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Original Article

Bioinformatics analysis of gene expression profiles in chronic lymphocytic leukemia

Lei Zhang[1], Aibin Liang[2], Wenjun Zhang[2], Hao Wang[1], Chen Wang[1]

Departments of [1]Advanced Medical Service, [2]Hematology, Tongji Hospital, Tongji University, Shanghai, China

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Abstract: Purpose: Chronic lymphocytic leukemia (CLL) is the most common type of leukemia. Due to the complex progression, the therapy is particularly challenging. This study aimed to find key genes and pathways related with CLL. Materials and Methods: The array data of GSE52774 was downloaded to analyze DEGs (differentially expressed genes) by limma package. GO (gene ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses for DEGs in PPI (protein-protein interaction) network were made by DAVID. Results: Transcription factors of key nodes in regulatory network were predicted, as well as the network modules analyses and functional modules enrichment analyses. *HSP90AA1* (Heat Shock Protein 90 kDa Alpha (Cytosolic), Class A Member 1) and *TP53* (Tumor Protein P53) with higher degrees in PPI network for up-regulated genes were important target genes regulated by many transcription factors. *UBE2I* (Ubiquitin-Conjugating Enzyme E2I), *CBLB* (Cbl Proto-Oncogene B, E3 Ubiquitin Protein Ligase) and *SIRT1* (Sirtuin 1) with higher degrees in PPI network for down-regulated genes were important target genes regulated by many transcription factors. Proteasome pathway enriched in PPI network for up-regulated genes was the most significant pathway in module 2. Conclusion: Therefore, *HSP90AA1, TP53, UBE2I, CBLB, SIRT1* and proteasome pathway may be key genes and pathway associated with CLL.

Keywords: Chronic lymphocytic leukemia, differentially expressed genes, pathway, protein-protein interaction network, modules

Introduction

Chronic lymphocytic leukemia (CLL), affecting B cell lymphocytes, is the most common type of leukemia in the western world with the probability of 5/100000 [1]. Furthermore, only 10%-15% of patients are diagnosed with this disease before 50 and the median age at diagnosis is 64 years old [2]. As CLL is a heterogeneous disease with an extremely variable course, the therapy of it is particularly challenging [1]. Because the management and control of this disease has become increasingly personalized, it is urgent to find detailed and useful knowledge of diagnostic methods and therapy options [1]. Understanding the molecular mechanisms of CLL may be able to give new outlooks for the treatment of this disease.

Damle et al. suggested that *CD38* (cluster of differentiation 38) expression and IgV_H (immunoglobulin heavy-chain variable-region) mutation status could serve as prognostic indicators in CLL [3]. Furthermore, Terry et al. suggested that *CD38* expression might vary in the progression of CLL [4]. The expression of *ZAP-70* (*Zeta*-Chain (TCR) Associated Protein Kinase 70 kDa) in CLL patients is relevant to the IgV_H mutational status and disease development [5], and *ZAP-70* is a predictor of CLL progression [6]. Besides, *Bcl-2* (B-Cell CLL/Lymphoma 2) family proteins are also predictor of CLL progression. *SF3B1* (Splicing Factor 3b, Subunit 1) mutations lead to mistakes in the splicing of some transcripts affecting the pathogenesis of CLL, thus *SF3B1* mutations are related with poor overall survival and faster development of CLL [7]. *STAT3* (Signal Transducer And Activator Of Transcription 3) can medicate C6-ceramide-induced cell death in CLL [8]. Moreover, Calin et al. indicated that unique microRNA signatures were related with prognostic factors and development of CLL [9]. In addition, some pathways, such as Wnt signaling pathway and PI3K/NF-Kb

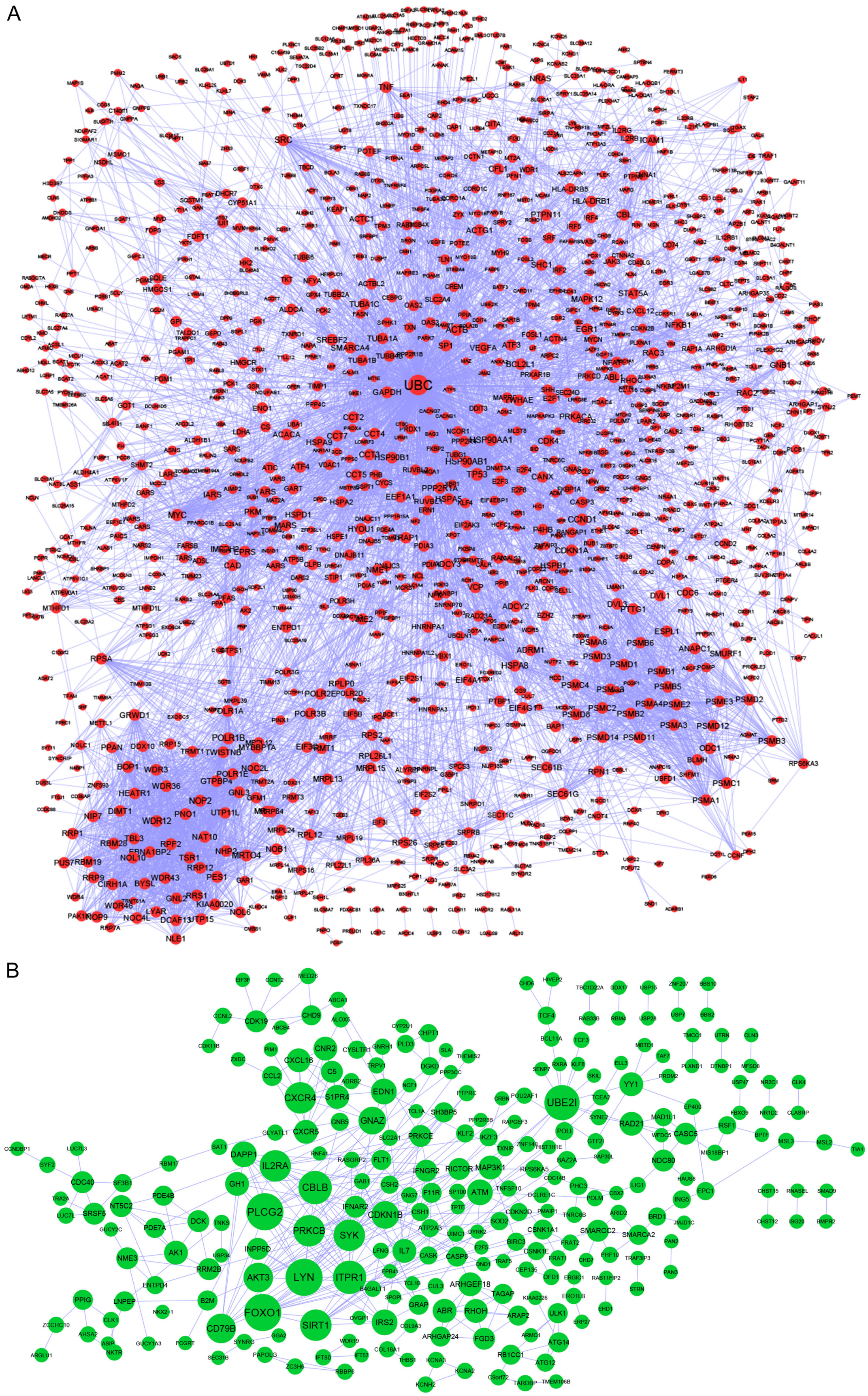

Figure 1. A: The PPI network for up-regulated genes, nodes: proteins coded by up-regulated genes; B: The PPI network for down-regulated genes, nodes: proteins coded by down-regulated genes.

Table 1. The first 15 nodes with higher degrees in PPI networks for up-regulated genes and down-regulated genes

	Degree	Gene
Up-regulated genes	704	UBC
	135	TP53
	104	HSP90AA1
	82	MYC
	74	CCND1
	73	MRTO4, POLR1B
	72	HSP90AB1
	71	SRC
	66	ACTB
	65	HSPA8, NOP2
	62	WDR12
	61	BOP1, CDKN1A
Down-regulated genes	13	FOXO1, LYN, PLCG2
	12	UBE2I
	11	ITPR1, CBLB
	10	SIRT1, SYK, PRKCB, IL2RA, CXCR4
	9	AKT3, CD79B
	8	GNAZ, CDKN1B

(Phosphoinositide 3-kinase/nuclear factor kappa-light-chain-enhancer of activated B cells) pathway are also associated with the development of CLL [10, 11]. In spite of the former researches, the present knowledge about the molecular mechanisms related with CLL seems to be insufficient. Thus, more efforts are needed to find oher important molecular mechanisms of CLL.

In the present study, the array data of GSE52774 was downloaded and the differentially expressed genes (DEGs) associated with CLL were identified. The protein-protein interaction (PPI) network was established, and functional enrichment analyses were performed for DEGs in PPI network. Furthermore, transcription factor of key nodes in regulatory network was searched. In addition, the network modules were analyzed and functional enrichment analyses for modules were also performed. We aimed to find significant genes and key pathways related with CLL, and then clarify the molecular mechanism of the disease.

Materials and methods

Microarray data

The array data of GSE52774, deposited by Bomben R and Gattei V, was downloaded from GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) database with the platform of GPL-6480 (Agilent-014850 Whole Human Genome Microarray 4x44K G4-112F, Aviano, Italy). A total of 16 CLL immobilized (CLL B cells co-stimulated with immobilized anti-IgM) and 16 CLL untreated (untreated CLL B cells) were included in the study, and the data were used to subsequent analysis.

Data preprocessing

The raw data were downloaded and were preprocessed by RMA (the robust multiarray average) algorithm with Affy package [12] in Bioconductor (http://www.bioconductor.org/). The probe of the microarray data was transformed to gene symbol and the probe not matched with gene symbol was eliminated. For several probes mapped to one gene, the mean value was set as the final gene expression level.

Screening of DEGs

The limma package [13] in Bioconductor was used to analyze DEGs in CLL immobilized samples compared with CLL untreated samples. The *p*-values of DEGs were calculated by t-test [14] in limma package. Then, the *p*-values were adjusted as FDR (false discovery rate) values with BH (Benjamini-Hochberg) [15]. $|\log_2 FC \text{ (fold change)}| \geq 0.58$ and FDR < 0.01 were set as cut-off criterion for DEGs.

PPI network

STRING (Search Tool for the Retrieval of Interacting Genes) [16] is a database used to recode information of biochemical interactions studies between proteins. Furthermore, neighborhood, gene fusion, co-occurrence, co-expression experiments, databases and textmining were prediction method of this database. In our present study, the input gene sets were DEGs and species were Homo sapiens. DEGs were mapped into PPIs and the combined score > 0.7 was set as the cutoff value. PPI networks were constructed with Cytoscape software [17]. Key nodes with higher degree in the network were screened.

Table 2. GO annotation and KEGG pathway enrichment analyses for up-regulated and down-regulated genes in PPI network

	Term	Description	Count	*P*-value
Up				
BP	GO:0043161	Proteasomal ubiquitin-dependent protein catabolic process	34	1.20E-13
	GO:0010498	Proteasomal protein catabolic process	34	1.20E-13
	GO:0034660	NcRNA metabolic process	53	1.73E-13
CC	GO:0005829	Cytosol	212	1.77E-29
	GO:0031974	Membrane-enclosed lumen	246	2.19E-22
	GO:0043233	Organelle lumen	241	8.02E-22
MF	GO:0000166	Nucleotide binding	271	3.83E-18
	GO:0017076	Purine nucleotide binding	238	3.65E-17
	GO:0032553	Ribonucleotide binding	226	1.55E-15
KEGG	hsa03050	Proteasome	24	3.28E-11
	hsa00900	Terpenoid backbone biosynthesis	11	2.98E-07
	hsa00970	Aminoacyl-tRNA biosynthesis	16	8.89E-06
Down				
BP	GO:0043067	Regulation of programmed cell death	39	1.07E-08
	GO:0010941	Regulation of cell death	39	1.18E-08
	GO:0042981	Regulation of apoptosis	38	2.67E-08
CC	GO:0044451	Nucleoplasm part	24	8.26E-06
	GO:0031981	Nuclear lumen	43	1.46E-05
	GO:0005654	Nucleoplasm	31	1.64E-05
MF	GO:0000166	Nucleotide binding	61	5.60E-05
	GO:0004672	Protein kinase activity	25	6.20E-05
	GO:0030554	Adenyl nucleotide binding	46	1.45E-04
KEGG	hsa04662	B cell receptor signaling pathway	9	3.43E-04
	hsa04666	Fc gamma R-mediated phagocytosis	9	1.67E-03
	hsa04062	Chemokine signaling pathway	12	4.35E-03

BP: biological process; CC: cellular component; MF: molecular function; Term: the identification number of GO or KEGG term; Description: the names of GO or KEGG term; Counts: the number of genes enriched in GO or KEGG terms.

GO (gene ontology) and pathway enrichment analyses in PPI network

GO, which includes MF (molecular function), BP (biological process), and CC (cellular component), is a tool that used for genes annotation [18]. KEGG (Kyoto Encyclopedia of Genes and Genomes) is a database used for revealing functions of genes or other molecules [19]. DAVID, an integrated biological knowledgebase and analytic tool, is used for analyzing gene lists [20].

In our present study, GO annotation and KEGG pathway enrichment analyses for up-regulated and down-regulated genes in PPI network were made by DAVID. *P*-value < 0.05 and gene counts ≥ 2 were set as threshold value.

Transcription factors of key nodes in regulatory network

ENCODE (Encyclopedia of DNA Elements) [21] is a database used to identify all functional elements in the human genome, and in this study the database was employed to extract all known human transcription factor binding sites on chromosomes. To increase the credibility of CIS binding sites, repeatability of every binding site were counted. Furthermore, CIS binding sites appeared in at least 2 independent samples were screened, and then used for subsequent analysis. Then, combined with the information of each gene promoter region (TSS upstream 1 kb to TSS downstream 0.5 kb), CIS regulatory regions overlapped with each gene promoter were screened, and set them as tran-

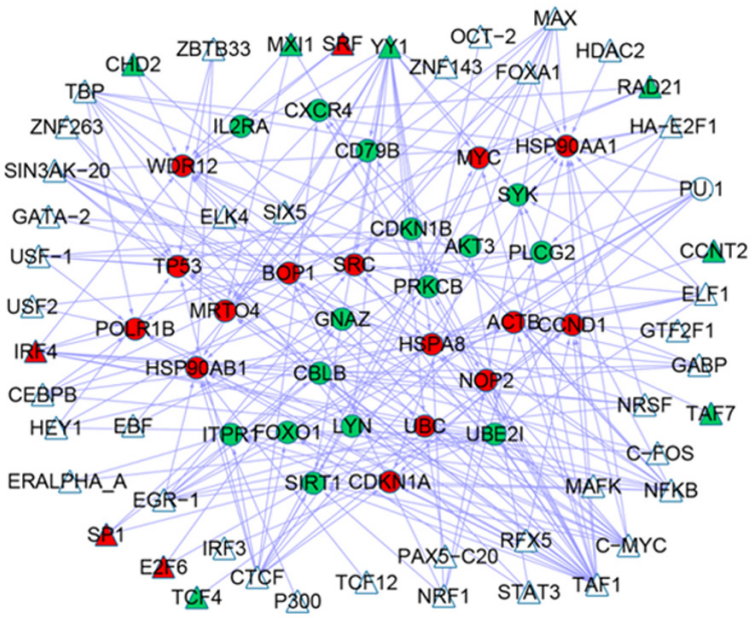

Figure 2. Transcription factors of key nodes in regulatory network. Red nodes: up-regulated genes; green nodes: down-regulated genes; triangle nodes: transcription factors; circular nodes: nodes with higher degrees.

Table 3. Totally 11 transcription factor that encoded by significantly differently expressed genes (DEGs)

Transcription factor	The number of target genes	logFC
MXI1	3	-0.9653558
YY1	16	-0.6544363
CCNT2	1	-0.6268253
TCF4	1	-0.622165
RAD21	3	-0.5975715
CHD2	2	-0.5894516
TAF7	1	-0.5866868
SRF	2	0.60777
E2F6	2	0.81319975
SP1	3	1.0483912
IRF4	8	2.0692659

scription factor-target gene pairs. Finally, transcription factors regulating key nodes of DEGs in PPI network were observed. Furthermore, key target genes nodes and transcription factors regulatory network were visualized by Cytoscape.

PPI network module analysis

The interaction of sub network modules in PPI network was complex and the sub network modules were tend to take part in common biological process. The network modules were analyzed by using ClusterONE [22], and the GO annotation and KEGG pathway enrichment analyses for modules were also performed. Modules that *P*-value < 9E-4 were set as significant modules.

Results

Data processing and DEGs analysis

A total of 2566 DEGs were identified in CLL immobilized samples compared with CLL untreated samples, among which 1664 genes were up-regulated and 902 genes were down-regulated.

PPI network

The PPI network for up-regulated genes was shown in **Figure 1A**. Totally 1197 nodes and 7101 protein pairs were included in the network. The PPI network for down-regulated genes was shown in **Figure 1B**. Totally 260 nodes and 361 protein pairs were included in the network. The higher the degree value was, the more likely it was to be the key node in the network. The first 15 nodes with higher degrees in 2 networks were selected respectively (**Table 1**). *HSP90AA1* (Heat Shock Protein 90 kDa Alpha (Cytosolic), Class A Member 1), *TP53* (Tumor Protein P53) with higher degrees were significantly enriched in PPI network for up-regulated genes, and *UBE2I* (Ubiquitin-Conjugating Enzyme E2I), *CBLB* (Cbl Proto-Oncogene B, E3 Ubiquitin Protein Ligase) and *SIRT1* (Sirtuin 1) with higher degrees were significantly enriched in PPI network for down-regulated genes.

GO and pathway enrichment analyses in PPI network

The results of GO annotation and KEGG pathway enrichment analyses for up-regulated and down-regulated genes in PPI network were shown in **Table 2**. Besides, proteasome pathway was enriched in PPI network with the highest counts (the number of genes enriched in KEGG terms) for up-regulated genes.

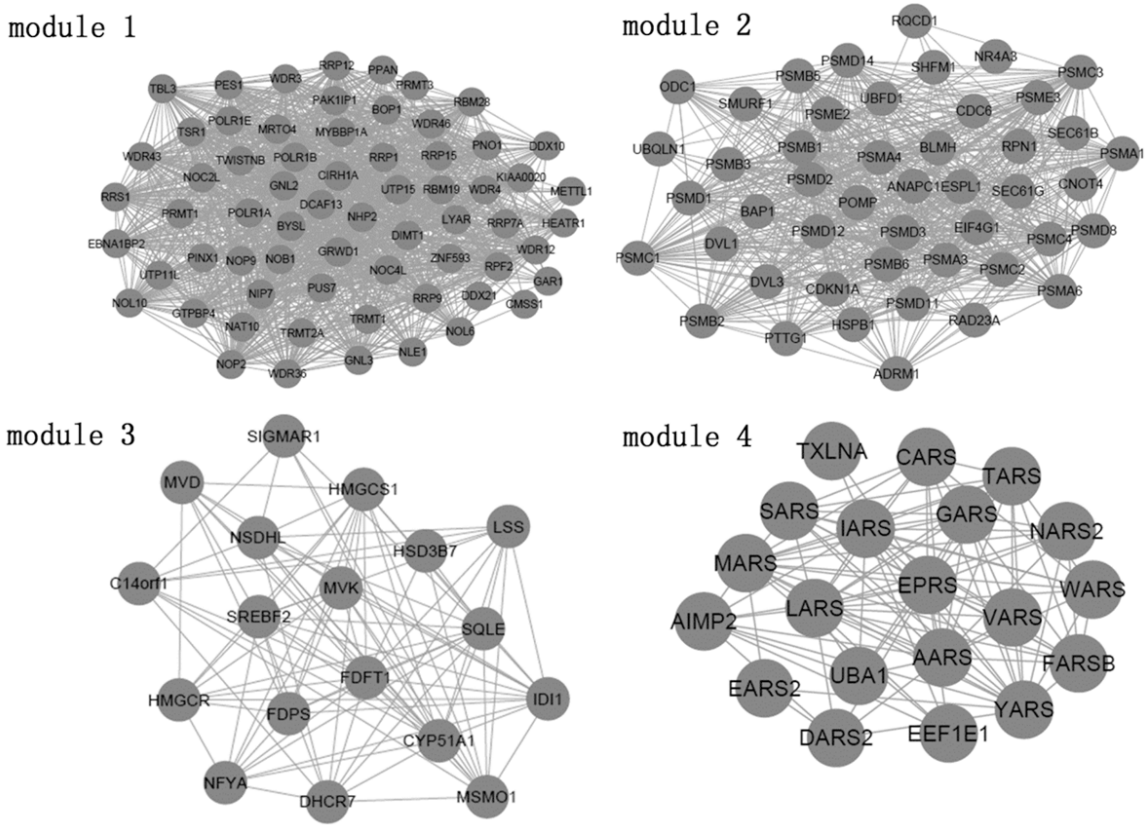

Figure 3. Sub network modules obtained from the PPI network for up-regulated genes.

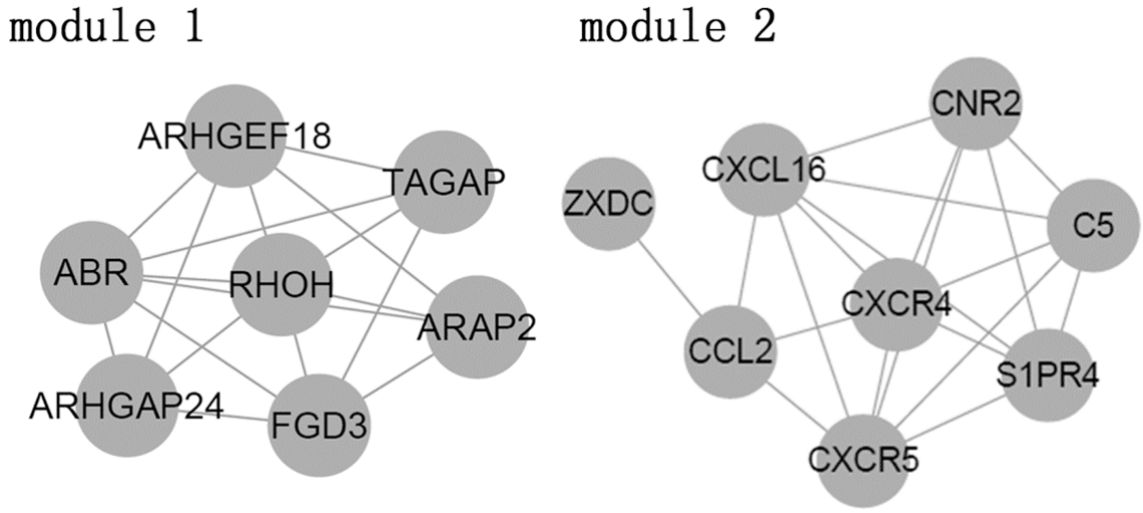

Figure 4. Sub network modules obtained from the PPI network for down-regulated genes.

Transcription factor of key nodes in regulatory network

Based on the PPI networks for up-regulated genes and down-regulated genes, we obtained a network including 30 nodes with higher degrees (**Figure 2**). A total of 49transcription factors and 30 target genes were included in this network. Combined with DEGs in CLL immobilized samples, we found 11 transcription factors encoded by significantly DEGs (**Table 3**).

Table 4. Significantly functional enrichment of module for up-regulated and down-regulated genes in PPI network

	Term	Description	Count	*P*-value
up-regulated genes				
Module 1				
BP	GO:0042254	Ribosome biogenesis	23	1.38E-35
	GO:0022613	Ribonucleoprotein complex biogenesis	23	1.31E-31
	GO:0034470	NcRNA processing	19	1.27E-23
CC	GO:0005730	Nucleolus	49	9.09E-58
	GO:0031981	Nuclear lumen	50	2.35E-44
	GO:0070013	Intracellular organelle lumen	50	5.81E-40
MF	GO:0003723	RNA binding	18	9.12E-14
	GO:0016423	TRNA (guanine) methyltransferase activity	3	5.86E-05
	GO:0034062	RNA polymerase activity	4	1.57E-04
KEGG	hsa03020	RNA polymerase	3	2.92E-05
	hsa00240	Pyrimidine metabolism	3	3.45E-04
	hsa00230	Purine metabolism	3	9.00E-04
Module 2				
BP	GO:0043161	Proteasomal ubiquitin-dependent protein catabolic process	26	4.48E-43
	GO:0010498	Proteasomal protein catabolic process	26	4.48E-43
	GO:0031145	Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	23	3.53E-41
CC	GO:0000502	Proteasome complex	27	6.68E-54
	GO:0005839	Proteasome core complex	9	4.27E-16
	GO:0005829	Cytosol	22	1.95E-11
MF	GO:0070003	Threonine-type peptidase activity	9	1.86E-16
	GO:0004298	Threonine-type endopeptidase activity	9	1.86E-16
	GO:0008233	Peptidase activity	14	1.95E-09
KEGG	hsa03050	Proteasome	24	2.04E-39
	hsa04110	Cell cycle	5	1.61E-02
Module 3				
BP	GO:0016126	Sterol biosynthetic process	14	1.01E-32
	GO:0006694	Steroid biosynthetic process	15	5.76E-30
	GO:0016125	Sterol metabolic process	15	7.76E-29
CC	GO:0005789	Endoplasmic reticulum membrane	8	9.43E-09
	GO:0042175	Nuclear envelope-endoplasmic reticulum network	8	1.37E-08
	GO:0044432	Endoplasmic reticulum part	8	5.46E-08
MF	GO:0016863	Intramolecular oxidoreductase activity, transposing C =C bonds	3	1.12E-04

	GO:0016860	Intramolecular oxidoreductase activity	3	8.55E-04
	GO:0000247	C-8 sterol isomerase activity	2	3.46E-03
KEGG	hsa00900	Terpenoid backbone biosynthesis	6	1.35E-10
	hsa00100	Steroid biosynthesis	6	2.77E-10
Module 4				
BP	GO:0043038	Amino acid activation	16	2.78E-35
	GO:0006418	tRNAaminoacylation for protein translation	16	2.78E-35
	GO:0043039	tRNAaminoacylation	16	2.78E-35
CC	GO:0005829	Cytosol	12	2.37E-07
	GO:0005625	Soluble fraction	7	2.98E-06
	GO:0000267	Cell fraction	7	2.78E-03
MF	GO:0016875	Ligase activity, forming carbon-oxygen bonds	16	2.67E-36
	GO:0004812	Aminoacyl-tRNA ligase activity	16	2.67E-36
	GO:0016876	Ligase activity, forming aminoacyl-tRNA and related compounds	16	2.67E-36
KEGG	hsa00970	Aminoacyl-tRNA biosynthesis	16	3.43E-32
	hsa00290	Valine, leucine and isoleucine biosynthesis	3	5.02E-04
Down-regulated genes				
Module 1				
BP	GO:0046578	Regulation of Ras protein signal transduction	4	3.60E-05
	GO:0051056	Regulation of small GTPase mediated signal transduction	4	6.21E-05
	GO:0035023	Regulation of Rho protein signal transduction	3	5.23E-04
MF	GO:0030695	GTPase regulator activity	7	8.76E-10
	GO:0060589	Nucleoside-triphosphatase regulator activity	7	1.00E-09
	GO:0005085	Guanyl-nucleotide exchange factor activity	4	3.07E-05
Module 2				
BP	GO:0007186	G-protein coupled receptor protein signaling pathway	7	2.10E-06
	GO:0006955	Immune response	6	6.56E-06
	GO:0007166	Cell surface receptor linked signal transduction	7	4.09E-05
CC	GO:0005887	Integral to plasma membrane	5	9.55E-04
	GO:0031226	Intrinsic to plasma membrane	5	1.04E-03
	GO:0044459	Plasma membrane part	5	9.83E-03
	GO:0008009	Chemokine activity	3	2.55E-04
	GO:0042379	Chemokine receptor binding	3	2.90E-04
	GO:0016494	C-X-C chemokine receptor activity	2	4.31E-03
KEGG	hsa04062	Chemokine signaling pathway	4	9.02E-04
	hsa04060	Cytokine-cytokine receptor interaction	4	2.41E-03

BP: biological process; CC: cellular component; MF: molecular function; Term: the identification number of GO or KEGG term; Description: the names of GO or KEGG term; Counts: the number of genes enriched in GO or KEGG terms.

The number of transcription factors regulating every target gene was observed by calculating degree value of the node. The target genes regulated by transcription factor with degree ≥ 9 were *HSP90AA1* (degree =16), *TP53* (degree =13), WDR12 (WD Repeat Domain 12, degree =13), *HSP90AB1* (Heat Shock Protein 90 kDa Alpha (Cytosolic), Class B Member 1, degree =12), *HSPA8* (Heat Shock 70 kDa Protein 8, degree =11), *UBE2I* (degree =10), *CBLB* (degree =9), *SIRT1* (degree =9), *UBC* (Ubiquitin C, degree =9) and *ACTB* (Actin, Beta, degree =9). The degree represents the number of transcription factor regulating target genes.

Network module analysis

A total of 4 sub network modules were obtained from the PPI network for up-regulated genes (**Figure 3**), and 2 sub network modules for down-regulated genes (**Figure 4**). The results of significantly functional enrichment of module were shown in **Table 4**. Furthermore, proteasome pathway was the most significant pathway with the highest counts (the number of genes enriched in KEGG terms) in module 2 for up-regulated genes.

Discussion

In this study, a total of 2566 DEGs including 1664 up-regulated and 902 down-regulated genes were identified in CLL immobilized samples compared with CLL untreated samples. Our results showed that *HSP90AA1* and *TP53* with higher degrees in PPI network for up-regulated genes were important target genes regulated by many transcription factors. Furthermore, *UBE2I*, *CBLB* and SIRT1 with higher degrees in PPI network for down-regulated genes were also important target genes regulated by many transcription factors. Besides, proteasome pathway enriched in PPI network for up-regulated genes was the most significant pathway in module 2.

It is reported that *TP53* (*p53*) mutations are strong predictors of refractoriness and poor survival in CLL [23], and are related with resistance to chemotherapy-based therapy and adverse outcomes in CLL [24]. Furthermore, in CLL, *TP53* mutations and dysregulations of this gene are considered as the most adverse prognostic factor [25]. Dufour et al. suggested that a complete disruption of *TP53* function could increase the risk of disease development in previously treated CLL patients [26]. Nadeu et al. indicated that clonal and subclonal mutations of *TP53* had influences on clinical outcome [27]. In addition, it is reported that miR-34a directly targeted by *p53* is related with the adverse outcome of CLL patients [28]. HSP90 is a member of the heat shock family of proteins. Castro et al. suggested that development of cancer in CLL and differences in clinical prognosis might be associated with the activity of HSP90, and activation of HSP90 might be able to protect aggressive CLL cells via stabilization of *ZAP-70* [29]. Furthermore, Hertlein showed that geldanamycin, one kind of HSP90 inhibitor, had preclinical efficacy in CLL therapy [30]. These studies showed that *HSP90AA1* and *TP53* might play important parts in the development of CLL, which was in line with our results.

Accumulation of mature B lymphocytes in secondary lymphoid organs, bone marrow and peripheral blood lead to the formation of CLL [31], and *UBE2I* takes part in regulation of cell cycle and apoptosis [32]. Furthermore, many studies showed that *UBE2I* interact with *TP53* [33, 34], and as above stated that *TP53* might be related with the development of CLL. Thus, *UBE2I* may be involved in the development of CLL. *CBLB* plays key parts in regulation of activation thresholds in mature lymphocytes [35]. Furthermore, *CBLB* associates with *ZAP-70* in CLL cells, and *ZAP-70* is a predictor of CLL progression [6]. Thus, *CBLB* may be related with CLL progression. *SIRT1*, a member of the sirtuin family of proteins, is an NAD^+-dependent deacetylase [36]. *SIRT1* levels were up-regulated in CLL cells [37]. *SIRT1* can deacetylate a key lysine residue and then inactivates *p53* (*TP53*) [38], and *TP53* mutations are strong predictors of poor survival in CLL [23]. Thus, *SIRT1*may be associated with CLL development by interacting with other genes. In our present study, *UBE2I*, *CBLB* and SIRT1 with higher degrees in PPI network for down-regulated genes were important target genes regulated by many transcription factors. Therefore, our present results are in accord with the former researches and suggest that *UBE2I*, *CBLB* and *SIRT1* may be involved in the progression of CLL.

In this study, proteasome pathway enriched in PPI network for up-regulated genes was the

most significant pathway in module 2. Proteasome inhibitors, such as NPI-0052 (Salinosporamide A) and bortezomib could induce apoptosis of lymphocytes in CLL patients or had proapoptotic activity in CLL cells [39]. Baou et al. suggested that combined with other agents influencing the levels of Mcl-1, NOXA (Phorbol-12-myristate-13-acetate-induced protein 1) could be an effective therapy for CLL patients [40]. Furthermore, activation of the TRAIL (TNF-related apoptosis-inducing ligand) apoptotic pathway can help to apoptosis induced by proteasome inhibitor in CLL cells [41]. Therefore, proteasome pathway may be associated with the development of CLL.

In conclusion, *HSP90AA1*, *TP53*, *UBE2I*, *CBLB* and *SIRT1* may be key genes associated with the development of CLL. Furthermore, proteasome pathway may play significant roles in the CLL progression. We infer that these genes and proteasome pathway possibly can be used as therapeutic targets for CLL in the future clinical trials. However, no experimental verification and relatively small sample size are our limitations, and further studies are needed to verify our results and find some other more genes or pathways related with CLL.

Disclosure of conflict of interest

None.

Address correspondence to: Aibin Liang, Department of Hematology, Tongji Hospital, Tongji University, Shanghai 200065, China. Tel: +86-21-6611-1016; Fax: +86-21-66111016; E-mail: liangaibbbb@hotmail.com

References

[1] Hallek M and Pflug N. Chronic lymphocytic leukemia. Ann Oncol 2010; 21: vii154-vii164.

[2] Andritsos L and Khoury H. Chronic lymphocytic leukemia. Curr Treat Options Oncol 2002; 3: 225-231.

[3] Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K and Kolitz J. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94: 1840-1847.

[4] Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK and Oscier DG. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood 2002; 99: 1023-1029.

[5] Crespo M, Bosch F, Villamor N, Bellosillo B, Colomer D, Rozman M, Marcé S, López-Guillermo A, Campo E and Montserrat E. ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. N Engl J Med 2003; 348: 1764-1775.

[6] Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG, Neuberg DS, Flinn IW, Rai KR and Byrd JC. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med 2004; 351: 893-901.

[7] Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS and Zhang L. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 2011; 365: 2497-2506.

[8] Doshi UA, Loughran TP and Kester M. STAT3 mediates C6-ceramide-induced cell death in chronic lymphocytic leukemia via the MEK/ERK and PKC pathway. Cancer Res 2015; 75: 2613-2613.

[9] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI and Fabbri M. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005; 353: 1793-1801.

[10] Lu D, Zhao Y, Tawatao R, Cottam HB, Sen M, Leoni LM, Kipps TJ, Corr M and Carson DA. Activation of the Wnt signaling pathway in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2004; 101: 3118-3123.

[11] Cuni S, Perez-Aciego P, Perez-Chacon G, Vargas J, Sanchez A, Martin-Saavedra F, Ballester S, Garcia-Marco J, Jorda J and Durantez A. A sustained activation of PI3K/NF-κB pathway is critical for the survival of chronic lymphocytic leukemia B cells. Leukemia 2004; 18: 1391-1400.

[12] Gautier L, Cope LBolstad BM and Irizarry RA. affy-analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004; 20: 307-315.

[13] Ritchie ME, Belinda P, Di W, Yifang H, Law CW, Wei S and Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.

[14] Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004; 3: Article 3.

[15] Ferreira JA. The Benjamini-Hochberg method in the case of discrete test statistics. Int J Biostat 2007; 3: Article 11.
[16] Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A and Tsafou KP. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2014; gku1003.
[17] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[18] Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS and Eppig JT. Gene Ontology: tool for the unification of biology. Nat Genet 2000; 25: 25-29.
[19] Kanehisa M and Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000; 28: 27-30.
[20] Huang DW, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.
[21] Consortium EP. The ENCODE (ENCyclopedia of DNA elements) project. Science 2004; 306: 636-640.
[22] Nepusz T, Yu H and Paccanaro A. Detecting overlapping protein complexes in protein-protein interaction networks. Nat Methods 2012; 9: 471-472.
[23] Rossi D, Khiabanian H, Spina V, Ciardullo C, Bruscaggin A, Famà R, Rasi S, Monti S, Deambrogi C and De Paoli L. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. Blood 2014; 123: 2139-2147.
[24] Pettitt AR, Jackson R, Carruthers S, Dodd J, Dodd S, Oates M, Johnson GG, Schuh A, Matutes E and Dearden CE. Alemtuzumab in combination with methylprednisolone is a highly effective induction regimen for patients with chronic lymphocytic leukemia and deletion of TP53: final results of the national cancer research institute CLL206 trial. J Clin Oncol 2012; 30: 1647-1655.
[25] Pospisilova S, Gonzalez D, Malcikova J, Trbusek M, Rossi D, Kater A, Cymbalista F, Eichhorst B, Hallek M and Döhner H. ERIC recommendations on TP53 mutation analysis in chronic lymphocytic leukemia. Leukemia 2012; 26: 1458-1461.
[26] Dufour A, Palermo G, Zellmeier E, Mellert G, Duchateau-Nguyen G, Schneider S, Benthaus T, Kakadia PM, Spiekermann K and Hiddemann W. Inactivation of TP53 correlates with disease progression and low miR-34a expression in previously treated chronic lymphocytic leukemia patients. Blood 2013; 121: 3650-3657.
[27] Nadeu F, Delgado J, Royo C, Baumann T, Stankovic T, Pinyol M, Jares P, Navarro A, Martín-García D, Beà S, Salaverria I, Oldreive C, Aymerich M, Suárez-Cisneros H, Rozman M, Villamor N, Colomer D, López-Guillermo A, González M, Alcoceba M, Terol MJ, Colado E, Puente XS, López-Otín C, Enjuanes A, Campo E. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1 and ATM mutations in chronic lymphocytic leukemia. Blood 2016; 127: 2122-30.
[28] Zenz T, Mohr J, Eldering E, Kater AP, Bühler A, Kienle D, Winkler D, Dürig J, van Oers MH and Mertens D. miR-34a as part of the resistance network in chronic lymphocytic leukemia. Blood 2009; 113: 3801-3808.
[29] Castro JE, Prada CE, Loria O, Kamal A, Chen L, Burrows FJ and Kipps TJ. ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia. Blood 2005; 106: 2506-2512.
[30] Hertlein E, Wagner AJ, Jones J, Lin TS, Maddocks KJ, Towns WH, Goettl VM, Zhang X, Jarjoura D and Raymond CA. 17-DMAG targets the nuclear factor-κB family of proteins to induce apoptosis in chronic lymphocytic leukemia: clinical implications of HSP90 inhibition. Blood 2010; 116: 45-53.
[31] Ghia P, Ferreri AJ and Caligaris-Cappio F. Chronic lymphocytic leukemia. Crit Rev Oncol Hematol 2007; 64: 234-246.
[32] Gogusev J, Murakami I, Doussau M, Telvi L, Stojkoski A, Lesavre P and Droz D. Molecular cytogenetic aberrations in autosomal dominant polycystic kidney disease tissue. J Am Soc Nephrol 2003; 14: 359-366.
[33] Bernier-Villamor V, Sampson DA, Matunis MJ and Lima CD. Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell 2002; 108: 345-356.
[34] Guven Maiorov E, Keskin O, Hatirnaz Ng O, Ozbek U and Gursoy A. Identification of interconnected markers for T-cell acute lymphoblastic leukemia. Biomed Res Int 2013; 2013: 210253.
[35] Bachmaier K, Krawczyk C, Kozieradzki I, Kong YY, Sasaki T, Oliveira-dos-Santos A, Mariathasan S, Bouchard D, Wakeham A and Itie A. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. Nature 2000; 403: 211-216.
[36] Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB and

Vu CB. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007; 450: 712-716.

[37] Audrito V, Vaisitti T, Rossi D, Gottardi D, D'Arena G, Laurenti L, Gaidano G, Malavasi F and Deaglio S. Nicotinamide blocks proliferation and induces apoptosis of chronic lymphocytic leukemia cells through activation of the p53/miR-34a/SIRT1 tumor suppressor network. Cancer Res 2011; 71: 4473-4483.

[38] Lee JT and Gu W. SIRT1 Regulator of p53 Deacetylation. Genes Cancer 2013; 4: 112-117.

[39] Ruiz S, Krupnik Y, Keating M, Chandra J, Palladino M and McConkey D. The proteasome inhibitor NPI-0052 is a more effective inducer of apoptosis than bortezomib in lymphocytes from patients with chronic lymphocytic leukemia. Mol Cancer Ther 2006; 5: 1836-1843.

[40] Baou M, Kohlhaas SL, Butterworth M, Vogler M, Dinsdale D, Walewska R, Majid A, Eldering E, Dyer MJ and Cohen GM. Role of NOXA and its ubiquitination in proteasome inhibitor-induced apoptosis in chronic lymphocytic leukemia cells. Haematologica 2010; 95: 1510-8.

[41] Kabore AF, Sun J, Hu X, McCrea K, Johnston JB and Gibson SB. The TRAIL apoptotic pathway mediates proteasome inhibitor induced apoptosis in primary chronic lymphocytic leukemia cells. Apoptosis 2006; 11: 1175-1193.