Pathology - Research and Practice 215 (2019) 152528

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Quantitative proteomics analysis of differentially expressed proteins in activated B-cell-like diffuse large B-cell lymphoma using quantitative proteomics

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ARTICLE INFO

Keywords:
Activated B-cell-like diffuse large B cell lymphoma
iTRAQ
Proteomics analysis
Pathology

ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with unclear pathogenesis. DLBCL accounts for 30%–35% of all non-Hodgkin lymphomas (NHLs) and is an aggressive subtype of mature B-cell neoplasm. At present, half of DLBCL cases can be cured, although one-third of patients experience recurrence after treatment and enter advanced tumor stage. This study aimed to investigate the differentially expressed proteins in activated B-cell-like-DLBCL (ABC-DLBCL) through quantitative proteomics (iTRAQ). Seven ABC-DLBCL experimental samples and eight control samples (reactive hyperplasia of the lymph node) were obtained from fresh tissues. The exclusion criteria were expressed as follows: (1) patients with other lymphoid diseases; and (2) patients undergoing chemical treatment. A total of 5974 proteins were identified. P value < 0.05 and multiple expressions were more than 1.2-fold. A total of 131 upregulated and 204 downregulated differentially expressed proteins were identified. Gene ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis were performed. Protein–protein interaction (PPI) network analysis was conducted. The expression levels of HSP90AB1, GNA13, LAMB2, LAMA5, YWHAZ, and IKBKB were evaluated through PRM and TCGA to validate the accuracy of iTRAQ and liquid chromatography–tandem mass spectrometry results. Results of differential multiple and *t*-test showed differences in the expression levels of six target proteins between the control and experimental groups. To the best of our knowledge, the present study is the first to identify proteins associated with ABC-DLBCL using iTRAQ technology. Our results provide new insights into the pathogenesis of ABC-DLBCL. The combination of ABC-DLBCL-associated signaling pathway proteins and targeted therapy to reverse drug resistance is of great significance in improving the comprehensive treatment of lymphoma and reducing mortality of affected individuals. The feasibility of the present study is limited due to the number of samples, and future studies are required to determine the function of proteins in ABC-DLBCL development.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin lymphoma (NHL) worldwide, and has huge heterogeneity in clinical manifestations, tissue morphology, immune typing, and prognosis. DLBCL accounts for 30%–35% of all NHLs and is an aggressive subtype of mature B-cell neoplasm [1]. DLBCL is subclassified into germinal center B-cell-like (GCB) and activated B-cell (ABC) subtypes based on cell-of-origin using the Hans algorithm [2]. The proportion of ABC-DLBCL patients in Asians is remarkably high, and the proportion of poor prognosis groups is higher than in Europeans and patients from other areas. Thus, ABC-DLBCL subtypes need to be the focus of clinical research [3]. At present, half of all DLBCL cases can be cured, although one-third of patients undergo recurrence after treatment and enter the advanced tumor stage because of the activation of various oncogenic pathways and drug resistance [4]. Activation of nuclear factor (NF)-κB, PI3K/AKT, and other signaling pathways in DLBCL has been confirmed [5]. NF-κB is an important set of transcription factors, and its abnormal activation induces the abnormal expression of a series of tumor-related genes, thereby inhibiting the

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https://doi.org/10.1016/j.prp.2019.152528
Received 22 March 2019; Received in revised form 10 June 2019; Accepted 5 July 2019
0344-0338/

apoptosis of tumor cells and directly participating in the occurrence and development of malignant tumors [6]. NF-κB activation may upregulate the expression levels of VEGF, MMPs, and uPA in tumor metastasis, thereby promoting tumor angiogenesis [7]. Meanwhile, NF-κB pathway is relevant to various kinds of cancer, such as B-cell neoplasm-like chronic lymphocytic leukemia (CLL). Alsagaby et al revealed that NF-κB activation inhibits apoptosis and induces survival of many kinds of CLL cells. NF-κB is continuously activated in CLL cells, and its activation is related to drug resistance. NF-κB activation is strongly correlated to advanced CLL stage, thereby suggesting that the activity of NF-κB is an independent predictor of prognosis [8]. PI3K/AKT signaling pathway is an important signal transduction pathway that promotes cell survival and maintains normal cell function. AKT protein kinase regulates cell proliferation, survival, and apoptosis, and its mediated phosphorylation can alter BCL-2 family members, NF-κB, and other transcription factors that initiate and inhibit apoptosis. Maladjustment of PI3K/AKT may lead to tumor formation, translocation, and resistance to chemotherapy [9,10]. Thus, many studies have used small molecular inhibitors to inhibit this signaling pathway. However, the induction of drug resistance of DLBCL is a multifactor, multisignal pathway, and multigene interaction process. Some limitations still exist because of the incomplete understanding of the DLBCL molecular mechanism. Thus, increasing studies have elucidated the causes and underlying molecular mechanisms of DLBCL.

At present, proteomics is an advanced method that allows global analysis of protein expression levels and provides a valuable opportunity for discovering disease-related proteins. Proteomics plays an important role in the detection of different proteins in hematological malignancies, such as DLBCL and CLL. A review of existing CLL proteomics indicates that proteomics is an advanced approach that enables large-scale characterizations of diff ;erent aspects of proteins, such as protein expression profiling, post translation modification, protein localization, and protein function [11]. A research showed that abnormal protein expression products has been reported to significantly influence the CLL behavior and clinical outcomes. Protein plays a role in BCR signal transduction in CLL cells [12]. Some overexpressed proteins in CLL cells are linked to mRNA processing and splicing. Some proteins (such as HSP27, ZAP-70, TCL-1, S100A8), which are potential therapeutic targets for CLL, may play an important role in the future [13]. Ryuetschi et al compared fresh frozen tumor tissues from DLBCL patients with early recurrence or refractory and long-term progression-free based on proteomics to obtain differentially expressed proteins. Progression-free patients show high expression levels of proteins involved in the actin cytoskeleton protein network and could be of functional importance in the development of sustained response to immunochemotherapy [14]. Researchers have used whole-exome and transcriptome sequencing to comprehensively define the landscape of 150 genetic drivers of DLBCL. Many oncogenes play a role in early pathogenesis or another cancer-related function [15]. Riby et al analyzed the serum proteome of DLBCL patients and found that AdipoQ, CD14, HSPG2, ECM1, and ACT are significantly increased in DLBCL patients. These proteins are related to tumor metastasis and immune regulation [16]. Furthermore, researchers have compared the differences in proteomics between GCB and nonGCB DLBCL. Bip/GRP 78, HSP 90, and cyclin B2 were enhanced in nonGCB DLBCL, thereby suggesting that they may be potential drug targets [17]. Subtype identification of DLBCL is based on different classifications. A group of researchers has divided DLBCL based on the shared genomic abnormalities. MCD and BN2 DLBCLs rely on chronic active B-cell receptor signaling that is amenable to therapeutic inhibition [18]. In our previous study, we used immunohistochemical (IHC) staining to detect the expression levels of BCL-6/PRDM1/Blimp1 proteins in 100 DLBCL cases. Differences were observed in the expression levels of PRDM1/Blimp1 in DLBCL with different immunological typing. The methylation status of PRDM1 in 100 patients with different subtypes of DLBCL was detected through MS-PCR. The methylation rate in ABC-DLBCL was higher than in GCB-DLBCL. PRDM1 was a tumor suppressor factor that is closely related to the differentiation and development of B lymphocytes and a downstream protein of NF- κB signaling pathway. The abnormal expression level of this gene may be involved in lymphoma development. We investigated ABC-DLBCL based on these findings. Genetic differences between lymph nodes and extranodal DLBCL were reported using proteomics and genome technology [19]. However, few proteomics studies used tissue samples of DLBCL patients to gain chemosensitivity-related proteins [20].

In this study, we evaluated the proteins and signaling pathways involved in ABC-DLBCL using iTRAQ to elucidate the causes and underlying molecular mechanisms of DLBCL for establishing novel diagnostic markers and providing a new therapeutic target for DLBCL treatment.

2. Materials and methods

2.1. Patients and samples

Fifteen samples, namely, 7 ABC-DLBCL experimental samples and 8 control samples (reactive hyperplasia of the lymph node), were gathered from fresh tissues, which were randomly divided into experimental and control groups, respectively. Each group contained three samples for a total of six samples. Sample details are shown in Table 1. Tissue

Table 1
Clinical characterization of 15 samples.

Grouping situation	Pathological number	Sex	Age(years)	Clinical stages	IPI score	B symptom	BMI
Experimental group							
nonGCB 1	2015-20144	Male	30	IV-B	1	Yes	No
	2017-16465	Female	72	III-A	3	No	No
nonGCB 2	2015-22809	Male	41	IV-A	2	No	No
	2017-14119	Male	69	IV-B	5	Yes	–
nonGCB 3	2016-06160	Female	34	II-A	0	No	No
	2016-19691	Male	53	III-B	3	Yes	–
	2017-10771	Male	74	III-A	3	No	Yes
Control group							
Control 1	2014-13712	Male	9	–	–	–	–
	2017-13689	Male	3	–	–	–	–
Control 2	2015-00937	Male	52	–	–	–	–
	2016-21447	Male	5	–	–	–	–
	2017-13686	Female	3	–	–	–	–
Control 3	2015-00938	Male	14	–	–	–	–
	2015-29323	Female	34	–	–	–	–
	2016-26284	Female	48	–	–	–	–

BMI : bone marrow involvement.

samples were immediately transferred to liquid nitrogen and stored at −80 °C until protein extraction. The exclusion criteria were expressed as follows: (1) patients with other lymphoid diseases, such as follicular lymphoma and primary central nervous system DLBCL; and (2) patients undergoing chemical treatment. The study was approved by the Ethics Committee of the Department of Medicine, First Affiliated Hospital of Xinjiang Medical University, and a written informed consent was obtained from all patients.

2.2. Protein extraction and iTRAQ labeling

The samples (human, mouse, and rat serum samples need to be removed from the high abundance protein in serum first) were extracted by SDT (4% (w/v) SDS, 100 mM Tris/HCl pH 7.6, 0.1 M DTT) cleavage method. Then, BCA was used for protein quantification. Enzymatic hydrolysis of trypsin was conducted through filter-aided proteome preparation. The proper amount of protein was obtained from each sample, and enzymatic hydrolysis peptide was desalted using C18 cartridge. The peptide was lyophilized and redissolved with 40 μL dissolution buffer. The peptide segment was quantified using OD280. Peptide fragment (100 μg) of each sample was labeled based on the instructions of AB SCIEX iTRAQ labeling kit.

2.3. SCX chromatographic classification

Each group of labeled peptides was mixed, and AKTA Purifier 100 was used for grading. Buffer solution A liquid was 10 mM KH_2PO_4, 25% ACN, pH 3, and B solution was 10 mM KH_2PO_4, 500 mM KCl, 25% ACN, pH 3.0. The chromatographic column was balanced with A liquid, and the sample was separated from the sampler to the chromatographic column at a flow rate of 1 mL/min. The gradient of liquid phase was expressed as follows: 0–25 min, the linear gradient of liquid B was from 0 to 10; 25–32 min, the linear gradient of liquid B was from 10% to 20%; 32–42 min, the linear gradient of liquid B was from 20% to 45%; 42–47 min, the linear gradient of liquid B was from 45% to 100%; and 47–52 and 52–60 min, the B solution was maintained at 100%. After 60 min, fluid B was reset to 0. The absorbance value at 214 nm was monitored during washing. Desalting was conducted with C18 cartridge after lyophilization. The elution components were collected at 1 min intervals.

2.4. LC–MS/MS data acquisition

Each fractionated sample was separated by a high-performance LC (HPLC) liquid phase system Easy nLC. Buffer A solution was 0.1% formic acid solution, and B solution was 0.1% formic acid acetonitrile aqueous solution (84% acetonitrile). The chromatographic column was balanced with 95% A liquid. The sample was collected from the automatic sampler to the sample column (Thermo Scientific Acclaim PepMap100, 100 μm*2 cm, nanoViper C18). After the analytical column separation (Thermo Scientific EASY column, 10 cm, ID75 μm, 3 μm, C18-A2), the velocity was 300 nL/min. The sample was separated through chromatographic separation, and a Q-Exactive mass spectrometer was used for mass spectrum analysis. Positive ion was used for detection, and parent ion scan ranged from 300 m/z to 1800 m/z. The resolution of first-order MS was 70,000 at 200 m/z, automatic gain control target was 1e6, maximum IT was 50 ms, and dynamic exclusion time was 60.0 s. The mass charge ratio of peptides and the fragments of peptides were collected in accordance with the following methods. Twenty fragments were collected after each full scan. MS2 activation type was HCD. Isolation window was 2 m/z, and second-order MS resolution was 17,500 at 200 m/z. Normalized collision energy was 30 EV, and underfill was 0.1%.

2.5. iTRAQ data analysis

Protein identifications were performed using the MASCOT2.2 search engine and Proteome Discoverer 1.4 for database identification. The relevant parameters and descriptions were expressed as follows: (1) Database, UniProt; (2)Taxonomy, Homo sapiens; (3) Enzyme, trypsin; (4)Fixed modifications, Carbamidomethyl(C), iTRAQ 4plex(N-term), iTRAQ 4plex(K); (5)Variable modifications, oxidation(M) iTRAQ 4/8 plex(Y); (6)Max missed cleavages, 2; (7)Peptide mass tolerance, ± 20 ppm; (8) Fragment mass tolerance, 0.1DA; (9)Peptide FDR, ≤0.01. Proteins with corrected $P < 0.05$ and a fold change of > 1.2 or < 0.83 were considered to be significantly and differentially expressed [21]. Data analysis was supported by Applied Protein Technology Co., Ltd. (Shanghai, China).

2.6. Bioinformatics analysis

Blast2GO was used to annotate the target protein set. The target protein set was annotated with the KEGG pathway using KEGG Automatic Annotation Server. CytoScape software (version number: 3.2.1) was used to generate network maps using the protein–protein interaction (PPI) data from the STRING database. For protein cluster analysis, a hierarchical clustering algorithm was used to analyze the differentially expressed proteins in the comparison group. The data were displayed in the form of a heatmap.

2.7. LC-PRM/MS detection and analysis

In this experiment, a new protein verification method, PRM, was selected. This is an ion monitoring technique based on high-resolution and high-precision mass spectrometry. It can selectively detect target proteins and target peptides (such as post translation modified peptides) to realize the quantification of target protein/peptide. The parent ion information of the target peptide was selectively detected in the first-stage MS (Q1) by using the selective detection ability of the four-stage mass analyzer, and the parent ion was broken by collision cell. A high-resolution, high-quality analyzer was used to detect the information of all fragments in the selected parent ion window in the second-order MS [22]. Seventeen target peptides of 6 kinds of target proteins were quantitatively analyzed through PRM. The peptide segment information suitable for PRM analysis was imported into the software Xcalibur to set up the PRM method. HPLC system was used for chromatographic separation. In each sample, the 10ug peptide fragment was taken and the 200 fmol PRTC labeled peptide was added into the sample (The selected internal standard peptide segment: ELGQSGVDTYLQTK) was used for detection. The samples separated by high- performance liquid chromatography (HPLC) were analyzed by PRM mass spectrometry with Q-Exactive HF mass spectrometer (Thermo Scientific). Six samples were detected by PRM, and the data of PRM files were analyzed by software Skyline 3.5.0.

2.8. Statistical analysis

For continuous variables, independent Student's *t*-test was used to compare the groups. Fisher's exact test was used to compare the categorical variables. $P < 0.05$ was considered statistically significant. All statistical analyses were performed on statistical software SPSS (version 22, SPSS, Chicago, Illinois, USA).

3. Results

3.1. Clinical information

Seven samples with ABC-DLBCL and eight samples with reactive hyperplasia of the lymph node were included in this study. The clinical data were obtained from medical records. These data are summarized

Table 2
Proteins significantly up-regulated in DLBCL.

No.	Protein	Accession no.	Gene name	Protein function	Fold change
1	Golgi transport 1 homolog B	G3V1U5	GOLT1B	vesicle-mediated transport	2.179
2	cDNA FLJ51108	B7Z6U5	N/A	integrin-mediated signaling pathway	2.103
3	Uncharacterized protein DKFZp686K18196	Q6N092	DKFZp686K18196	Unclassified	2.013
4	cDNA FLJ96580	B2RDE8	N/A	Unclassified	1.829
5	La-related protein 4	F8W1I4	LARP4	RNA binding	1.764
6	Importin subunit alpha	A8K7D9	N/A	nuclear import signal receptor activity	1.722
7	Adenylate kinase isoenzyme 6	D6RDH4	AK6	adenylate kinase activity	1.678
8	Presenilins-associated rhomboid-like protein, mitochondrial	F8WCQ4	PARL	Unclassified	1.645
9	Mitochondrial import inner membrane translocase subunit Tim8 B	Q9Y5J9	TIMM8B	metal ion transmembrane transporter activity	1.640
10	Nucleoplasmin-3	O75607	NPM3	RNA binding	1.595

in Table 1. The samples were enrolled from 2013 to 2017.

3.2. Identification of differentially expressed proteins

This study adopted iTRAQ quantitative proteomics technology. Six labeled samples were mixed and divided into 15 groups, and we performed 15 runs. A total of 5974 proteins were identified in *H. sapiens*. Proteins with corrected $P < 0.05$ and a fold change of > 1.2 or < 0.83 were considered significantly and differentially expressed. A total of 131 upregulated and 204 downregulated differentially expressed proteins were identified. Gene ontology (GO) function and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analysis showed that the functions of these differentially expressed proteins were binding, catalytic activity, structural molecule activity, molecular function (MF) regulator, and transporter activity. These differentially expressed proteins were mainly involved in important biological processes (BPs), such as cellular process, metabolic process, biological regulation, BP regulation, and response to stimulus. The 10 most significantly upregulated and the 10 most significantly downregulated proteins are shown in Tables 2 and 3, respectively. The top 20 most significantly enriched pathways are shown in Fig. 1.

3.3. Classification of differentially expressed proteins

GO is a standardized functional classification system that provides a dynamic updated and standardized vocabulary and describes the attributes of genes and gene products in organisms in three means, namely, BP, MF, and cellular component. The results of the analysis are shown in Fig. 2. Hierarchical clustering of protein expression profiles classified nonGCB versus the control group (Fig. 3).

3.4. Protein interaction network analysis

We constructed a PPI network of 335 differentially expressed proteins. In general, a greater degree of connectivity of a protein leads to a greater disturbance in the entire system when the protein changes. The protein may be the key to maintaining the balance and stability of the system. PPI network analysis revealed that PDCD11, NOP2, and POLR1B had a high degree of protein connectivity and participated in various BPs, such as RNA binding, rRNA processing, and DNA binding (Fig. 4).

3.5. Validation of differentially expressed proteins identified using iTRAQ

HSP90AB1, GNA13, LAMB2, LAMA5, YWHAZ, and IKBKB expression levels were evaluated through PRM and TCGA to validate the accuracy of iTRAQ and LC–MS/MS results. The traditional protein verification methods are westernblot (WB) and immunohistochemical staining. However, these methods have several disadvantages, such as antibody required, verification of protein quantity, and do not agree with the experimental results. The new protein verification method, PRM, can quantify multiple proteins simultaneously and can accurately and specifically analyze the target proteins/peptides in complex samples. The changing trend of six proteins screened through PRM in the experiment was consistent with the results of iTRAQ, thereby indicating the reliability of the experiment. HSP90AB1 and GNA13 were upregulated, whereas other proteins were downregulated in ABC-DLBCL.

4. Discussion

In recent years, the incidence of lymphoma has increased in many countries [23]. Lymphoma is a heterogeneous disease, and its pathogenesis and drug resistance mechanism remain unclear. DLBCL is a common B-NHL subtype, and one-third of patients experience refractory disease or relapse after treatment. Although autologous stem cell transplantation is the standard salvaging treatment for patients with relapsed or refractory DLBCL, its success rates are poor [24,25]. The genetic differences of lymph node and extranodular DLBCL were compared by using proteomics and genomic techniques. However, few proteomics studies have been conducted on differentially expressed proteins in tissue samples of DLBCL patients.

Patients with ABC-DLBCL and with tumors located at MYC translocations may have high risk for disease relapse after standard immunochemotherapy. Patients with GCB-DLBCL have better prognoses

Table 3
Proteins significantly down-regulated in DLBCL.

No.	Protein	Accession no.	Gene name	Protein function	Fold change
1	C-C motif chemokine 21	Q5VZ73	CCL21	immune response	0.331
2	Alcohol dehydrogenase 1B	P00325	ADH1B	alcohol dehydrogenase activity	0.393
3	Placenta-specific 8, isoform CRA_b	D6RA24	PLAC8	Unclassified	0.421
4	Beta globin (Fragment)	O95408	HBB	oxygen carrier activity	0.428
5	Kelch repeat and BTB domain-containing protein 11	O94819	KBTBD11	protein ubiquitination	0.433
6	Histone H2B type 3-B	Q8N257	HIST3H2BB	protein heterodimerization activity	0.454
7	Collagen alpha-1(II) chain	P02458	COL2A1	collagen fibril organization	0.465
8	Embryonic stem cell-specific 5-hydroxymethylcytosine-binding protein (Fragment)	D6RAI0	HMCES	peptidase activity	0.485
9	Putative histone H2B type 2-D	Q6DRA6	HIST2H2BD	DNA binding	0.489
10	cDNA FLJ52487	B7Z2D3	N/A	transcription factor activity	0.496

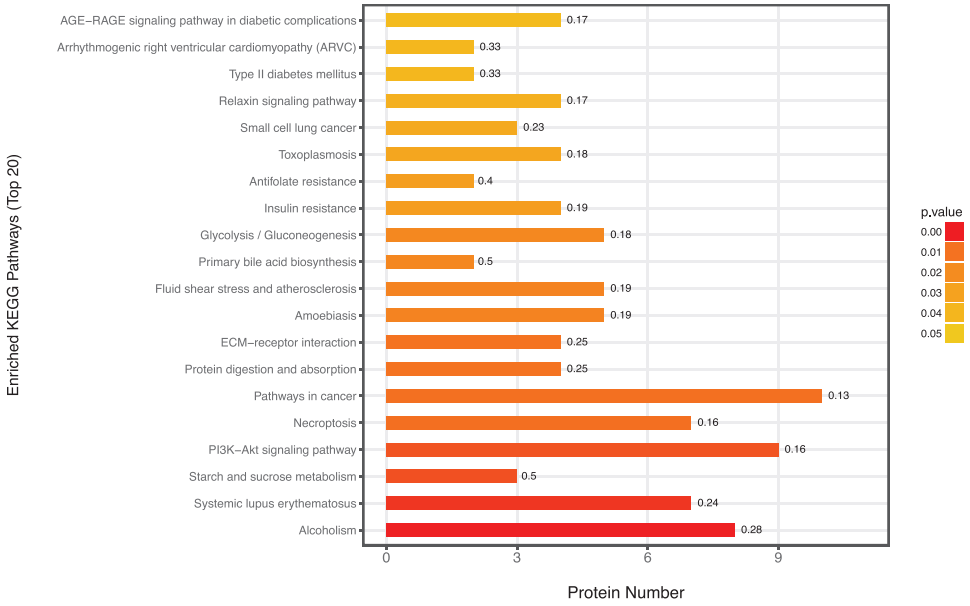

Fig. 1. The top 20 most significantly enriched pathways in DLBCL.

than those with ABC-DLBCL when treated with R-CHOP [26]. The pathogenic hallmark of ABC-DLBCL is the constitutive activation of the NF-κB pathway, and the PI3K/AKT pathway is activated by various mechanisms across B-cell malignancies. The production of DLBCL resistance is a multifactor, multisignal pathway, and multigene interaction process [27,28].

Lam et al. inhibited the activation of NF-κB in ABC-DLBCL cells by using IKK inhibitor Ps-1145 and its related complexes, thereby resulting in toxicity to tumor cells. NF-κB activation can increase multidrug resistance mediated by P-gp in resistance genes [29]. Uddin et al. confirmed that AKT in DLBCL is phosphorylated and inhibits PI3K-induced dephosphorylation of activated AKT, FOXO transcription factor, and GSK3 in LY sensitive cell lines. After treatment, the level of apoptosis inhibitor XIAP in DLBCL cell line sensitive to LY294002 decreased, and the XIAP level in the drug-resistant DLBCL cell line LY294002 remained constant. The results suggested that the level of AKT phosphorylation in

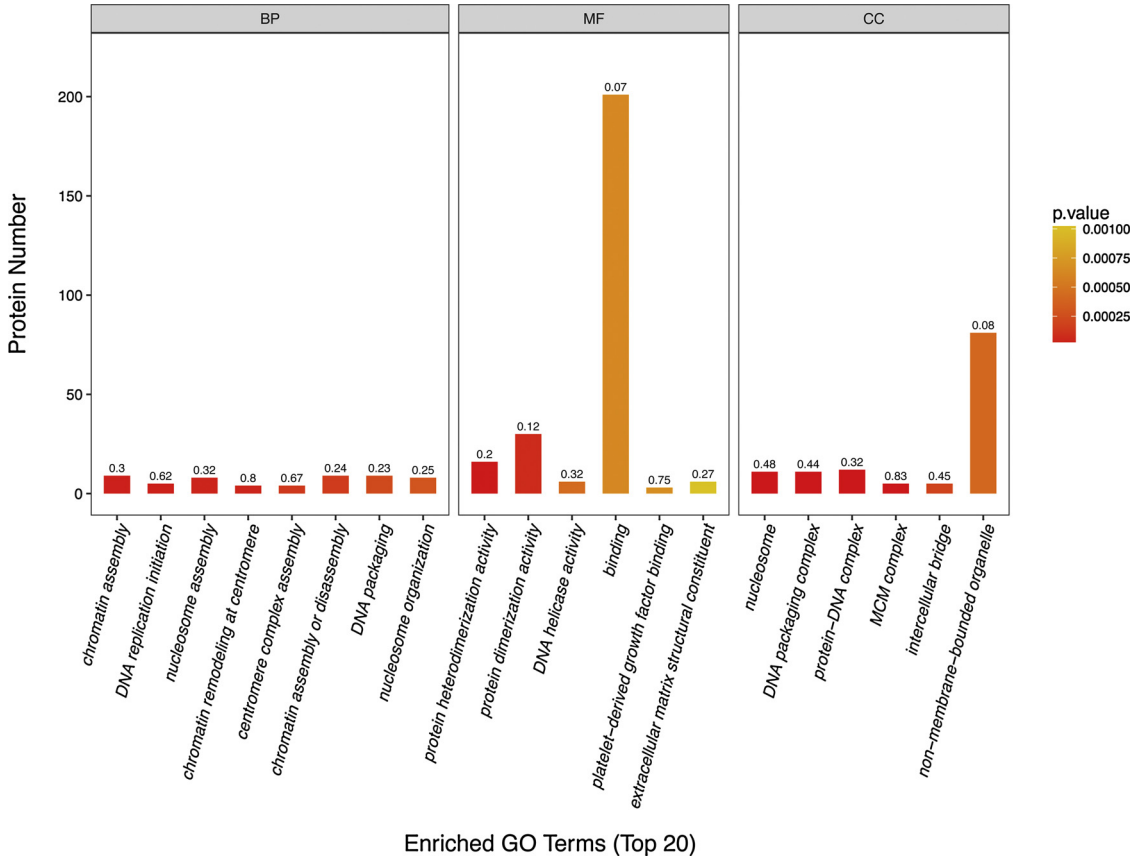

Fig. 2. The top 20 most significantly enriched GO terms in DLBCL.

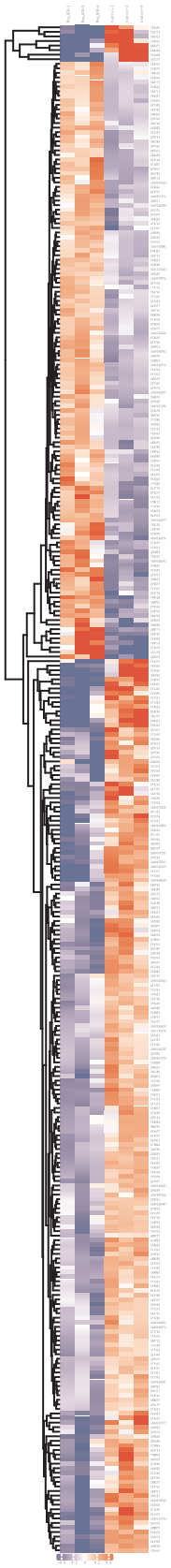

Fig. 3. Hierarchical clustering of the protein expression.

DLBCL cells is higher than that in sensitive DLBCL cells, and the inhibition of PI3K enables most DLBCL tumor cells to release cytochrome c and activate its downstream apoptotic enzymes [30]. We used Fisher's exact test method to analyze the differential expression protein of the experimental and control groups through KEGG pathway enrichment analysis. The results showed that the important pathways, such as alcoholism, systemic lupus erythematosus, PI3K/AKT signaling pathway, and starch and sucrose metabolism were significantly changed. The PI3K/AKT signaling pathway occupied large differentially expressed proteins (Fig. 5). The differentially expressed proteins of the NF-κB signaling pathway was 4, and the p value of the NF-κB signaling pathway in KEGG pathway enrichment analysis was 0.061 and was statistically insignificant.

In KEGG pathway enrichment analysis, alcoholism is the most upregulated pathway. Han et al. evaluated 575 female NHL patients and concluded that wine and liquor drinkers have decreased and increased risk of negative outcomes for DLBCL patients, respectively, compared with patients who do not drink [31]. In another study, high young adult BMI was associated with increased DLBCL risk, and lifetime alcohol consumption was inversely associated with risk among men [32]. Ollberding NJ et al suggested that a high dietary pattern in meat, fats, and sweets may be associated with an increased risk of NHL [33]. These findings may reveal the underlying connection between the pathogenesis of DLBCL and the mode of life. Meanwhile, the signaling pathways, such as PI3K/AKT, Wnt, Notch, and Jak/STAT in cancer occupied the highest protein number (Fig. 6).

In the GO function enrichment analysis, the functions of differentially expressed proteins were mainly binding, catalytic activity, structural molecule activity, MF regulator, and transporter activity (Fig. 7). These differentially expressed proteins were mainly involved in important BPs, such as cellular process, metabolic process, biological regulation, BP regulation, and stimulus response. PPI network analysis revealed that PDCD11, NOP2, and POLR1B had a high degree of protein connectivity and participated in various BPs, such as RNA binding, rRNA processing, and DNA binding. PDCD11 is an NF-κB binding protein that colocalizes with U3 RNA in the nucleolus and is required for rRNA maturation and generation of 18S rRNA [34]. NOP2, also known as NOL1, is highly expressed in the majority of human tumor cells and is associated with catalytically active telomerase. NOL1 is a new means for the telomerase to activate cyclinD1 gene transcription, thereby maintaining cell proliferation [35]. DNA-directed RNA polymerase I subunit RPA2, coded by POLR1B, also named as RPA135 combined with RPA194 are associated and form the active center of Pol I. RPA194 is unstable in the absence of RPA135, and small molecule BMH-21 can inhibit rRNA synthesis in cancer cells through the degradation of RPA194 [36,37].

HSP90AB1, GNA13, LAMB2, LAMA5, YWHAZ, and IKBKB were confirmed through PRM and TCGA. The results of differential multiple and *t*-test showed that some differences were present between the expression levels of six target proteins of the control and experimental groups. HSP90AB1 is a member of the large family of HSPs that function as molecular chaperones. HSPs play an important role in maintaining cell growth and promoting tumor formation and cancer cell proliferation. HSPs are frequently upregulated in cancer and may result in drug resistance. HSP110 sustains chronic NF-κB signaling in ABC-DLBCL through MyD88 stability. In this study, HSP90AB1 is related to PI3K/AKT signaling pathway (Fig. 8). Future investigations should be conducted to elucidate their biological mechanisms. GNA13 is a G protein involved in modulating tumor proliferative capacity, infiltration, metastasis, and migration. GNA13 loss, in combination with MYC overexpression, promotes lymphoma in mice. GNA13 mutations are linked to worse prognosis in R−CHOP-treated ABC-DLBCL patients. Thus, the role of GNA13 in untreated ABC-DLBCL should be determined.

YWHAZ is reportedly a prognostic marker for various tumors and plays a role in many oncogenic processes, such as proliferation, migration, and invasion [38]. YWHAZ is included in the 14-3-3 family of proteins, which is a family of evolutionarily highly conserved acidic proteins expressed in all eukaryotic organisms. YWHAZ may be

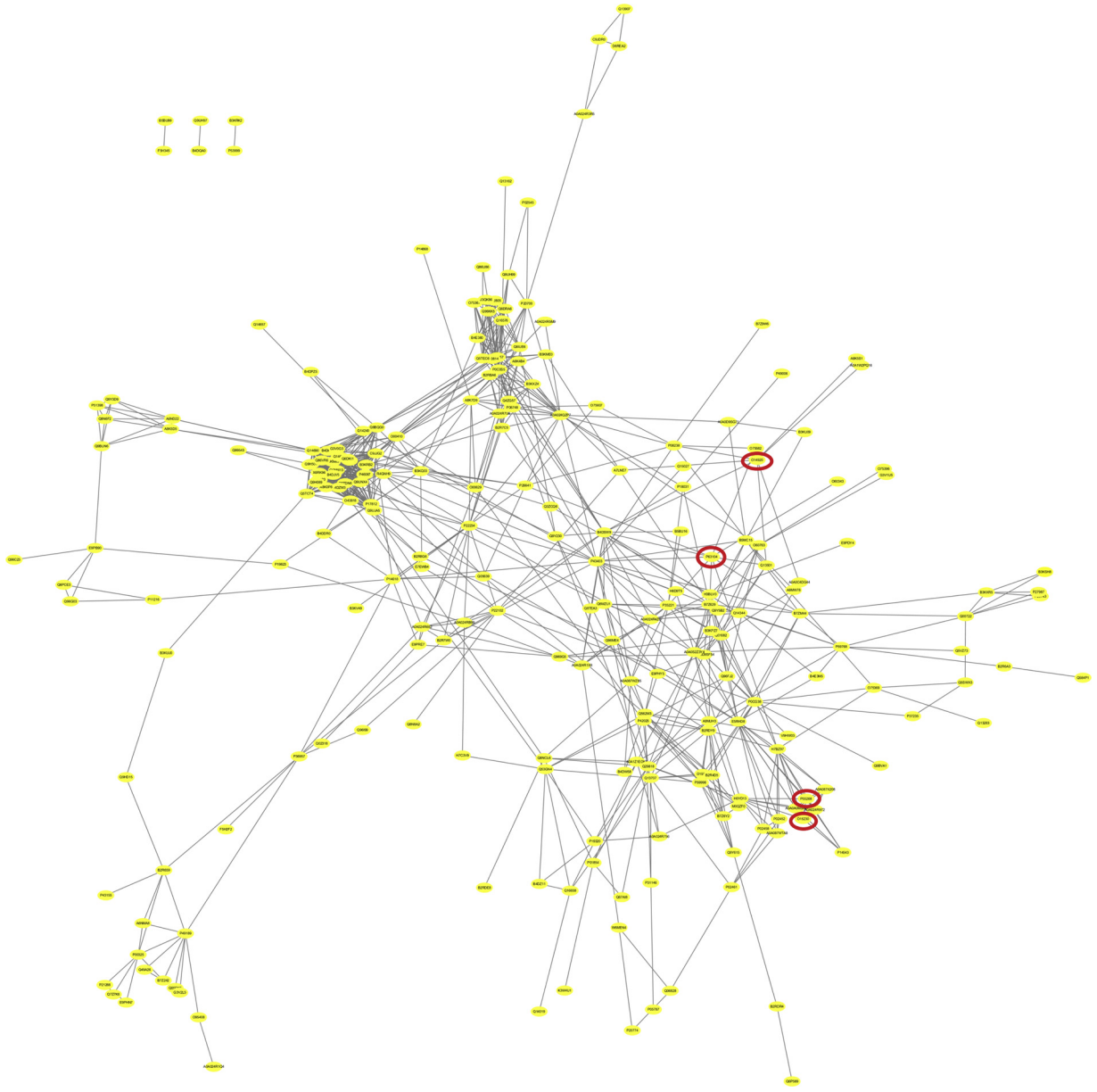

Fig. 4. Protein-Protein interaction (PPI) network of 335 differently expressed proteins between Non-GCB and control group.

frequently overexpressed in gastric cancer lines and primary gastric cancers [39,40]. The overexpression of YWHAZ is a poor prognostic factor that is independent of other prognostic factors. A research found that p17 variants (vp17 s) in HIV-1-seropositive patients with lymphoma are endowed with B-cell clonogenicity through the activation of the PTEN/PI3K/Akt signaling pathway, and different molecules, such as YWHAZ, are involved in apoptosis inhibition [41]. YWHAZ plays an important role in ASC (human antibody-secreting cells) survival. Few studies have been reported on the clinical significance of YWHAZ in patients with ABC-DLBCL.

IKBKB is an important regulator of NF-κB and is implicated in survival, proliferation, and apoptosis resistance of lymphoma cells [42]. Ayad et al found that IKBKB perturbation by ML120B leads to the synergistic enhancement of vincristine cytotoxicity in lymphoma [43]. Mutant IKBKB molecules are constitutively active in an activation-loop phosphorylation-independent manner [44]. Inhibition of IKK and IKK-related kinases is considered a therapeutic option for the treatment of inflammatory diseases and cancer [45]. In our study, we found that laminin is related to ECM-receptor interaction signaling pathway (Fig. 9). However, LAMB2 and LAMA5 have been rarely investigated in DLBCL. Laminin is implicated in various BPs, such as cell adhesion, differentiation, migration, signaling, neurite outgrowth, and metastasis. Its functions in DLBCL remain unclear and should be investigated.

5. Conclusions

The present study is the first to identify proteins associated with ABC-DLBCL using iTRAQ technology. The results of this study may provide new insights into the pathogenesis of ABC-DLBCL. The combination of the tumor signaling pathway proteins and targeted therapy to reverse drug resistance is of great significance in improving the comprehensive treatment of lymphoma and reduction of its mortality. The present study had limited sample size, which may lead to a high false positive rate. This study lacked IHC and WB experimental verification. We used PRM to validate six important proteins. Future studies should be conducted to determine the function of differentially expressed proteins in ABC-DLBCL development.

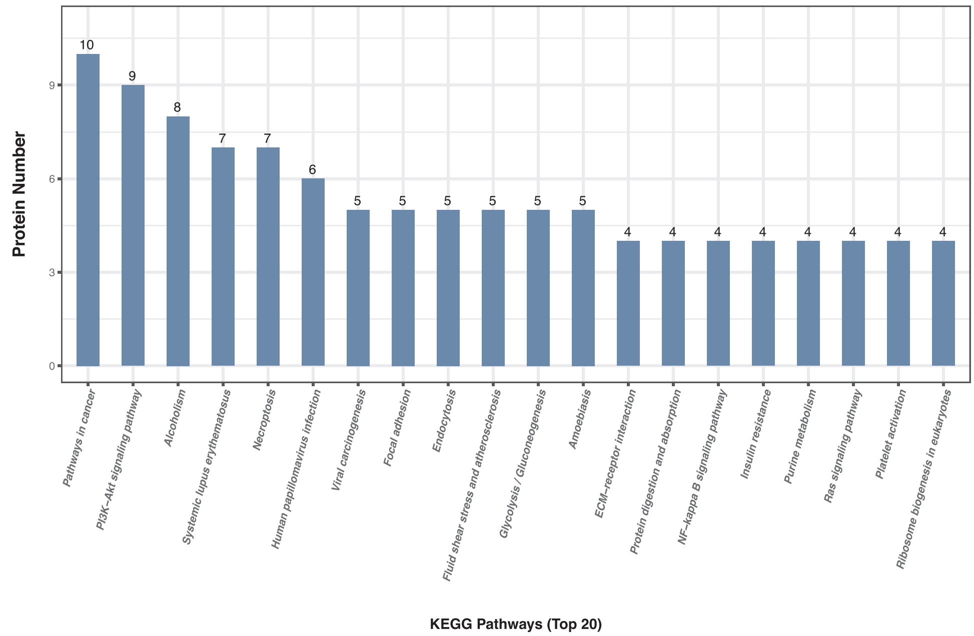

Fig. 5. The top 20 KEGG Pathways with the most protein numbers in DLBCL.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

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

Funding

This work was supported by the project of National Natural Science

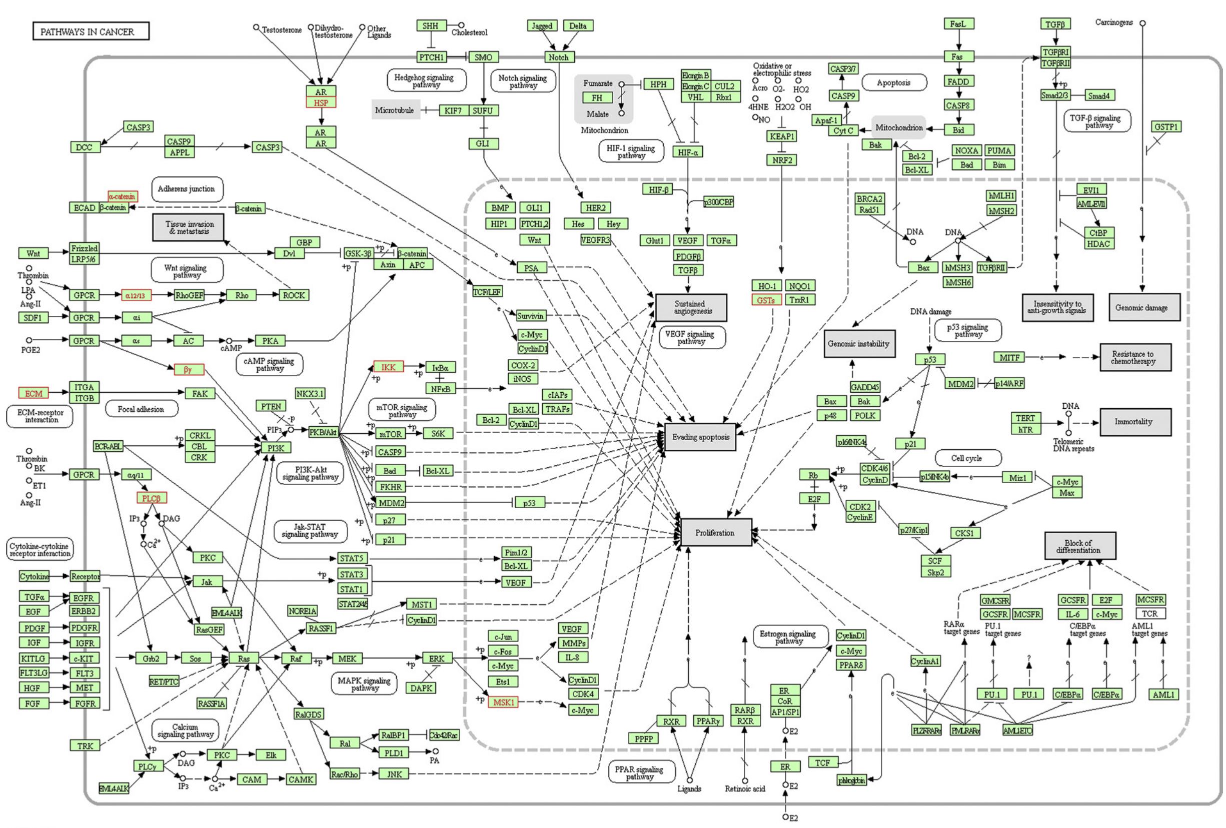

Fig. 6. Pathways in cancer.

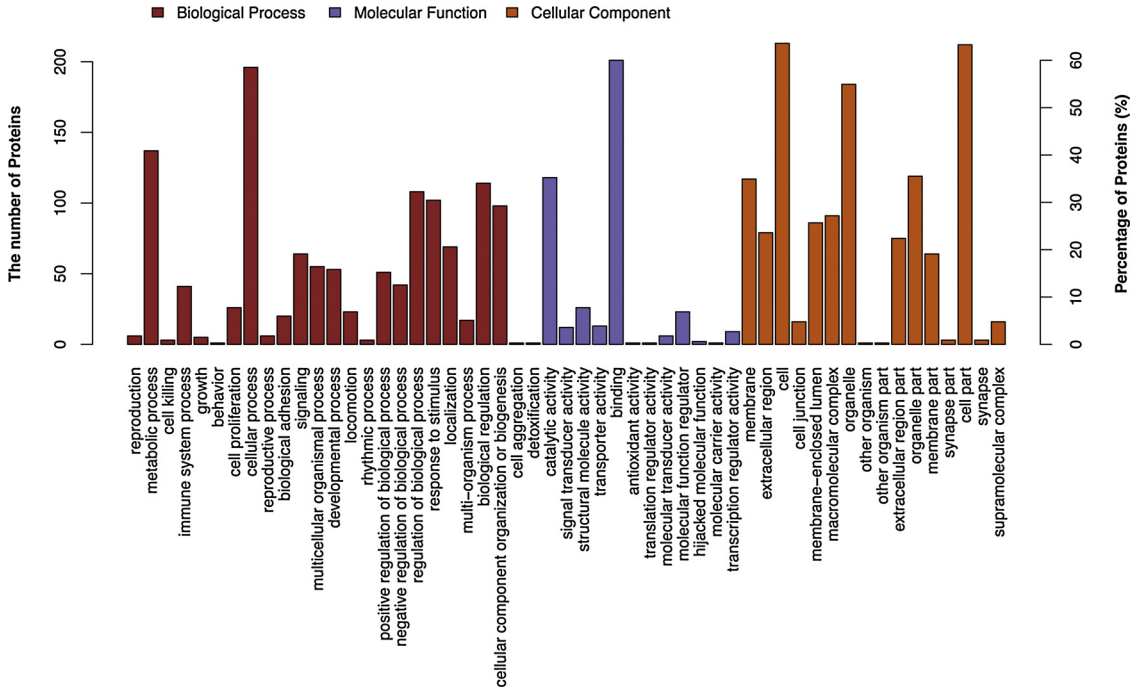

Fig. 7. GO function analysis.

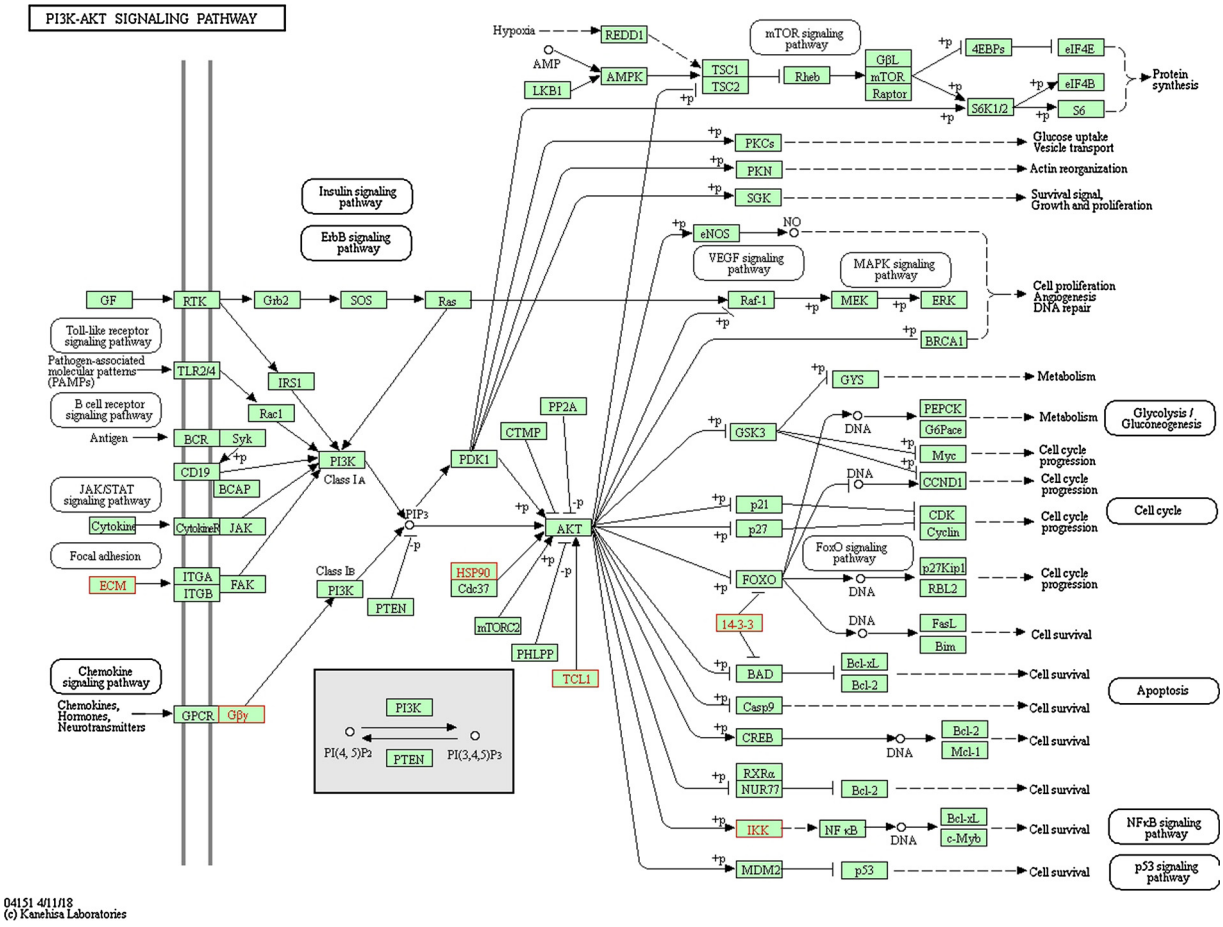

Fig. 8. Pathways in PI3K-Akt.

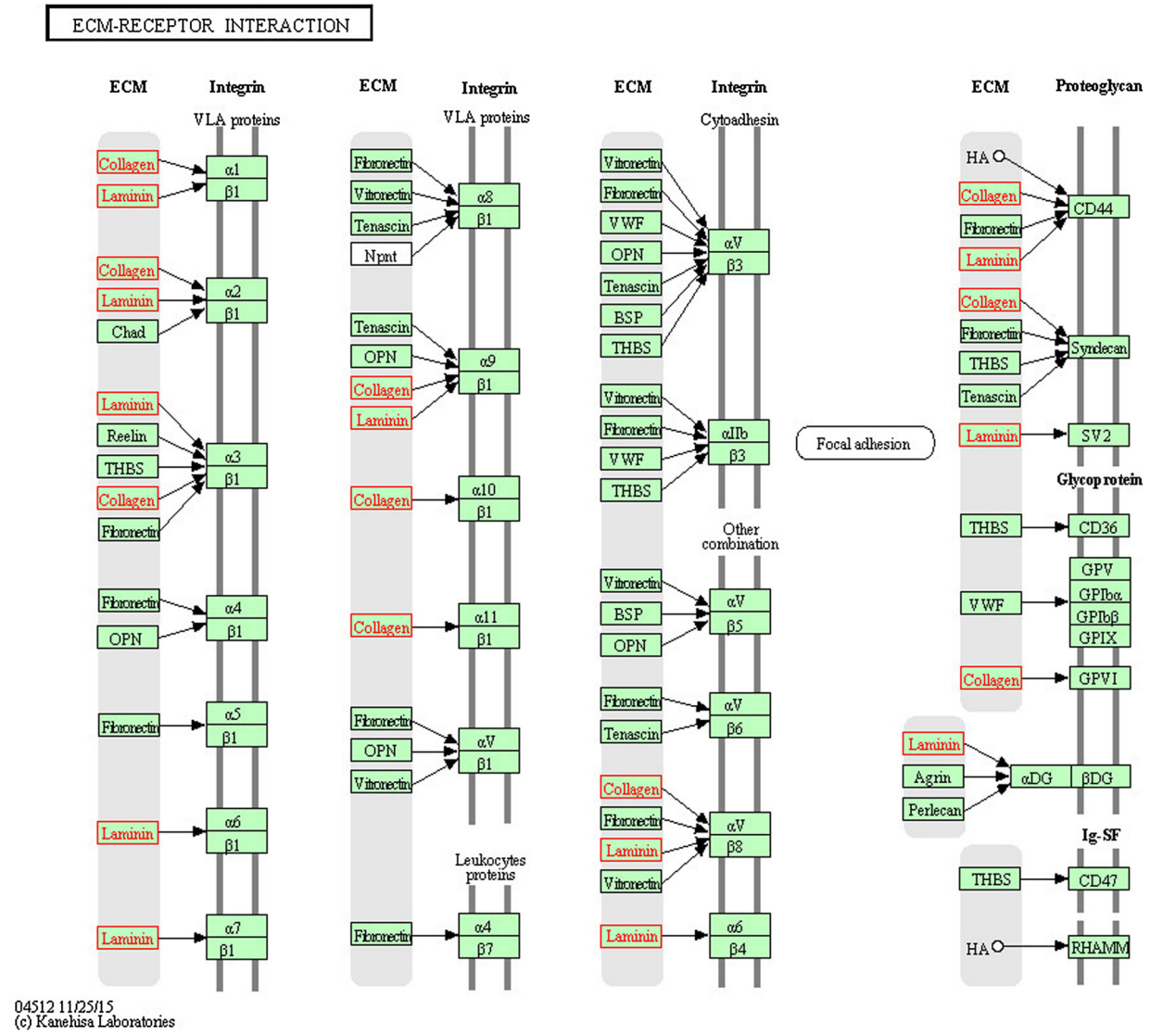

Fig. 9. ECM-receptor interaction signaling pathway.

Foundation of China under Grant No. 81660036.

Acknowledgement

Not applicable.

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