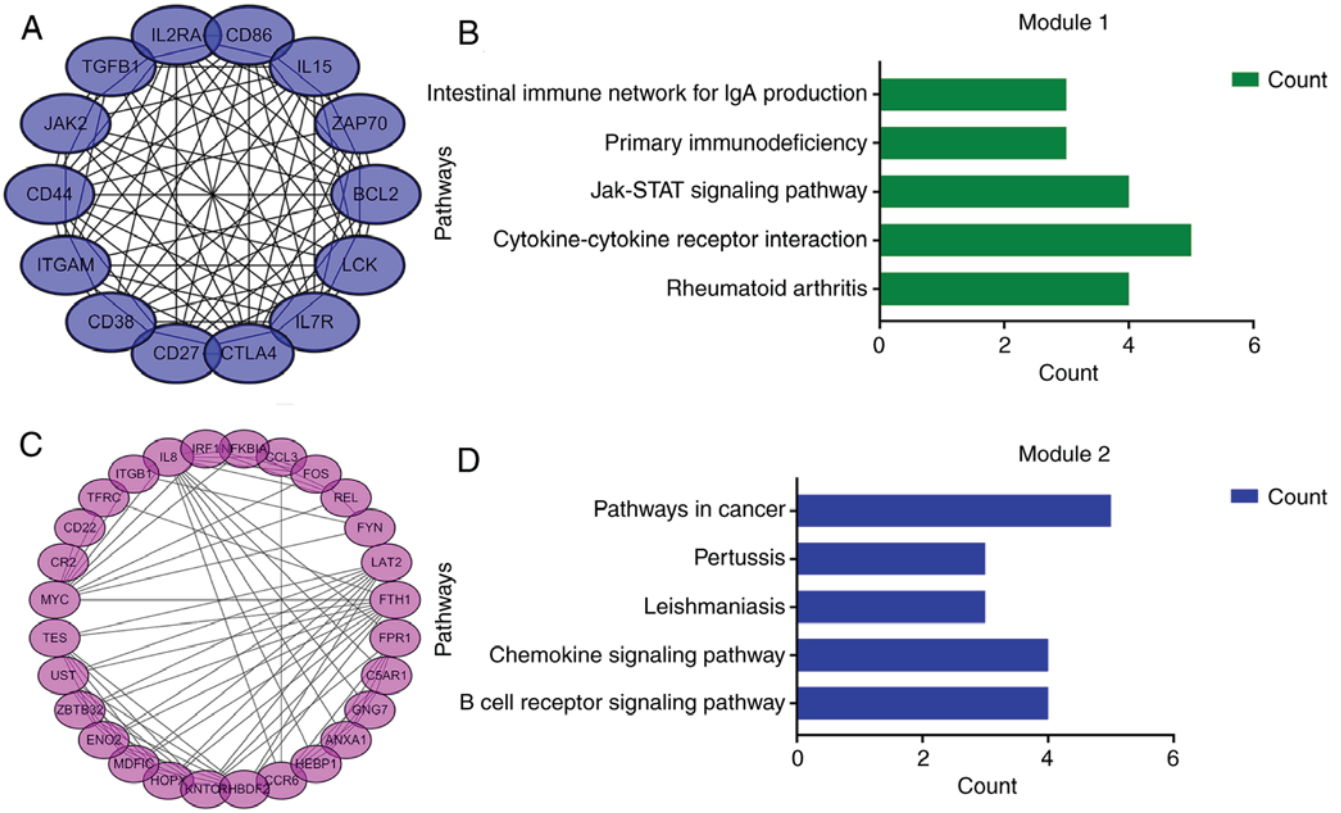

Figure 4. Top 2 modules from the protein-protein interaction network analysis. (A) Module 1 and (B) the top five most significantly enriched pathways based on the P-value. (C) Module 2 and (D) the top five most significantly enriched pathways based on the P-value.

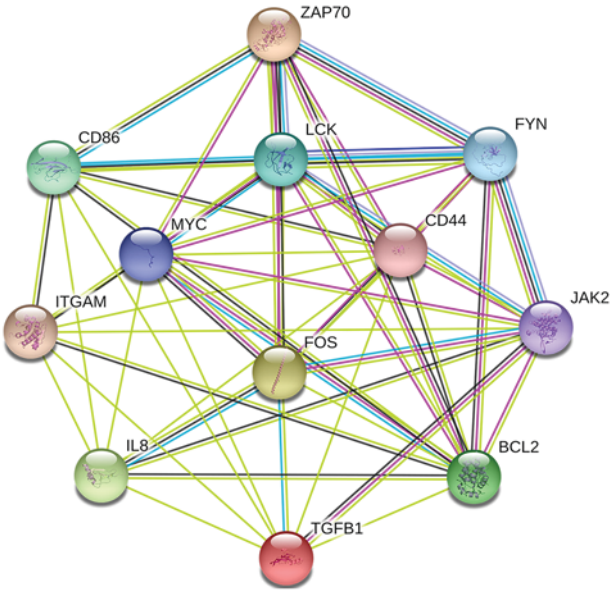

Figure 5. Protein-protein interaction network of the top 12 hub genes in chronic lymphocytic leukemia. Colored lines indicate the type of interaction evidence.

maintenance of basic cellular processes including proliferation, differentiation, migration and survival (27). A previous study demonstrated that dysregulation of the MAPK signaling pathway is a common mechanism in the pathogenesis of CLL (28,29). These results indicate that the development and progression of cancer cells are consistent with the close association between cell cycle/cell proliferation regulation and the dysfunction of cancer cells.

In addition, a study reported that nodes with a high degree of connectivity serve an important role in maintaining the entire PPI network and are indispensable (30-32). The 12 hub genes screened from the PPI network are the 12 most central nodes in the network and are considered to be the key genes associated with the development of CLL. Based on the significant association between the node structure and its functional importance in the PPI network (32), the 12 hub genes could be used to gain further insight into therapeutic studies of CLL at the molecular level.

As a gene involved in the induction of apoptosis, *MYC* was identified as one of the mutant genes that demonstrated the highest degree of connectivity in the present study. It is known that *MYC* serves an oncogenic role in many cancer types, including prostate and bladder cancers (33,34), and serves an important role in leukemia and lymphomas (35,36). In the present study, *MYC* was differentially expressed and was enriched in the transcriptional dysregulation of cancer and chronic myelogenous leukemia. *BCL2* is the first discovered inhibitor of apoptosis and serves a key role in promoting cell survival. Many studies have demonstrated that *BCL2* is one of the most important oncogenes involved in cancer (37-39), inducing the development of lymphoma, together with *c-MYC* overexpression, in particular (40). In the present study, *MYC* and *BCL2* were identified as the top-ranking regulated candidates among the differentially expressed genes in CLL, as previously demonstrated (41,42).

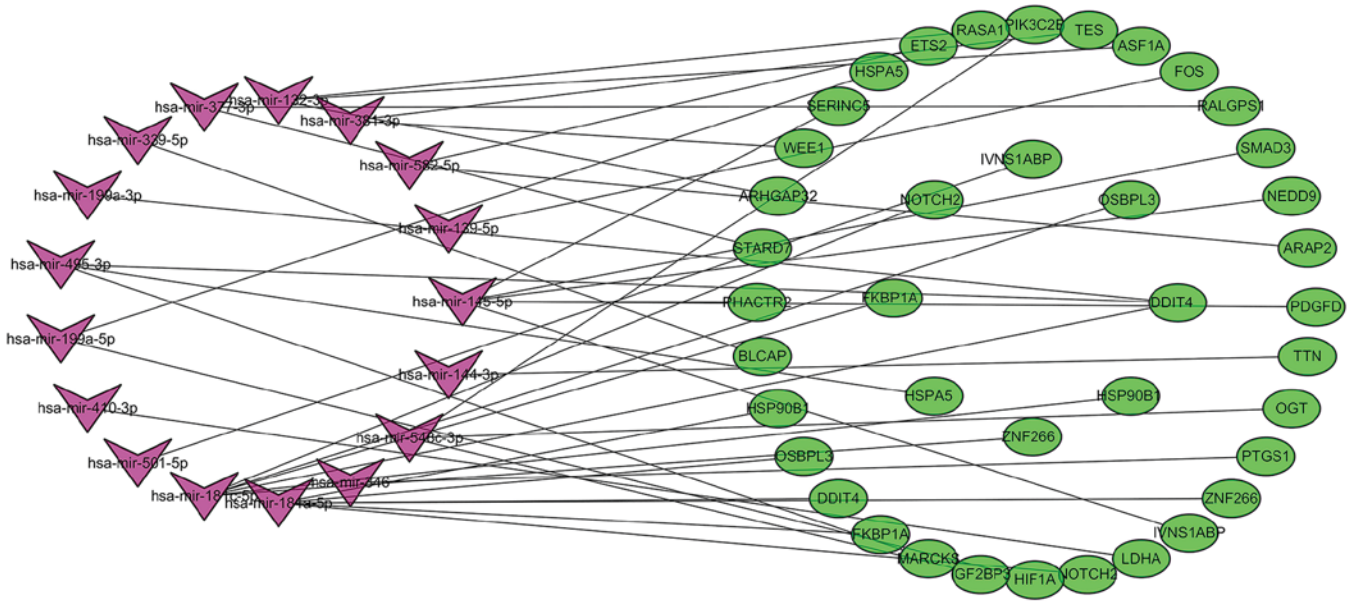

Figure 6. miRNA-mRNA regulatory network of chronic lymphocytic leukemia. Purple corresponds to the differentially expressed miRNAs and green to the differentially expressed genes (mRNAs) screened. miR, microRNA.

A persistent inflammatory response is the result of excessive production of inflammatory mediators, leading to inflammatory diseases and cancer (43). A study demonstrated that several CLL-associated cytokines, such as *IL8*, are associated with disease stage, and the correlation of *IL-8* expression levels has been described as one of the prognostic factors for CLL (44). It was also demonstrated that *IL8* is differentially expressed in CLL and can serve as a biomarker, further demonstrating that the present results have a certain degree of accuracy. *CD44*, another member of the adhesion factor family, participates in the process of cell proliferation, differentiation, adhesion and migration (45). Studies have demonstrated that anti-CD44 antibodies can interfere with the formation of B lymphocytes and that the downregulation of *CD44* leading to the arrest of cell cycle at the G0/G1 phase, resulting in the decrease of proliferation of K562 cells (46-48). In addition, the expression of *CD86* in many types of cancerous tissues was significantly lower than that in paracancerous tissues and normal tissues (49). As a member of the Janus family of tyrosine kinases, *JAK2* is a key component of chemokine signaling of B lymphocytes in CLL (50). Previous studies demonstrated that inhibition of *JAK2* activity in leukemia cells can promote oncogene silencing (51). The mutation of *JAK2* activates the JAK-STAT signaling pathway and is involved in the development of myeloproliferative neoplasms (52), suggesting that upregulation of the JAK pathway may be a key factor in the progression of CLL. In addition, a series of studies demonstrated that dysregulated expression of *TGFB1, ITGAM, LCK* and *FYN* are involved in the occurrence of cancer and play an important role in tumor progression (53-56).

As a tyrosine protein kinase, *ZAP-70* is involved in the signal transduction of T cells (57). Under normal conditions, B cells have a very low expression of *ZAP-70*, but the expression of *ZAP-70* in B cells can be used as a specific and sensitive indicator of B cell diseases, particularly CLL (58). In the present study, the expression of *ZAP-70* was upregulated, which is consistent with previous studies (57,59). Gene transcription is regulated by the transcription factor complex, which is widely involved in cell proliferation, differentiation and regulation of transformation (60). As a nuclear proto-oncogene, *FOS* encoded protein is an important transcription factor, which can induce the transcription and protein expression of its downstream gene that is involved in the regulation of cell proliferation and apoptosis (61). The abnormal expression of *FOS* can induce cell transformation and tumor formation (62). In the present study, the dysregulation of *FOS* expression was observed in CLL patients, which may be involved in abnormal gene transcription in CLL.

Module analysis of the 12 central genes from the PPI network revealed that 8 genes belonged to module 1, and 4 belonged to module 2. Functional and pathway enrichment analysis of genes in module 1 indicated that multiple pathways contribute to the development of CLL, including the NF-κB signaling pathway, which is involved in immune and inflammatory responses, and the phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) signaling pathway, which is related to chronic myelocytic leukemia resistance and cell proliferation regulation (63). In module 2, the genes were mainly involved in the B cell receptor signaling pathway and the Toll-like receptor signaling pathway. The results suggest that dysregulation of these molecular pathways in CLL may serve a role in its pathogenesis and may eventually serve as additional biomarkers to facilitate early diagnosis and treatment.

The aforementioned analyses demonstrated that these genes have a variety of biological functions in tumorigenesis and progression. A previous study demonstrated the presence of a regulatory control network between miRNAs and mRNAs (64), in which miRNAs could silence mRNA translation through sequence-specific targeting; therefore, it was reasonable to investigate the pathogenesis and treatment of tumors by studying the specific miRNA-mRNA

Table IV. A total of 42 miRNA-mRNA pairs that identified, including 16 positively associated target pairs and 26 negatively associated target pairs.

Category	miRNA	mRNA
Positively associated target pairs	hsa-mir-145	PDGFD
	hsa-mir-145	PHACTR2
	hsa-mir-548c-3p	OGT
	hsa-mir-548c-3p	IGF2BP3
	hsa-mir-181a	NOTCH2
	hsa-mir-377	STARD7
	hsa-mir-381	WEE1
	hsa-mir-145	SERINC5
	hsa-mir-132	ASF1A
	hsa-mir-377	RALGPS1
	hsa-mir-132	RASA1
	hsa-miR-181c-3p	ZNF266
	hsa-mir-181a	FKBP1A
	hsa-mir-181a	DDIT4
	hsa-mir-181a	OSBPL3
	hsa-mir-181a	HSP90B1
Negatively associated IVNS1ABP target pairs	hsa-mir-145	IVNS1ABP
	hsa-mir-501-5p	IVNS1ABP
	hsa-mir-410	LDHA
	hsa-mir-181a	ZNF266
	hsa-miR-181c-3p	NOTCH2
	hsa-mir-199a-5p	HIF1A
	hsa-mir-495-3p	MARCKS
	hsa-miR-181c-3p	FKBP1A
	hsa-miR-181c-3p	DDIT4
	hsa-mir-199a-3p	DDIT4
	hsa-mir-495	DDIT4
	hsa-miR-181c-3p	OSBPL3
	hsa-miR-181c-3p	HSP90B1
	hsa-mir-339-5p	BLCAP
	hsa-mir-132	ARHGAP3
	hsa-mir-199a-5p	HSPA5
	hsa-mir-495	HSPA5
	hsa-mir-582-5p	ETS2
	hsa-mir-548c-3p	PIK3C2B
	hsa-mir-381	TES
	hsa-mir-139-5p	FOS
	hsa-mir-145	SMAD3
	hsa-mir-145	NEDD9
	hsa-mir-582-5p	ARAP2
	hsa-mir-144	TTN
	hsa-mir-346	PTGS1

miRNA, microRNA.

co-regulation effect. In the present study, 34 DEMs were identified, including 29 upregulated and 5 downregulated miRNAs in CLL. Among them, miR-582 was the most significantly upregulated miRNA in the GSE62137 dataset, whereas miR-144 and miR-181a were the most significantly downregulated. A total of 42 miRNA-mRNA pairs were identified, including 17 differential miRNAs and 32 differential mRNAs and comprising 16 positively correlated and 26 negatively correlated target pairs.

miR-144 is expressed at low levels in various tumors, such as hepatocellular carcinoma (65), B-cell lymphoma (66) and chronic lymphocytic leukemia (67). In the present study, *TTN* was predicted to be the target of miR-144, which was upregulated in CLL according to the GSE22529 and GSE39411 datasets. *TTN* may be a good predictor of disease progression, survival, and treatment response in metastatic prostate cancer (68). Other studies have demonstrated that miR-582 was highly expressed in a variety of tumors, such as colorectal cancer (69) and non-small cell lung carcinoma (70), and that the upregulation of miR-582 contributed to an increase in the proliferation of prostate cancer cells (71) and to the reduction of human bladder cancer proliferation and invasion by suppressing the expression of target genes (72). In the present study, miR-582 was significantly overexpressed in CLL cells compared to normal samples and that it targeted the downregulated *ETS2* and *ARAP2* genes. There are few reports regarding the role of *ETS2* and *ARAP2* in cancer. Some studies indicated that the expression of *ETS2* might be associated with the progression of CML (73). Further study is needed to determine the role of *ETS2* and *ARAP2* in the development of CLL. The results of a previous study confirmed that miR-181a was significantly downregulated in CLL (74). NOTCH2, one of the target genes of miR-181a, is involved in the regulation of cellular processes including differentiation, proliferation and apoptosis (75), and dysregulation of *NOTCH2* signaling is involved in aberrant *CD23* expression in B-cell CLL (76).

As miRNAs can participate in complex biological functions in the body, they can influence a variety of biological processes and pathways through miRNA-mRNA regulatory networks (77,78). Providing that each miRNA potentially regulates a large number of targets, understanding the function of miRNAs in tumorigenesis may provide insight into the key questions that remain in cancer research. The miRNA-mRNA regulatory network highlighted in the present study provides new theoretical guidance for further exploring the mechanism of CLL and provides a novel perspective for understanding the underlying biological processes of CLL and miRNA-targeted therapy.

Taken together, the present data demonstrated that the molecular mechanisms of CLL can be understood and that biomarker prediction can be achieved through data mining and integration. Overall, 488 candidate genes and 32 different miRNAs from multiple datasets were screened by bioinformatics analysis. From the PPI network of DEGs, 12 major altered hub genes and multiple pathways associated with the development of CLL were identified, such as the Jak-STAT signaling pathway, the PI3K-Akt signaling pathway and the MAPK signaling pathway. In addition, the comprehensive bioinformatics analysis of DEGs and the construction of miRNA-mRNA regulatory network provides a bioinformatics approach to further study the pathogenesis of CLL and lay a solid foundation for the personalized treatment of CLL in the

future. However, further studies are needed to validate these results.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

CG, CS and CZ conceived and designed the study. JZ and LL performed data analysis. JW, CL, and HL integrated the data and created the figures. CZ reviewed and revised the article. 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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