Identification of key genes related to dexamethasone-resistance in acute lymphoblastic leukemia

Qiuni Chen^{1,2,&}, Shixin Chen^{3,&}, Yuye Shi^{1,2}, Shandong Tao^{1,2}, Wei Chen⁴, Chunling Wang^{1,2}, Liang Yu^{1,2,*}

1. Department of Hematology, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an 223300, Jiangsu Province, PR.China.
2. Key Laboratory of Hematology of Nanjing Medical University, Nanjing, 210029, Jiangsu Province, PR.China.
3. Department of Nursing, Huai'an Fourth Hospital, Huai'an 223300, Jiangsu Province, PR.China.
4. Department of Respiratory Medicine, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an 223300, Jiangsu Province, PR.China.

*Correspondence to: Liang Yu, email: yuliangha@163.com

&These authors contributed equally to this work.

Abstract

Drug resistance is the main cause of poor chemotherapy response in acute leukemia. Despite the extensive use of dexamethasone(DEX) in the treatment of acute lymphoblastic leukemia for many years, the mechanisms of dexamethasone – resistance has not been fully understood. We choose GSE94302 from GEO database aiming to identify key genes that contribute to the DEX resistance in acute lymphoblastic

leukemia. Differentially expressed gene(DEGs) are selected by using GEO2R tools. A total of 837 DEGs were picked out, including 472 up-regulated and 365 down-regulated DEGs. All the DEGs were underwent gene ontology(GO) analysis and Kyoto Encyclopedia of Gene and Genome(KEGG) pathway analysis. In addition, the DEGs-encoded protein-protein interaction (PPI) was screened by using Cytoscape and Search Tool for the Retrieval of Interacting Genes(STRING). Total 20 genes were found as key genes related to DEX resistance with high degree of connectivity, including *CDK1*, *PCNA*, *CCNB1*, *MYC*, *KPNA2*, *AURKA*, *NDC80*, *HSPA4*, *KIF11*, *UBE2C*, *PIK3CG*, *CD44*, *CD19*, *STAT1*, *DDX41*, *LYN*, *BCR*, *CD48*, *JAK1* and *ITGB1*. They could be used as biomarkers to identify the DEX-resistant acute lymphoblastic leukemia.

Keywords: acute lymphoblastic leukemia; gene; drug resistance; bioinformatics analysis

Introduction

Acute lymphoblastic leukemia(ALL) is a heterogeneous hematologic malignant disorder characterized by the proliferation of immature lymphoid cells in the bone marrow, peripheral blood, and other organs. It is the most common childhood cancer and a major cause of death of adult leukemia^[1].The treatment of ALL comprises supportive therapy, anti-leukemia chemo-therapy, immunotherapy and other target therapy. The anti-leukemia chemo-therapy regime includes the administration of a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and at least one other agent (usually asparaginase and anthracycline) ^[2]. With the use of intensive

chemotherapy regimen, the complete remission rates of adult ALL were 85 to 90%, but the long-term survival rates are only 30 to 50%^[3]. Compared to the childhood ALL, the adult ALL patients have poorer prognosis, and the resistance to chemotherapeutic drugs, including dexamethasone(DEX) - resistance, is one of the most important cause. The mechanism of DEX-resistance was not fully understood and needed further investigation^[4].

Recently, gene expression profiling has been proven to be valuable in the pathogenesis, diagnosis and prognosis of many tumors including ALL^[5]. Molecular analysis of the data from some public databases is a promising method to explore the disease related biomarkers. This article is aimed to identify the key genes and pathways relating to the DEX - resistant ALL patients, and it will be helpful to investigate the mechanisms of DEX resistance.

Methods

Microarray Data

GSE94302 from Gene Expression Omnibus(GEO) was chosen for this research, which is a public and freely available database (<https://www.ncbi.nlm.nih.gov/geo/>) ^[6].

GSE94302 is based on the following platform: GPL10558(Illumina HumanHT-12 V4.0 expression beadchip, Illumina Inc, San Diego, CA, USA). The preparation of samples was described in a public website (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94302>). The dataset

includes 99 samples, containing 58 DEX sensitive samples, 18 DEX resistant and 23

unknown ones. The known response samples were divided into two groups according to their regulation.

Identification of differentially expressed genes (DEGs)

GEO2R(<http://www.ncbi.nlm.nih.gov/geo/geo2r>) was used to detect DEGs in sensitive and resistant samples. DEGs were defined as $p < 0.05$ and $|\log FC| > 1$ as the cut-off criterion. 837 DEGs were found, including 472 up-regulated and 365 down-regulated genes, and the top 10 genes with high degree of connectivity were chosen as key candidate genes in each group.

Gene Ontology and Pathway Enrichment Analysis

The Gene Ontology(GO) concept was intended to use a common vocabulary to annotate the homologous gene and protein sequences in multiple organisms, thus we could query and retrieve genes and proteins based on their shared biology^[7]. GO analysis and pathways analysis were carried out by KEGG PATHWAYS(<http://www.kegg.jp/>)^[8] and DAVID(<https://david.ncifcrf.gov/>)^[9] with $p < 0.05$ as the cut-off criterion.

Protein-Protein Interaction (PPI) Network and Module Analysis

The online database STRING(<https://string-db.org/>) was used to develop DEGs-encoded proteins and protein-protein interaction network. Search Tool for the Retrieval of Interacting Genes (STRING) collects experimental and predictable information associated with the interactions of protein pairs in a given cell context via calculating the combined score of PPIs^[10]. The DEGs were mapped into STRING, and the confidence score ≥ 0.4 , maximum number of interactors = 0 are set

as the cut-off criterions. In addition, Cytoscape software^[11] was used to screen the modules of PPI network.

Results

Identification of DEGs and key genes

To identify the genes may be related to the dexamethasone sensitivity, DEGs between sensitive and resistant samples are screened using the GEO2R online analysis tool with p value < 0.05 and $|\log FC| > 1$. A total of 837 DEGs were identified, including 472 up-regulated and 365 down-regulated genes(Fig 1). Besides, 10 key genes in each group with high degree of connectivity were picked out (Table1).

Fig 1: Distribution of DEGs.

X-axis: $-\log p$ value, y-axis: $-\log|FC(\text{fold change})|$. DEGs were marked as blue dot.

Table 1:

A:

Name	Degree	pvalue
<i>CDK1</i>	68	0.000000264
<i>PCNA</i>	60	0.0000076
<i>CCNB1</i>	56	2.07E-08
<i>MYC</i>	50	0.0000623
<i>KPNA2</i>	41	0.000777
<i>AURKA</i>	39	2.29E-08
<i>NDC80</i>	37	2.52E-14
<i>HSPA4</i>	36	7.54E-12
<i>KIF11</i>	34	3.11E-13
<i>UBE2C</i>	33	0.0000639

B:

Name	Degree	pvalue
<i>PIK3CG</i>	32	3.15E-08
<i>CD44</i>	29	0.00776
<i>CD19</i>	23	0.000117
<i>STAT1</i>	21	2.52E-11
<i>DDX41</i>	17	8.72E-17
<i>LYN</i>	17	0.0114
<i>BCR</i>	16	0.000804
<i>CD48</i>	14	1.24E-05
<i>JAK1</i>	13	1.35E-08
<i>ITGB1</i>	13	2.47E-12

Table1: TOP 10 key genes with higher

degree of connectivity in two groups. A represents up-regulated DEGs. B represents down-regulated DEGs.

GO function and KEGG pathway enrichment analysis

In order to understanding the DEGs well, function and pathway enrichment analysis were conducted. DEGs were divided into two groups according to the criterion whether genes are up-regulated or down-regulated. All the up-regulated genes were put into DAVID software, the functions of up-regulated genes are screened (Fig 2 A). The results showed that the up-regulated genes were particularly enriched in

biological process(BP), including cell division, while in cell component(CC), they were enriched in following aspects: nucleus, cytosol, cytoplasm. For molecular function(MF), they were enriched in protein binding, poly(A) RNA binding (Table2 A). The same method was used in the down-regulated genes. The functions of the down-regulated DGEs were shown in Fig 2 B. These DGEs are enriched in BP, including interferon-gamma signaling pathway, cellular response to zinc ion and cadmium ion, negative regulation of growth, type I interferon signaling pathway. In terms of CC, the down-regulated genes were enriched in cell-cell adhering junction parts and membrane. For molecular function(MF), they were enriched in cadherin binding which involved in cell-cell adhesion (Table2 B).

Fig 2: Gene ontology analysis of DEGs.

A:

B:

A represents the functional annotation of up-regulated genes, and B represents the down-regulated genes.

Table 2:

A:

Category	Term	Cou nt	%	PValu e	FDR
GOTERM_BP_DIR ECT	GO:0051301~cell division	25	0.0343 26	6.49E- 06	0.0112 77
GOTERM_CC_DIR ECT	GO:0005634~nucleus	178	0.2443 98	1.19E- 07	1.65E- 04

GOTERM_CC_DIR ECT	GO:0005829~cytosol	120	0.1647 63	4.41E- 07	6.15E- 04
GOTERM_CC_DIR ECT	GO:0005654~nucleoplasm	103	0.1414 21	1.63E- 06	0.0022 79
GOTERM_CC_DIR ECT	GO:0005737~cytoplasm	166	0.2279 22	4.40E- 06	0.0061 41
GOTERM_MF_DIR ECT	GO:0005515~protein binding	283	0.3885 65	6.14E- 13	9.04E- 10
GOTERM_MF_DIR ECT	GO:0044822~poly(A) RNA binding	53	0.0727 7	5.06E- 06	0.0074 4

B :

Table 2: Gene ontology analysis of DEGs. A represents the up-regulated DEGs, and B

Category	Term	Count	%	PValue	FDR
GOTERM_BP_DI RECT	GO:0060333~interferon-gamma-mediated signaling pathway	11	0.0196 58	8.56E- 07	0.0014 4
GOTERM_BP_DI RECT	GO:0071294~cellular response to zinc ion	7	0.0125 1	9.70E- 07	0.0016 33
GOTERM_BP_DI RECT	GO:0071276~cellular response to cadmium ion	6	0.0107 23	1.21E- 05	0.0203 93
GOTERM_BP_DI RECT	GO:0045926~negative regulation of growth	6	0.0107 23	2.21E- 05	0.0371 42
GOTERM_BP_DI RECT	GO:0060337~type I interferon signaling pathway	9	0.0160 84	2.67E- 05	0.0448 96
GOTERM_CC_DI RECT	GO:0005913~cell-cell adherens junction	21	0.0375 29	2.23E- 06	0.0030 23
GOTERM_CC_DI RECT	GO:0016020~membrane	67	0.1197 35	3.02E- 05	0.0409 95
GOTERM_MF_DI RECT	GO:0098641~cadherin binding involved in cell-cell adhesion	19	0.0339 55	6.40E- 06	0.0092 67

represents the down-regulated DEGs.

GO: Gene Ontology; FDR: False Discovery Rate; BP: biological process; CC: cell component; MF: molecular function

In addition, KEGG pathway analysis was also employed. As it's shown in Table 3, the up-regulated DEGs were enriched in cell cycle, biosynthesis of antibiotics, and p53 signaling pathway, while down-regulated DEGs were enriched in mineral absorption, platelet activation, hematopoietic cell lineage, actin cytoskeleton regulation, Epstein-Barr virus infection, graft-versus-host disease, peroxisome and fc gamma R-mediated phagocytosis signaling pathway.

Table 3:

Category	Term	Count	%	PValue	Gene
KEGG_P ATHWAY	hsa04110: Cell cycle	13	0.01 7849	1.29E- 04	<i>CCNB1, CDK1, YWHAG, RAD21, CCNH, CDKN2D, PCNA, BUB1B, CDK6, GADD45B, YWHAЕ, MYC, BUB3</i>
KEGG_P ATHWAY	hsa01130: Biosynthesis of antibiotics	12	0.01 6476	0.0295 2	<i>ACAA2, LDHB, ISYNA1, ALDH7A1, UAPI, ASS1, ALDOC, HK1, CAT, OAT, AK6, PCCA</i>
KEGG_P ATHWAY	hsa04115: p53 signaling pathway	6	0.00 8238	0.0350 94	<i>CCNB1, CDK1, EI24, CD82, CDK6, GADD45B</i>
KEGG_P ATHWAY	hsa04978: Mineral absorption	7	0.01 251	5.71E- 04	<i>MT1A, ATOX1, MT1E, MT1H, MT1X, MT1G, MT1F</i>
KEGG_P ATHWAY	hsa04611: Platelet activation	10	0.01 7871	0.0028 31	<i>PIK3CG, F2RL3, PRKCZ, LYN, VAMP8, GUCY1A3, ITGB1, MYLK, ITPR1, GP9</i>
KEGG_P ATHWAY	hsa04640: Hematopoiet ic cell lineage	7	0.01 251	0.0128 75	<i>CD9, CD19, CD44, HLA-DRB1, ITGA5, HLA-DRB5, GP9</i>
KEGG_P ATHWAY	hsa04810: Regulation of actin	11	0.01 9658	0.0224 84	<i>PIK3CG, VAV3, GSN, ITGA5, FGF9, DIAPH2, IQGAP2,</i>

	cytoskeleton				<i>ITGB1, MYLK, FGD3, VCL</i>
KEGG_P	hsa05169:	10	0.01	0.0297	<i>PIK3CG, CD19, CD44,</i>
ATHWAY	Epstein-Barr virus infection		7871	67	<i>LYN, CD58, HSPB1, JAK1, HLA-E, SPN, HLA-F</i>
KEGG_P	hsa05332:	4	0.00	0.0387	<i>HLA-DRB1, HLA-</i>
ATHWAY	Graft-versus-host disease		7148	82	<i>DRB5, HLA-E, HLA-F</i>
KEGG_P	hsa04146:	6	0.01	0.0409	<i>AGPS, PEX2, PEX16,</i>
ATHWAY	Peroxisome		0723	3	<i>MPV17, SLC27A2, ACAA1</i>
KEGG_P	hsa04666:	6	0.01	0.0427	<i>PIK3CG, VAV3, LYN,</i>
ATHWAY	Fc gamma R-mediated phagocytosis		0723	49	<i>GSN, BIN1, PRKCD</i>

Table3: KEGG pathway analysis of DEGs. The up-regulated DEGs are marked in blue background, the others are down-regulated DEGs.

KEGG: Kyoto Encyclopedia of Genes and Genomes

PPI network and module analysis

Using the STRING online database and Cytoscape, the PPI network of the key genes from two groups (Table1) were made (shown in Fig 3). The key genes are selected by the criterion of higher connectivity. Based on the GO analysis and KEGG pathway analysis, the results show that the key genes from up-regulated gene group, including *CCNBI*, *CDKI*, *PCNA* and *MYC*, were associated with cell cycle pathway. For those from down-regulated DEGs, including *PIK3CG*, *CD19*, *CD44*, *LYN* and *JAK1*, were enriched in Epstein-Barr virus infection pathway.

Fig 3: The protein -protein interaction network of DEGs.

A:

B:

A: The protein-protein interaction network of top 10 hub gene in up-regulated DEGs.

B represents the network of hub genes from down-regulated ones

Discussion

Chemotherapy is the main treatment for pediatric and adult ALL, the therapeutic

regimen contains induction, consolidation and maintenance therapy phases ^[12]. The induction therapy agents typically include glucocorticoid (prednisone or dexamethasone), vincristine and asparaginase, with or without anthracycline. According to previous studies, remission-induction treatment can eradicate the initial leukemic cell burden and restore the normal hematopoiesis (get complete remission, CR) in 96–99% of children and 78–92% of adult ALL^[13], but there were only 30% to 50% adult ALL patients achieve clinical cure^[3,14]. The drug resistance is the main cause of the poor prognosis of adult ALL. Therefore, early prediction of the resistant-drug had an essential role in guiding ALL therapy.

Dexamethasone – resistance is the common cause of the poor prognosis of adult ALL. This study was focused on identifying the key genes related to dexamethasone - resistance in ALL, The GSE94302 from GEO database was chosen in the study. A total of 837 DEGs were picked up, including 472 up-regulated and 365 down-regulated genes. Then the DAVID software was used to analysis the DEGs in three aspects (MF, BP and CC). For deeper understanding, the up- and down-regulated genes are further clustered based on functions signaling pathways with significant enrichment analysis. The protein-protein interaction(PPI) network of DEGs is developed, and the top 10 genes were selected with the higher degree of connectivity.

The results shown that the up-regulated DEGs, including *CCNBI*, *CDKI*, *PCNA* and *MYC*, are biologically related to cell cycle regulation. Regulation of cell cycle has been proven to be closely related to tumor development and drug resistance^[15].Cohen Y et al. found that *CCNBI* was shown to be down-regulated in multiple myeloma and acute

myeloid leukemia cells^[16]. In this study, we found that high expression of *CCNB1* lead to ALL cells sensitive to dexamethasone treatment. *CDK1*(cyclin dependent kinase 1) is a member of the *Serine/Threonine* protein kinase family. Previous studies showed that *CDK1* was related to many types of tumors growth and the patients survival ^[17-21]. Based on our analysis, *CDK1* was identified as the key gene in up-regulated DEGs, and GO annotations related to nucleoplasm, Ub1 conjunction cytoskeleton and cell division. The association of *CDK1* between drug resistance hasn't been well explained. Therefore, our results could be helpful to explore its role in drug resistance deeply. *CCNB1* and *CDK1* have been found to regulated drug sensitiveness not only through cell cycle pathway, but p53 pathway according to our analysis. Previous study also found that *PCNA* was associated with cancers development ^[22, 23] and patients prognosis ^[24, 25]. This study found that *PCNA* could serve as a biomarker indicating dexamethasone sensitivity through cell cycle pathway based on our analysis. *MYC* gene was first discovered in Burkitt lymphoma patients, it has been proven to play key role in the development of many tumors^[26-28]. In our study, the level of *MYC* expression was related to the sensitiveness of dexamethasone treatment, and *MYC* regulated the drug sensitiveness through cell cycle pathway. This result need to be further validated in future studies.

The other key genes in up-regulated DEGs except for those enriched in cell cycle, including *KPNA2*, *AURKA*, *NDC80*, *HSPA4*, *KIF11*, *UBE2C*. Based on GO analysis, it was found that *KPNA2*, *AURKA*, *NDC80*, *HSPA4*, and *UBE2C* are enriched in protein binding. Drug's performance can be enhanced or reduced by protein binding^[29]. Recent

research has found *KPNA2* is significantly associated with tumor differentiation, tumor depth, lymph node metastasis, venous invasion, recurrence and clinical response through L-type amino acid transporter 1^[30]. In addition, *KPNA2* has been proven to be a potential marker of prognosis and therapeutic sensitivity in colorectal cancer patients^[31]. But its mechanism needs further investigation. Our study also indicated that *KPNA2* could be a biomarker for ALL prognosis. *AURKA* plays an important role in the development of pediatric glioblastoma (pGBM), and its inhibitor is considered as the effective therapy of pGBM^[32]. *AURKA*, is a type of Aurora kinases. Recently, several Aurora kinase inhibitors were being investigated as novel anticancer therapy in breast cancer^[33]. The importance of *AURKA* in ALL has not been studied deeply, our analysis will provide a new angle for further investigation. *NDC80* is overexpressed in a variety of human cancers^[34], its GO analysis suggests it is functional in protein binding, cell cycle, nucleus and cell division. The value of *NDC80* in leukemia has not been reported in previous studies. *HSPA4*, *KIF11* and *UBE2C* all have relationships with many carcinomas^[35-37], but their roles in leukemia need to be further studied.

The down-regulated genes are dominantly related to Epstein-Barr virus infection, platelet activation, hematopoietic cell lineage and Fc gamma R-mediated phagocytosis, for example, *PIK3CG*, *LYN*, *CD19* and *CD44*. *PIK3CG* encodes a protein that belongs to the pi3/pi4-kinase family of proteins. The product of it is an enzyme which is thought to play a pivotal role in the regulation of cytotoxicity in NK cells. *PIK3CG* has been proven contributed to B cell development and maintenance, transformation, and

proliferation^[38]. The finding in this study indicted that *PIK3CG* regulated DEX sensitiveness through platelet activation, regulation of actin cytoskeleton, Epstein-Barr virus infection, and fc gamma R-mediated phagocytosis pathway. *LYN* has been a biomarker in many cancers^[39, 40]. In this study, *LYN* also could be a sign on the response to dexamethasone. *CD19* is located on the surface of B lineage cells, except for plasma cells and on follicular dendritic cells. It has been used to diagnose cancers that arise from this type of cell - notably B-cell lymphomas^[41]. *CD19* has also been proven to be a useful treatment target of B cell malignance. *CD19* may regulate drug resistance in ALL by hematopoietic cell lineage and Epstein-Barr virus infection pathway. *CD44* participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis and tumor metastasis. *CD44* expression is an indicative marker for effector-memory T-cells. The high levels of the adhesion molecule *CD44* on leukemic cells were essential to generate leukemia^[42]. *CD44* was considered as cancer stem cell-like marker^[43]. However, the value upon drug resistance of *CD44* in neoplasms remains controversial^[44]. Therefore, it is necessary to identify the exact role of it in cancers.

STAT1, *DDX41*, *BCR*, *CD48*, *JAK1* and *ITGB1* are related to phosphoprotein according to the GO annotation. *STAT1* is a member of the STAT protein family. In mammals, the *JAK/STAT* pathway is the principal signaling mechanism for a wide array of cytokines and growth factors. *STAT1* and *JAK1* participate in the biological process via participating in type I interferons binding. *STAT1* is a key regulatory gene in autoimmune diseases and metastatic melanoma^[45, 46]. Studies found that *JAK/STAT*

pathway genes may play roles in lymphomagenesis, but they still need further investigation^[47-49]. It has been shown that germline mutations in *DDX41* gene in several leukemia families, and *DDX41* mutation could develop neoplasia through acquisition of another somatic mutation. The recognition of *DDX41* mutated cases may have implications for surveillance, assessment of prognosis, and the design of targeted therapies^[50]. Breakpoint cluster region(*BCR*) is at the chromosome 9 breakpoint. Although the *BCR-ABL* fusion protein has been extensively studied, the function of the normal *BCR* gene product is not clear. In our analysis, *BCR* may take part in DEX resistance, but its specific mechanism is unclear. *CD48* is a B-lymphocyte activation marker, expressed on all peripheral blood lymphocytes (PBL) including T cells, B cells, Null cell and thymocytes ^[51-53]. *CD48* is being investigated among other markers in research on disease markers, and our study will be useful for research. *ITGB1*(integrin beta1) is associated with adverse prognosis of prostate cancer through regulating Caveolin-1 (*CAVI*), *CAVI* was over-expressed in prostate cancer and predicts adverse prognosis^[54]. *ITGB1* was indicated to regulated drug resistance via platelet activation and regulation of actin cytoskeleton pathway according to our study, the relationships between *ITGB1* and leukemia need further research.

Conclusion

In conclusion, this study identified several potential molecular targets that might contribute to the DEX resistance in ALL, including *CDKI*, *PCNA*, *CCNBI*, *MYC*,

KPNA2, AURKA, NDC80, HSPA4, KIF11, UBE2C, PIK3CG, CD44, CD19, STAT1, DDX41, LYN, BCR, CD48, JAK1 and *ITGB1*, which may function via cell cycle pathway, platelet activation pathway, or Epstein-Barr virus infection pathway. However, the finding here should be taken prudently.

Acknowledgements

We are grateful to the researchers who provided their data for this analysis, and it is our pleasure to acknowledge their contributions.

References

- [1] Hunger SP, Mullighan CG. Acute Lymphoblastic Leukemia in Children. *N Engl J Med*. 2015. 373(16): 1541-52.
- [2] Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006. 354(2): 166-78.
- [3] Kantarjian H, Stein A, Gökbuget N, et al. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. *N Engl J Med*. 2017. 376(9): 836-847.
- [4] Paugh SW, Bonten EJ, Savic D, et al. NALP3 inflammasome upregulation and CASP1 cleavage of the glucocorticoid receptor cause glucocorticoid resistance in leukemia cells. *Nat Genet*. 2015. 47(6): 607-14.
- [5] Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. *Nat Genet*. 2013. 45(3): 242-52.
- [6] Davis S, Meltzer PS. GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor.

- Bioinformatics. 2007. 23(14): 1846-7.
- [7] Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000. 25(1): 25-9.
- [8] Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017. 45(D1): D353-D361.
- [9] Sherman BT, Huang dW, Tan Q, et al. DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis. BMC Bioinformatics. 2007. 8: 426.
- [10] Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013. 41(Database issue): D808-15.
- [11] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003. 13(11): 2498-504.
- [12] Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet. 2008. 371(9617): 1030-43.
- [13] Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013. 381(9881): 1943-55.
- [14] Intermesoli T, Krishnan S, MacDougall F, Jenner M, Lister A, Rohatiner A. Efficacy of an intensive post-induction chemotherapy regimen for adult patients with Philadelphia chromosome-negative acute lymphoblastic leukemia, given predominantly in the out-patient setting. Ann Hematol. 2011. 90(9): 1059-65.
- [15] Liu P, Begley M, Michowski W, et al. Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus. Nature. 2014. 508(7497): 541-5.
- [16] Cohen Y, Gutwein O, Garach-Jehoshua O, Bar-Haim A, Kornberg A. The proliferation arrest of primary tumor cells out-of-niche is associated with widespread downregulation of mitotic and transcriptional genes.

- Hematology. 2014. 19(5): 286-92.
- [17] Yang W, Cho H, Shin HY, et al. Accumulation of cytoplasmic Cdk1 is associated with cancer growth and survival rate in epithelial ovarian cancer. *Oncotarget*. 2016. 7(31): 49481-49497.
- [18] Lin PC, Yang YF, Tyan YC, et al. Identification of Phosphorylated Cyclin-Dependent Kinase 1 Associated with Colorectal Cancer Survival Using Label-Free Quantitative Analyses. *PLoS One*. 2016. 11(7): e0158844.
- [19] Bednarek K, Kiwerska K, Szaumkessel M, et al. Recurrent CDK1 overexpression in laryngeal squamous cell carcinoma. *Tumour Biol*. 2016. 37(8): 11115-26.
- [20] Bae T, Weon KY, Lee JW, Eum KH, Kim S, Choi JW. Restoration of paclitaxel resistance by CDK1 intervention in drug-resistant ovarian cancer. *Carcinogenesis*. 2015. 36(12): 1561-71.
- [21] Luo Y, Wu Y, Peng Y, Liu X, Bie J, Li S. Systematic analysis to identify a key role of CDK1 in mediating gene interaction networks in cervical cancer development. *Ir J Med Sci*. 2016. 185(1): 231-9.
- [22] Wang X, Wang D, Yuan N, et al. The prognostic value of PCNA expression in patients with osteosarcoma: A meta-analysis of 16 studies. *Medicine (Baltimore)*. 2017. 96(41): e8254.
- [23] Venturi A, Piaz FD, Giovannini C, Gramantieri L, Chieco P, Bolondi L. Human hepatocellular carcinoma expresses specific PCNA isoforms: an in vivo and in vitro evaluation. *Lab Invest*. 2008. 88(9): 995-1007.
- [24] Xing X, Guo J, Wen X, et al. Analysis of PD1, PDL1, PDL2 expression and T cells infiltration in 1014 gastric cancer patients. *Oncoimmunology*. 2018. 7(3): e1356144.
- [25] Juríková M, Danihel Ľ, Polák Š, Varga I. Ki67, PCNA, and MCM proteins: Markers of proliferation in the diagnosis of breast cancer. *Acta Histochem*. 2016. 118(5): 544-52.
- [26] Jovanović KK, Roche-Lestienne C, Ghobrial IM, Facon T, Quesnel B, Manier S. Targeting MYC in multiple myeloma. *Leukemia*. 2018 .
- [27] Wang J, Liu Z, Wang Z, et al. Targeting c-Myc: JQ1 as a promising option for c-Myc-amplified esophageal

- squamous cell carcinoma. *Cancer Lett.* 2018. 419: 64-74.
- [28] Rickman DS, Schulte JH, Eilers M. The Expanding World of N-MYC-Driven Tumors. *Cancer Discov.* 2018. 8(2): 150-163.
- [29] Scheife RT. Protein binding: what does it mean. *DICP.* 1989. 23(7-8 Suppl): S27-31.
- [30] Altan B, Kaira K, Watanabe A, et al. Relationship between LAT1 expression and resistance to chemotherapy in pancreatic ductal adenocarcinoma. *Cancer Chemother Pharmacol.* 2018. 81(1): 141-153.
- [31] Takada T, Tsutsumi S, Takahashi R, et al. KPNA2 over-expression is a potential marker of prognosis and therapeutic sensitivity in colorectal cancer patients. *J Surg Oncol.* 2016. 113(2): 213-7.
- [32] Kogiso M, Qi L, Braun FK, et al. Concurrent Inhibition of Neurosphere and Monolayer Cells of Pediatric Glioblastoma by Aurora A Inhibitor MLN8237 Predicted Survival Extension in PDOX models. *Clin Cancer Res.* 2018 .
- [33] Han EH, Min JY, Yoo SA, et al. A small-molecule inhibitor targeting the AURKC-I κ B α interaction decreases transformed growth of MDA-MB-231 breast cancer cells. *Oncotarget.* 2017. 8(41): 69691-69708.
- [34] Huang LY, Lee YS, Huang JJ, et al. Characterization of the biological activity of a potent small molecule Hec1 inhibitor TAI-1. *J Exp Clin Cancer Res.* 2014. 33: 6.
- [35] Webster AF, Williams A, Recio L, Yauk CL. Gene expression analysis of livers from female B6C3F1 mice exposed to carcinogenic and non-carcinogenic doses of furan, with or without bromodeoxyuridine (BrdU) treatment. *Genom Data.* 2014. 2: 117-22.
- [36] Hitti E, Bakheet T, Al-Souhibani N, et al. Systematic Analysis of AU-Rich Element Expression in Cancer Reveals Common Functional Clusters Regulated by Key RNA-Binding Proteins. *Cancer Res.* 2016. 76(14): 4068-80.
- [37] Zhang Y, Tian S, Li X, Ji Y, Wang Z, Liu C. UBE2C promotes rectal carcinoma via miR-381. *Cancer Biol*

- Ther. 2018. 19(3): 230-238.
- [38] Beer-Hammer S, Zebedin E, von HM, et al. The catalytic PI3K isoforms p110gamma and p110delta contribute to B cell development and maintenance, transformation, and proliferation. *J Leukoc Biol.* 2010. 87(6): 1083-95.
- [39] Kim YJ, Hong S, Sung M, et al. LYN expression predicts the response to dasatinib in a subpopulation of lung adenocarcinoma patients. *Oncotarget.* 2016. 7(50): 82876-82888.
- [40] Nguyen PH, Fedorchenko O, Rosen N, et al. LYN Kinase in the Tumor Microenvironment Is Essential for the Progression of Chronic Lymphocytic Leukemia. *Cancer Cell.* 2016. 30(4): 610-622.
- [41] Scheuermann RH, Racila E. CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy. *Leuk Lymphoma.* 1995. 18(5-6): 385-97.
- [42] Quéré R, Andradottir S, Brun AC, et al. High levels of the adhesion molecule CD44 on leukemic cells generate acute myeloid leukemia relapse after withdrawal of the initial transforming event. *Leukemia.* 2011. 25(3): 515-26.
- [43] Li S, Xu F, Zhang J, et al. Tumor-associated macrophages remodeling EMT and predicting survival in colorectal carcinoma. *Oncoimmunology.* 2018. 7(2): e1380765.
- [44] Li X, Ma X, Chen L, et al. Prognostic value of CD44 expression in renal cell carcinoma: a systematic review and meta-analysis. *Sci Rep.* 2015. 5: 13157.
- [45] Sezin T, Vorobyev A, Sadik CD, Zillikens D, Gupta Y, Ludwig RJ. Gene Expression Analysis Reveals Novel Shared Gene Signatures and Candidate Molecular Mechanisms between Pemphigus and Systemic Lupus Erythematosus in CD4+T Cells. *Front Immunol.* 2017. 8: 1992.
- [46] Wang LX, Li Y, Chen GZ. Network-based co-expression analysis for exploring the potential diagnostic biomarkers of metastatic melanoma. *PLoS One.* 2018. 13(1): e0190447.

- [47] Chen Y, Lan Q, Zheng T, et al. Polymorphisms in JAK/STAT signaling pathway genes and risk of non-Hodgkin lymphoma. *Leuk Res.* 2013. 37(9): 1120-4.
- [48] Lollies A, Hartmann S, Schneider M, et al. An oncogenic axis of STAT-mediated BATF3 upregulation causing MYC activity in classical Hodgkin lymphoma and anaplastic large cell lymphoma. *Leukemia.* 2018. 32(1): 92-101.
- [49] Moffitt AB, Ondrejka SL, McKinney M, et al. Enteropathy-associated T cell lymphoma subtypes are characterized by loss of function of SETD2. *J Exp Med.* 2017. 214(5): 1371-1386.
- [50] Maciejewski JP, Padgett RA, Brown AL, Müller-Tidow C. DDX41-related myeloid neoplasia. *Semin Hematol.* 2017. 54(2): 94-97.
- [51] Yokoyama S, Staunton D, Fisher R, Amiot M, Fortin JJ, Thorley-Lawson DA. Expression of the Blast-1 activation/adhesion molecule and its identification as CD48. *J Immunol.* 1991. 146(7): 2192-200.
- [52] Henniker AJ, Bradstock KF, Grimsley P, Atkinson MK. A novel non-lineage antigen on human leucocytes: characterization with two CD-48 monoclonal antibodies. *Dis Markers.* 1990. 8(4): 179-90.
- [53] Vaughan HA, Thompson CH, Sparrow RL, McKenzie IF. Hu Ly-M3--a human leukocyte antigen. *Transplantation.* 1983. 36(4): 446-50.
- [54] Pellinen T, Blom S, Sánchez S, et al. ITGB1-dependent upregulation of Caveolin-1 switches TGF β signalling from tumour-suppressive to oncogenic in prostate cancer. *Sci Rep.* 2018. 8(1): 2338.