Pathology - Research and Practice 216 (2020) 152799

Contents lists available at ScienceDirect

Pathology - Research and Practice

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

Original article

TCL1 as a hub protein associated with the PI3K/AKT signaling pathway in diffuse large B-cell lymphoma based on proteomics methods

Hai-Xia Gao[a,b], Si-Jing Li[a,b], Jing Niu[a,b], Zhi-Ping Ma[a], Aijiang Nuerlan[a,b], Jing Xue[a,b], Meng-Bo Wang[a,b], Wen-Li Cui[a], Gulinaer Abulajiang[a], Wei Sang[a], Wei Zhang[a], Xin-Xia Li[a,*]

[a] Department of Pathology, The First Affiliated Hospital of Xinjiang Medical University, No. 137 Liyushan Southern Road, Urumqi, the Xinjiang Uygur Autonomous Region of China, 830054, PR China
[b] Xinjiang Medical University, No. 393 Xinyi Road, Urumqi, the Xinjiang Uygur Autonomous Region of China, 830011, PR China

ARTICLE INFO

Keywords:
DLBCL
iTRAQ
Pathways
TCL1
Clinicopathologic characteristics
Immunotherapeutic target

ABSTRACT

This study aimed to investigate the hub protein related to the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway in diffuse large B-cell lymphoma (DLBCL). We used proteomics methods (iTRAQ) to explore the differentially expressed proteins in the non-germinal center B-cell -like (non-GCB) DLBCL in our previous study. In this study, a total of 137 formalin-fixed paraffin-embedded DLBCL tissue samples were analyzed via immunohistochemistry to verify the expression of TCL1, AKT1 + 2+3, IKKβ and to determine the differentially expressed proteins associated with the PI3K/AKT signaling pathway. Spearman correlation was used to analyze the relationship between these proteins, and survival analysis was used to investigate their effects on prognosis. Immunohistochemistry analysis indicated that TCL1, AKT1 + 2+3, and IKKβ were highly positively expressed in DLBCL. Results showed that the expression of TCL1 was related to ethnicity ($p = 0.022$), primary site ($p = 0.045$), Ann Arbor stage ($p = 0.037$), the International Prognostic Index ($p = 0.005$), β2-microglobulin ($p = 0.030$), BCL2 expression ($p < 0.001$), and Ki-67 expression ($p = 0.008$). A positive correlation was found between TCL1 and AKT1 + 2+3 ($p < 0.001$; $r = 0.475$). A positive correlation was also found between AKT1 + 2+3 and IKKβ ($p < 0.001$; $r = 0.342$). In survival analysis, anemia, non-treatment with R-CHOP, positive TCL1 expression, and Ki-67 expression ≥50% independently predicted short progression-free survival and overall survival in the total cohort ($p < 0.05$). Thus, TCL1 as a hub protein is associated with the PI3K/AKT signaling pathway in DLBCL. TCL1 expression indicated a poor prognosis in patients with DLBCL. With further studies, TCL1 may be established as a reliable prognostic biomarker and potential immunotherapeutic target for improving therapeutic efficacy for DLBCL in the future.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of B-cell non-Hodgkin lymphoma in adults and is known to be heterogeneous in clinical manifestations, tissue morphology, immune typing, and prognosis [1,2]. The subclassification of DLBCL based on cell-of-origin by using the Hans algorithm [3] is useful in predicting prognosis because germinal center B-cell like (GCB) subtypes have better outcomes than non-GCB DLBCL subtypes [4]. The five-year overall survival is approximately 60%–70% with standard chemotherapy of rituximab plus cyclo-phosphamide, doxorubicin, vincristine, and prednisone (R – CHOP). However, this disease is known to have a recurrence rate of 30%–50% shortly after treatment and progresses to the advanced stage, possibly because of the activation of a wide variety of oncogenic pathways and drug resistance [5]. The phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway is an important signal transduction pathway that promotes cell survival and maintains normal cell function. Deregulating the PI3K/AKT signaling pathway may lead to tumor formation, translocation, and resistance to chemotherapy in DLBCL [6]. Several studies have used small molecular inhibitors to inhibit the signaling pathway, but the induction of drug resistance of DLBCL is a multi-factor, multi-signal pathway, multi-gene interaction process, and some limitations still exist due to the incomplete understanding of the molecular mechanism of DLBCL [7]. Therefore, the pathogenesis of DLBCL and effective therapeutic drugs for this disease are still under active exploration.

Proteomics is an advanced approach that enables large-scale characterizations of diff ;erent aspects of proteins, such as protein

* Corresponding author.
E-mail address: lxx-patho@163.com (X.-X. Li).

https://doi.org/10.1016/j.prp.2019.152799
Received 24 September 2019; Received in revised form 9 December 2019; Accepted 20 December 2019

expression profiling, posttranslational modification, protein localization and protein function [8]. Proteomics plays an important role in detecting differential proteins in hematological malignancies and provides a valuable opportunity to discover disease-related proteins. Proteins are the ultimate effector of phenotype, and they have been shown to not always correlate well with mRNA levels [9]. As the functional molecules in cells are proteins, proteome analysis may have an advantage over cDNA microarray in clinical use [10]. Furthermore, using tissue samples from patients may be the most direct and effective way to find biomarkers and therapeutic targets for tumors via a proteomics approach [10]. Proteomics-based strategies for identifying proteins relevant to leukemia [11]and lymphomas [12] are available. The above studies indicate that using tissue samples from patients is necessary for studying the changes of proteins related to the PI3K/AKT signaling pathway in non-GCB DLBCL, because this pathway has an important role in DLBCL and non-GCB has a worse prognosis than GCB DLBCL.

We employed proteomics methods via iTRAQ in our previous study to explore and quantitate differentially expressed proteins between non-GCB DLBCL tissues and reactive hyperplasia of lymph nodes [13]. In the study, 15 fresh tissue samples, including 7 samples of non-GCB DLBCL and 8 samples of reactive hyperplasia of lymph nodes, were analyzed via iTRAQ. Gene ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway analyses were performed. A total of 5974 proteins were identified via iTRAQ. P-value < 0.05 and the expression multiple was more than 1.2 fold. A total of 131 differentially expressed proteins were upregulated, and 204 were downregulated. The top 20 terms of KEGG pathway enrichment of the differential proteins were presented [13]. In our previous study, the PI3K/AKT signaling pathway was associated with differentially expressed proteins [13], including T cell leukemia/lymphoma 1A(TCL1/TCL1A), inhibitor of nuclear factor kappa B kinase subunit beta(IKKβ), heat shock protein 90 alpha family class B member 1(HSP90αB1), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (14-3-3ζ), G protein subunit gamma 2(Gβγ2), laminin subunit alpha 5(LAMα5), laminin subunit beta 2(LAMβ2), collagen type I alpha 1 chain(CO1α1), collagen type II alpha 1 chain(CO2α1). In the present study, the expression levels of TCL1, AKT1 + 2+3 and IKKβ associated with the PI3K/AKT signaling pathway were verified via immunohistochemical staining. Spearman correlation was used to analyze the relationship between the aforementioned proteins, and survival analysis was conducted to reveal the effects of the proteins on prognosis associated with the PI3K/AKT signaling pathway. TCL1, AKT1 + 2+3 and IKKβ were all highly positively expressed in DLBCL, as indicated by the immunohistochemistry. TCL1 expression was significantly associated with AKT1 + 2+3 ($p < 0.001$; $r = 0.475$). A correlation was found between AKT1 + 2+3 and IKKβ ($p < 0.001$; $r = 0.342$). In the survival analysis, anemia, non-treatment with R-CHOP, Ki-67 ≥ 50% expression, and the positive expression of TCL1 independently predicted short progression-free survival (PFS) and overall survival (OS) in the total cohort ($p < 0.05$).

In the current study, we evaluated the expression levels of TCL1, AKT1 + 2+3 and IKKβ in DLBCL. We found that the positive expression of TCL1 indicated a poor prognosis in patients with DLBCL. With further investigation, TCL1 may serve as a reliable prognostic biomarker and therapeutic target for improving therapeutic efficacy for DLBCL in the future.

2. Materials and methods

2.1. Patients and formalin-fixed paraffin-embedded (FFPE) tissue samples

For immunohistochemical analysis, 137 DLBCL FFPE tissue samples were collected from the First Affiliated Hospital of Xinjiang Medical University in July 2010–August 2018. Clinicopathological information was obtained from clinical records and pathology reports. The pathological classification was based on the 2016 revised 4th edition of the World Health Organization classification and was further subtyped using the Hans algorithm [3] by experienced hematopathologists. Written informed consent was obtained from all patients and their family members. This study was approved by the Ethics Committee of the Department of Medicine, the First Affiliated Hospital of Xinjiang Medical University.

2.2. Tissue microarray (TMA) construction

Hematoxylin and eosin stained slides for each case were reviewed to confirm the original diagnosis and select the most representative sections. TMA was constructed using 2 mm diameter cores derived from the representative areas of the FFPE tissue blocks from each case and from the normal reactive hyperplasia of lymph nodes for controls by our hospital.

2.3. Immunohistochemical staining and assessment

All of the immunohistochemical reactions were carried out in an automated immunostainer (Bench Mark Ultra). TMAs were sectioned at a thickness of 4-μm and then stained. Appropriate positive and negative controls were also included. The antibody markers included TCL1, AKT1 + 2+3, IKKβ, CD10, Bcl-2, Bcl-6, Mum-1, C-myc, P53, CD5, CD30 and Ki-67. The details of the markers are listed in Table 1.

Two senior pathologists evaluated all slides of the immunostained sections and scored the percentage of cells (labeling index). TCL1 was scored in non-malignant immune cells and in tumor B-cells. TCL1 was considered positively expressed in tumor B-cells or non-malignant immune cells if nuclear staining alone or nuclear and cytoplasmic staining was present. The evaluation criteria of TCL1 immunohistochemical staining in the paraffin-embedded tissue sections of the B cell lymphoma specimens, adopted from those of a previous study [14]. In the study, the researchers investigated the expression of the TCL1 protein in various types of B-cell lymphomas, and the paraffin-embedded tissue sections of the lymphoma specimens were subjected to TCL1 immunohistochemistry. The results were scored via a three-tiered scale: − (< 25% cells), + (25–50% cells), and + + (> 50% cells). AKT1 + 2+3 was considered to be positively expressed in tumor B-cells if cytoplasmic or cytomembrane staining was present. IKKβ was considered positively expressed in tumor B-cells if cytoplasmic or membrane staining was present. Tumor B-cells were distinguished from non-malignant immune cells on the basis of histological features, such as nuclear enlargement and atypism, and a comprehensive interpretation with other immunohistochemical markers, including CD20, BCL2, BCL6, CD10 and MUM1.

2.4. Statistical analysis

For immunohistochemical analysis, the relationship between clinical and pathological characteristics was determined using Chi square

Table 1
Immunohistochemical reagents and source.

Antibodies	Clone	Vendor	dilution
TCL1	EPR3949	Abcam	1:700
AKT1 + 2+3	Bs-6951R	Bioss	1:800
IKKβ	EPR6043	Abcam	1:500
CD10	C6D1	Celnovte	1 : 150
BCL-6	EP278	Celnovte	1 : 70
MUM1	EP190	Celnovte	ready-to-use
BCL-2	EP124	Celnovte	1 : 150
C-myc	C-myc	ZSGB	1:50
P53	DO7	Celnovte	1:100
CD5	GR020	Celnovte	1:90
CD30	C5E10	Celnovte	1:100
Ki-67	MIB-1	Dako	ready-to-use

or Fisher's exact test. Pearson's correlation test was used to determine the association between immunohistochemical expressions. Survival time was estimated using OS and PFS. PFS is the time interval between the starting date of treatment and the date of progression of a radiologically verified progressive disease based on positron-emission tomography computed tomography (CT) or CT, refractoriness to first-line therapy in a response assessment after the second cycle or completion of treatment, and relapse or death. OS is the time interval between a patient's diagnosis of DLBCL and the date of the last follow-up or death. Univariate and multivariate analyses were utilized in the survival analysis by the Kaplan–Meier method and Cox hazard regression analysis. The Kaplan–Meier method was used to analyze the clinicopathological parameters related to the prognosis of DLBCL. Cox hazard regression analysis was used to analyze the independent factors correlated with the prognosis of DLBCL on the basis of the results of the Kaplan–Meier method. Survival curves were constructed accordingly. Probability values of less than 0.05 were considered statistically significant. Data were processed and analyzed using SPSS Statistics 17.0 software.

3. Results

3.1. Clinicopathological characteristics

The characteristics of 137 patients with DLBCL were summarized (Table 2). Our cohort consisted predominantly of the non-GCB subtype (66.4%; 91/137) and partly of the GCB DLBCL subtype (33.6%; 46/137). The total cohort mainly included cases with non-bulky masses (94.9%; 130/137), absence of B symptoms (76%; 104/137), normal serum lactate dehydrogenase (73.7%; 101/137), high Ann Arbor stage (72.3%; 99/137), extranodal primary sites (67.2%; 92/137), and high International Prognostic Index (IPI; 63.5%; 87/137). Most of the patients received R−CHOP chemotherapy (64.2%; 88/137). Compared to patients with GCB and non-GCB-DLBCL subtypes had age ($p = 0.046$), B symptoms ($p = 0.039$), which did reach statistical significance.

3.2. The expression levels of TCL1, AKT1 + 2+3, IKKβ and association between TCL1, AKT1 + 2+3, IKKβ protein markers

In 137 cases of DLBCL, immunohistochemistry analysis indicated that TCL1 was negatively expressed in 43 tumors (31.4%) but positively expressed in 94 tumors (68.6%). AKT1 + 2+3 was positive in 111 patients (81.0%) and negative in 26 patients (19.0%). IKKβ was negatively expressed in 21.2% (29/137) of the patients, but was positively expressed in 78.8% (108/137) of the patients. The expression levels of the protein markers are exhibited in Fig. 1.

We performed correlation analysis (Table 3) to clarify the associations between TCL1, AKT1 + 2+3 and IKKβ protein markers. We found that TCL1 expression was associated with AKT1 + 2+3 ($p < 0.001$; $r = 0.475$) and that AKT1 + 2+3 and IKKβ were correlated ($p < 0.001$; $r = 0.342$).

3.3. Association between the expression levels of TCL1, AKT1 + 2+3, IKKβ and clinicopathological features

We investigated the expression levels of TCL1, AKT1 + 2+3, and IKKβ and their relationship with clinicopathological parameters among DLBCL patients (Table 4). The expression of TCL1 was related to ethnicity ($p = 0.022$), primary site ($p = 0.045$), Ann Arbor stage ($p = 0.037$) and IPI ($p = 0.005$), β2-microglobulin ($p = 0.030$), BCL2 expression ($p < 0.001$), and Ki-67 expression ($p = 0.008$). AKT1 + 2+3 was associated with age ($p = 0.045$), primary site ($p = 0.010$), IPI ($p = 0.041$), β2-microglobulin ($p = 0.001$), BCL2 expression ($p < 0.001$), and Ki-67 expression ($p = 0.003$). The expression of IKKβ was correlated with P53 ($p < 0.001$).

3.4. Survival analysis

Survival analysis was conducted in the total DLBCL cohort (Table 5). In the univariate survival analysis via the Kaplan–Meier method, the following prognostic factors exhibited an effect on OS (Fig. 2): age (> 60 vs. ≤ 60 years old; $p = 0.001$), primary site (extranodal vs. nodal; $p = 0.007$), Ann Arbor stage (III–IV vs. II−I; $p < 0.001$), the Eastern Cooperative Group Performance Status (≥2 vs. < 2; $p = 0.001$), serum lactate dehydrogenase (elevated vs. normal; $p < 0.001$), IPI (3–5 vs. 0–2; $p < 0.001$), peripheral lymphocyte counts at diagnosis (decreased vs. normal; $p = 0.002$), Hb (anemia vs. normal; $p = 0.023$), β2-microglobulin (elevated vs. normal; $p = 0.003$), treatment regimen (R−CHOP vs. others; $p < 0.001$), BCL2 expression (expression ≥70% vs. expression 0–70%; $p = 0.004$), Ki-67 expression (expression ≥50% vs. expression 0–50%; $p < 0.001$), CD30 expression (positive vs. negative; $p = 0.007$), CD5 expression (positive vs. negative; $p = 0.020$), TCL1 expression (positive vs. negative; $p = 0.003$), AKT1 + 2+3 expression (positive vs. negative; $p = 0.029$). The results of the multivariate analysis (Cox's proportional hazards regression model) of OS that can be the independent prognostic factors for OS were as follows: age (HR: 2.426; 95% CI: 1.140–5.162; $p = 0.021$), Ann Arbor stage (HR: 4.475; 95% CI: 1.360–14.726; $p = 0.014$), Hb (HR: 2.194; 95% CI: 1.156–4.165; $p = 0.016$), treatment regimen (HR: 0.239; 95% CI: 0.113–0.505; $p = 0.001$), Ki-67 expression (HR: 2.556; 95% CI: 1.120–5.833; $p = 0.026$), TCL1 expression (HR: 3.804; 95% CI: 1.553–9.361; $p = 0.003$). In the univariate survival analysis and multivariate analysis of PFS (Fig. 3), most prognostic factors were the same as those of OS, and only the primary site (extranodal vs. nodal; $p = 0.120$) was not a prognostic factor. Age and Ann Arbor stage were not independent prognostic factors for PFS.

The results showed that the independent factors that predicted short OS and PFS in the total DLBCL cohort were not receiving R−CHOP, a positive TCL1 expression, Ki-67 expression≥50% and anemia.

4. Discussion

The incidence of lymphoma has increased in many countries in recent years [15]. DLBCL is a heterogeneous disease whose pathogenesis remains unclear. Despite the majority of patients being treated with chemoimmunotherapy, up to 30% will eventually succumb to the disease [16]. Patients with non-GCB DLBCL had a worse prognosis than patients with GCB DLBCL after R−CHOP therapy, as indicated by molecular diagnosis [17]. The pathogenic hallmark of DLBCL is the constitutive activation of the NF-κB pathway, which is activated by various mechanisms across B cell malignancies [18]. Proteomics has become an extensively used tool for characterizing proteins which are the ultimate effector of phenotypes. Therefore, elucidating non-GCB DLBCL associated with the PI3K/AKT signaling pathway proteins is necessary for the comprehensive treatment of DLBCL and for providing new insights into the pathogenesis of the disease.

In the present study, we analyzed the differentially expressed proteins of experimental and control groups via iTRAQ. Through the KEGG pathway enrichment analysis, we found that important pathways, such as the pathways in cancer, the PI3K-AKT signaling pathway, alcoholism (Fig. 4), systemic lupus erythematosus and necroptosis were significantly changed. Alcoholism is a pathway of interest because its role in DLBCL is not clear. Han et al. revealed that among patients with DLBCL, wine and liquor drinkers have an increased risk of negative outcomes relative to patients who do not drink [19]. A study showed that medical history, lifestyle, family history, and occupational risk factors are associated with DLBCL, and they confirmed that high young adult body mass index is related to increased DLBCL risk while lifetime alcohol consumption is inversely associated with such risk among men [20]. We believe that an underlying connection exists between the pathogenesis of DLBCL and lifestyle, such as drinking wine and liquor. The functions of alcoholism in DLBCL remain unclear and should be

Table 2
Clinicopathologic characteristics according to Hans classification in DLBCL patients(n = 137).

Clinicopathologic characteristics	Total(137)	GCB(46)	Non-GCB(91)	p-value
Age				
≤ 60	76(76/137, 55.5%)	31(31/46, 67.4%)	45(45/91, 49.5%)	0.046#
> 60	61(61/137, 44.5%)	15(15/46, 32.6%)	46(46/91, 50.5%)	
Sex				
Male	81(81/137, 59.1%)	28(28/46, 60.9%)	53(53/91, 59.1%)	0.768#
Female	56(56/137, 40.9%)	18(18/46, 39.1%)	38(38/91, 41.8%)	
Ethnic				
Han	89(89/137, 65.0%)	28(28/46, 60.9%)	61(61/91, 67.0%)	0.475#
Others	48(48/137, 35.0%)	18(18/46, 39.1%)	30(30/91, 33.0%)	
Primary site				
Nodal	45(45/137, 32.8%)	17(18/46, 37%)	28(28/91, 30.8%)	0.466#
Extranodal	92(92/137, 67.2%)	29(29/46, 63%)	63(63/91, 69.2%)	
B symptoms				
Absent	104(104/137, 76.0%)	40(40/46, 87.0%)	64(64/91, 70.3%)	0.039#
Present	33(33/137, 24.0%)	6(6/46, 13.0%)	27(27/91, 29.7%)	
ECOG PS(the Eastern Cooperative Group Performance Status)				
< 2	70(70/137, 51.1%)	27(27/46, 58.7%)	43(43/91, 47.3%)	0.206#
≥ 2	67(67/137, 48.9%)	19(19/46, 41.3%)	48(48/91, 52.7%)	
Ann Arbor stage				
I–II	38(38/137, 27.7%)	13(13/46, 28.3%)	25(25/91, 27.5%)	0.922#
III–IV	99(99/137, 72.3%)	33(33/46, 71.7%)	66(66/91, 72.5%)	
International prognostic index				
0-2	50(50/137, 36.5%)	18(18/46, 39.1%)	32(32/91, 35.2%)	0.649#
3-5	87(87/137, 63.5%)	28(28/46, 60.9%)	59(59/91, 64.8%)	
Serum lactate dehydrogenase				
Normal	101(101/137, 73.7%)	34(34/46, 73.9%)	67(67/91, 73.6%)	0.971#
Elevated	36(36/137, 26.3%)	12(12/4, 26.1%)	24(24/91, 26.4%)	
β2-microglobulin				
Normal	46(46/137, 33.6%)	18(18/46, 39.1%)	28(28/91, 30.8%)	0.328#
Elevated	91(91/137, 66.4%)	28(28/46, 60.9%)	63(63/91, 69.2%)	
Bulky mass (cm)				
< 10	130(130/137, 94.9%)	43(43/46, 93.5%)	87(87/91, 95.6%)	0.687†
≥10	7(7/137, 5.1%)	3(3/46, 6.5%)	4(4/91, 4.4%)	
Different kinds of peripheral blood cells counts at diagnose(10^9/L)				
Lymphocyte count				
Decreased	22(22/137, 16.1%)	5(5/46, 10.9%)	17(17/91, 18.7%)	0.250†
Normal (0.8-4*10^9/L)	110(110/137, 80.3%)	38(38/46, 82.6%)	72(72/91, 79.1%)	
Elevated	5(5/137, 3.6%)	3(3/46, 6.5%)	2(2/46, 2.2%)	
Monocyte count				
Decreased	4(4/137, 2.9%)	1(1/46, 2.2%)	3(3/91, 3.3%)	0.738†
Normal(0.12-0.8*10^9/L)	116(116/137, 84.7%)	38(38/46, 85.7%)	78(78/91, 85.7%)	
Elevated	17(17/137, 12.4%)	7(7/46, 15.2%)	10(10/91, 11.0%)	
Neutrophil count				
Decreased	2(2/137, 1.5%)	1(1/46, 2.2%)	1(1/91, 1.1%)	0.419†
Normal(2-7*10^9/L)	116(116/137, 84.7%)	41(41/46, 89.1%)	75(75/91, 82.4%)	
Elevated	19(16/137, 13.9%)	4(4/46, 8.7%)	15(15/91, 16.5%)	
Hb(g/L)				
Normal(Male120-160,Famale110-150,Newborn170-200)	98(98/137, 71.5%)	37(37/46, 80.4%)	61(61/91, 67.0%)	0.101#
Anemia	39(38/137, 28.5%)	9(9/46, 19.6%)	30(30/91, 33.0%)	
Treatment regimen				
Others	49(49/137, 35.8%)	12(12/46, 26.1%)	37(37/91, 40.7%)	0.093#
R-CHOP	88(88/137, 64.2%)	34(34/46, 73.9%)	54(54/91, 59.3%)	
CD10 expression				
Negative	107(107/137, 78.1%)	16(16/46, 34.8%)	91(91/91, 100%)	< 0.001†
Positive	30(30/137, 21.9%)	30(30/46, 65.2%)	0(0/91, 0%)	
MUM1 expression				
Negative	22(22/137, 16.1%)	21(21/46, 45.7%)	1(1/91, 1.1%)	< 0.001†

(*continued on next page*)

Table 2 (*continued*)

Clinicopathologic characteristics	Total(137)	GCB(46)	Non-GCB(91)	p-value
Positive	115(115/137, 83.9%)	25(25/46, 54.3%)	90(90/91, 98.9%)	
BCL6 expression				
Negative	25(25/137, 18.2%)	3(3/46, 6.5%)	22(22/91, 24.2%)	0.018†
Positive	112(112/137, 81.8%)	43(43/46, 93.5%)	69(69/91, 75.8%)	
BCL2 expression				
Expression(< 70%)	80(80/137, 58.4%)	26(26/46, 56.5%)	54(54/91, 59.3%)	0.752#
Expression(≥70%)	57(57/137, 41.6%)	20(20/46, 43.5%)	37(37/91, 40.7%)	
Ki-67 expression				
Expression(< 50%)	54(54/137, 39.4%)	22(22/46, 47.8%)	32(32/91, 35.2%)	0.152#
Expression(≥50%)	83(83/137, 60.6%)	24(24/46, 52.2%)	59(59/91, 64.8%)	
C-myc expression				
Expression(< 40%)	84(84/137, 61.3%)	28(28/46, 60.9%)	56(56/91, 61.5%)	0.939#
Expression(≥40%)	53(53/137, 38.7%)	18(18/46, 39.1%)	35(35/91, 38.5%)	
P53 expression				
Negative	58(58/137, 42.3%)	18(18/46, 39.1%)	40(40/91, 44.0%)	0.589#
Positive	79(79/137, 57.7%)	28(28/46, 60.9%)	51(51/91, 56.0%)	
CD5 expression				
Negative	109(109/137, 79.6%)	38(38/46, 82.6%)	71(71/91, 78.0%)	0.155#
Positive	28(28/137, 20.4%)	8(8/46, 17.4%)	20(20/91, 22.0%)	
CD30 expression				
Negative	112(112/137, 81.8%)	41(41/46, 89.1%)	71(71/91, 78.0%)	0.112#
Positive	25(25/137, 18.2%)	5(5/46, 10.9%)	20(20/91, 22.0%)	

Abbreviations: GCB : germinal center B cell; R−CHOP: rituximab with cyclophosphamide–doxorubicin–vincristine–prednisone, p-values were calculated by using Fisher's exact test (2-sided)†, Pearson's Chisquare test (2-sided)#.

investigated because of the high prevalence of drinking. If drinking is linked to DLBCL, changes in lifestyle could help reduce the risk of DLBCL. In our previous study, TCL1, AKT, IKKβ, HSP90, 14-3-3, Gβγ and ECM (laminin, collagen), as extinct differential proteins, were associated with the PI3K/AKT signaling pathway [13], studying the role of differentially expressed proteins in DLBCL is necessary. In the present study, we confirmed the expression levels of TCL1, AKT1 + 2+3 and IKKβ, which are proteins associated with the PI3K/AKT signaling pathway via immunohistochemistry on the basis of the proteomics method described in our previous study. We looked for core proteins associated with this signaling pathway for our further study.

Carlo Croce's group discovered the T cell leukemia/lymphoma 1A oncogene (TCL1 or TCL1A) in the 1980s. TCL1 is a 13 kDa protein

Table 3
Relationship between TCL1, AKT1 + 2+3 and IKKβ expression.

Protein	Expression	TCL1 Negative	Positive	r	P-value
AKT1 + 2+3	Negative	20(46.5%)	6(6.4%)	0.475	< 0.001
	Positive	23(53.5%)	88(93.6%)		
IKKβ	Negative	13(30.2%)	16(17.0%)	0.150	0.079
	Positive	30(69.8%)	78(83.0%)		
Protein	**Expression**	**AKT1 + 2+3** Negative	Positive	**r**	**P-value**
IKKβ	Negative	13(50.0%)	16(14.4%)	0.342	< 0.001
	Positive	13(50.0%)	95(85.6%)		

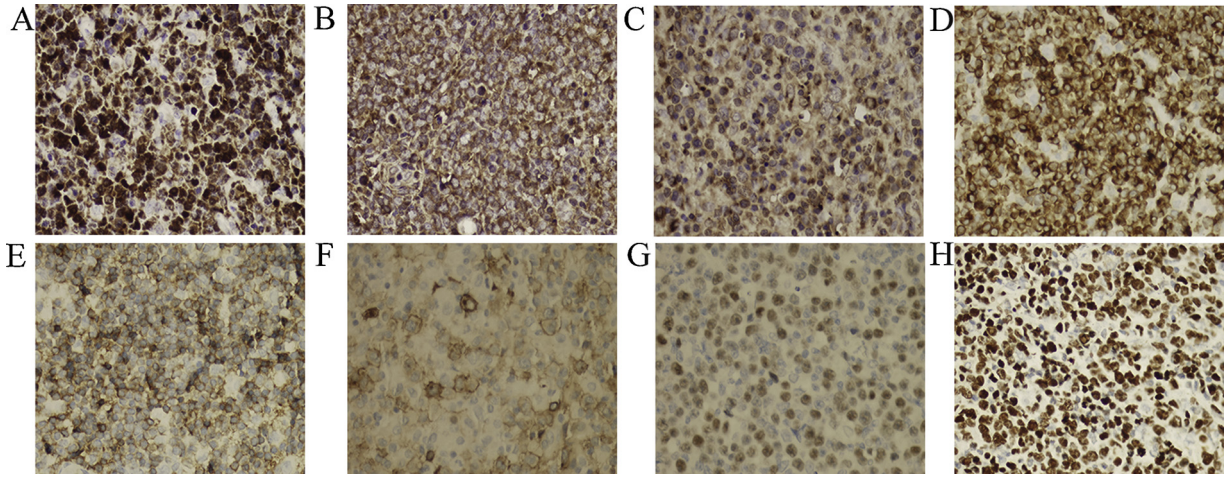

Fig. 1. Results of immunohistochemistry in DLBCL. (A) The expression of TCL1 (200×). (B) The expression of AKT1 + 2+3 (200×). (C) The expression of IKKβ (200×). (D) The expression of BCL2 (200×). (E) The expression of CD5 (200×). (F) The expression of CD30 (200×). (G) The expression of C-myc (200×). (H) The expression of P53 (200×).

Table 4
Clinicopathologic characteristics according to protein expression of TCL1, AKT1 + 2+3, IKKβ in diffuse large B cell lymphoma patients(n = 137).

Clinicopathologic characteristics	TCL1			AKT1 + 2+3			IKKβ		
	Negative	Positive	p-value	negative	Positive	p-value	negative	Positive	p-value
Age									
≤ 60	29(67.4%)	47(50.0%)	0.057#	19(73.1%)	57(51.4%)	0.045#	16(55.2%)	60(55.6%)	0.971#
> 60	14(32.6%)	47(50.0%)		7(26.9%)	54(48.6%)		13(44.8%)	48(44.4%)	
Sex									
Male	32(74.4%)	49(52.1%)	0.054#	19(73.1%)	62(55.9%)	0.108#	20(69.0%)	61(56.5%)	0.225#
Female	11(25.6%)	45(47.9%)		7(26.9%)	49(44.1%)		9(31.0%)	47(43.5%)	
Ethnic									
Han	22(51.2%)	67(71.3%)	0.022#	15(57.7%)	74(66.7%)	0.388#	18(62.1%)	71(65.7%)	0.713#
Others	21(48.8%)	27(28.7%)		11(42.3%)	37(33.3%)		11(37.9%)	37(34.3%)	
Primary site									
Nodal	9(20.9%)	36(38.3%)	0.045#	3(11.5%)	42(37.8%)	0.010#	18(62.1%)	18(62.1%)	0.713#
Extranodal	34(79.1%)	58(61.7%)		23(88.5%)	69(62.2%)		18(62.1%)	18(62.1%)	
B symptoms									
Absent	35(81.4%)	69(73.4%)	0.310#	22(84.6%)	82(73.9%)	0.249#	22(75.9%)	82(75.9%)	0.994#
Present	8(18.6%)	25(26.6%)		4(15.4%)	29(26.1%)		7(24.1%)	26(24.1%)	
ECOG PS(the Eastern Cooperative Group Performance Status)									
< 2	23(53.5%)	47(50.0%)	0.705#	14(53.8%)	56(50.5%)	0.755#	13(44.8%)	57(52.8%)	0.447#
≥ 2	20(46.5%)	47(50.0%)		12(46.2%)	55(49.5%)		16(55.2%)	51(47.2%)	
Ann Arbor stage									
I–II	17(39.5%)	21(22.3%)	0.037#	9(34.6%)	29(26.1%)	0.384#	7(24.1%)	31(44.8%)	0.626#
III–IV	26(60.5%)	73(77.7%)		17(65.4%)	82(73.9%)		22(75.9%)	77(71.3%)	
International prognostic index									
0-2	23(53.5%)	27(28.7%)	0.005#	14(53.8%)	36(32.4%)	0.041#	8(27.6%)	42(38.9%)	0.262#
3-5	20(46.5%)	67(71.3%)		12(46.2%)	75(67.6%)		21(72.4%)	66(61.1%)	
Bulky mass (cm)									
< 10	41(95.3%)	89(94.7%)	0.869†	25(96.2%)	105(94.6%)	0.745†	28(96.6%)	102(94.4%)	1.000†
≥10	2(4.7%)	5(5.3%)		1(3.8%)	6(5.4%)		1(3.4%)	6(5.6%)	
Serum lactate dehydrogenase(LDH)									
Normal	36(83.7%)	65(69.1%)	0.072#	23(88.5%)	78(70.3%)	0.082†	21(72.4%)	80(74.1%)	0.857#
Elevated	7(16.3%)	29(30.9%)		3(11.5%)	33(29.7%)		8(27.6%)	28(25.9%)	
β2-microglobulin									
Normal	20(46.5%)	26(27.7%)	0.030#	16(61.5%)	30(27.0%)	0.001#	13(44.8%)	33(30.6%)	0.148#
Elevated	23(53.5%)	68(72.3%)		10(38.5%)	81(73.0%)		16(55.2%)	75(69.4%)	
Hans classification									
GCB	16(37.2%)	30(31.9%)	0.543#	12(46.2%)	34(30.6%)	0.131#	9(31.0%)	37(34.3%)	0.744#
Non-GCB	27(62.8%)	64(68.1%)		14(53.8%)	77(69.4%)		20(69.0%)	71(65.7%)	
Different kinds of peripheral blood cells counts at diagnose($10^{9/L}$)									
Lymphocyte count									
Normal	33(82.5%)	77(83.7%)	0.865#	21(87.5%)	89(82.4%)	0.764†	25(86.2%)	85(82.5%)	0.638†
Decreased	7(17.5%)	15(16.3%)		3(12.5%)	19(17.6%)		4(13.8%)	18(17.5%)	
Monocyte count									
Normal	39(92.9%)	77(84.6%)	0.266†	25(96.2%)	91(85.0%)	0.192†	26(89.7%)	90(86.5%)	0.764†
Elevated	3(7.1%)	14(15.4%)		1(3.8%)	16(15.0%)		3(10.3%)	14(13.5%)	
Neutrophil count									
Normal	39(92.9%)	76(82.6%)	0.181†	24(92.3%)	92(84.4%)	0.367†	25(89.3%)	91(85.0%)	0.566†
Elevated	3(7.1%)	16(17.4%)		2(7.7%)	17(15.6%)		3(10.7%)	16(15.0%)	
Hb(g/L)									
Normal	30(69.8%)	68(72.3%)	0.757#	23(88.5%)	75(67.6%)	0.051†	22(75.9%)	76(70.4%)	0.561#
Anemia	13(30.2%)	26(27.7%)		3(11.5%)	36(32.4%)		7(24.1%)	32(29.6%)	
BCL2 expression									
Expression (< 70%)	31(72.1%)	45(47.9%)	< 0.001#	21(80.8%)	52(46.8%)	< 0.001#	18(62.1%)	62(57.4%)	0.651#
Expression (≥70%)	12(27.9%)	49(52.1%)		5(19.2%)	59(53.2%)		11(37.9%)	46(42.6%)	
Ki-67 expression									
Expression (< 50%)	24(55.8%)	30(31.9%)	0.008#	17(65.4%)	37(33.3%)	0.003#	10(34.5%)	44(40.7%)	0.540#
Expression (≥50%)	19(44.2%)	64(68.1%)		9(34.6%)	74(66.7%)		19(65.5%)	64(59.3%)	

(*continued on next page*)

Table 4 (*continued*)

Clinicopathologic characteristics	TCL1			AKT1 + 2+3			IKKβ		
	Negative	Positive	p-value	negative	Positive	p-value	negative	Positive	p-value
C-myc expression									
Expression (< 40%)	30(69.8%)	54(57.4%)	0.169#	20(76.9%)	64(57.7%)	0.069#	22(75.9%)	62(57.4%)	0.070#
Expression (≥40%)	13(30.2%)	40(42.6%)		6(23.1%)	47(42.3%)		7(24.1%)	46(42.6%)	
P53 expression									
Negative	20(46.5%)	36(38.3%)	0.169#	17(65.4%)	39(35.1%)	0.005#	21(72.4%)	35(32.4%)	< 0.001#
Positive	23(53.5%)	58(61.7%)		9(34.6%)	72(64.9%)		8(27.6%)	73(67.6%)	
CD5 expression									
Negative	35(81.4%)	74(78.7%)	0.719#	22(84.6%)	87(78.4%)	0.596#	23(79.3%)	86(79.6%)	0.970#
Positive	8(18.6%)	20(21.3%)		4(15.4%)	24(21.6%)		8(20.7%)	22(20.4%)	
CD30 expression									
Negative	36(83.7%)	76(80.9%)	0.687#	24(92.3%)	88(79.3%)	0.162†	25(86.2%)	87(80.6%)	0.596†
Positive	7(16.3%)	18(19.1%)		2(7.7%)	23(20.7%)		4(13.8%)	21(19.4%)	

Abbreviations: GCB : germinal center B cell; R−CHOP: rituximab with cyclophosphamide–doxorubicin–vincristine–prednisone, p-values were calculated by using Fisher's exact test (2-sided)†, Pearson's Chisquare test (2-sided)#.

whose function requires it to form homodimers. TCL1 is normally expressed in fetal tissues and early developmental stage lymphocytes. TCL1 acts as co-activator of AKT kinases, and when physiologically expressed it mediates normal growth and survival signals; however, it causes lymphomagenesis and cancer progression when dysregulated [21]. TCL1 also plays a central role in lymphomagenesis as a co-activator of other elucidated interacting protein partners, such as IκB [22], ATM [23], HSP70 [24], TP63 [25], ROR1 [26]and many others [27]. The expression of TCL1 has been described in follicular lymphoma (FL), Burkitt lymphoma (BL), DLBCL and chronic lymphocytic leukemia [21]. The increased and prolonged expression of TCL1 in the late phases of thymocyte development causes T cell prolymphocytic leukemia. The dysregulation of TCL1 in T cells is due to a chromosomal translocation that brings TCL1 (on chromosome 14q31.2) under T cell receptor enhancer elements [27]. However, the precise mechanisms underlying the overexpression of TCL1 in B cell tumors remain unclear, because neither translocations nor Epstein–Barr virus infection were involved [28], thus, studies should be undertaken to determine the precise mechanisms underlying the overexpression of TCL1 in B cell tumors. Transcriptional events and altered epigenetic signals might underlie the abnormal expression of TCL1 in B cell malignancies [29]. In the present study, immunohistochemistry analysis conducted among 137 cases of DLBCL showed that TCL1 was negatively expressed in 43 tumors (31.4%) and positively expressed in 94 tumors (68.6%). TCL1 had a high positive expression in DLBCL, as shown in the germinal centroblast (GC), centrocyte, and post-GC memory B cells and in tumors arising from the germinal center such as FL, BL and DLBCL [21]. Clinicopathologic variables indicated the negative expression of TCL1 in GCB (37.2%) and non-GCB (62.8%) and the positive expression in GCB (31.9%) and non-GCB (68.1%). Thus, TCL1 was more positively expressed in non-GCB than in GCB. Non-GCB DLBCL subtype was associated with a high positive expression of TCL1 ($p = 0.543$), but the association did not reach statistical significance. TCL1 positive expression was related to ethnicity ($p = 0.022$). The positive expression of TCL1 was more frequent in primary extranodal than in nodal DLBCL ($p = 0.045$)). However, the present study has a limited sample size, which may lead to a high false-positive rate; thus, further studies are needed. The expression of TCL1 was associated with BCL2 expression ($p < 0.001$) and Ki-67 expression ($p = 0.008$). The positive expression of TCL1 was associated with the high expression of BCL2 and Ki-67; we speculated that TCL1 may be related to apoptosis and proliferation in DLBCL. Laine et al were the first to demonstrate a physical interaction between TCL1 and AKT and proposed a mechanism by which TCL1 enhances AKT phosphorylation with consequent phosphorylation of downstream targets, thereby increasing cell proliferation and survival [30]. We found that the positive expression of TCL1 was positively associated with AKT1 + 2+3 ($p < 0.001$; $r = 0.475$), and the results were consistent with those of previous studies. Shin SJ et al suggested that the TCL1 expression profile may have a role in the prediction of overall outcomes in patients with mantle cell lymphoma. They also showed that TCL1 expression was associated with significantly short OS ($p = 0.006$). Multivariate analysis identified TCL1 expression ($p = 0.003$), high-risk MIPI ($p = 0.027$), and anemia ($p = 0.018$) as adverse prognostic factors [31]. Ramuz O et al showed that TCL1A immunoexpression was correlated with either high relapse ($p = 0.02$) or low five-year OS ($p = 0.009$) rates in DLBCL [32]. Through our univariate survival analysis, we found that TCL1 expression was associated with a significantly short OS and PFS. The multivariate analysis, meanwhile, revealed that TCL1 expression was an independent factor that predicted short OS and PFS in the total DLBCL cohort. TCL1 is emerging as a new target for anticancer therapy because of its involvement in important signaling pathways that are upregulated in hematologic malignancies. The available literature describing the biology, functions, and structure of TCL1 has convinced academic researchers and biotechnology companies to attempt the synthesis of chemical inhibitors [27]. TCL1 peptides for immunotherapy have been patented (patent WO 2,013,075,105 A3). They bind to MHC I (HLA-A2) on tumor cells or other antigen-presenting cells and are recognized by T cell receptors on T cells. Weng et al put forward an interesting study describing an epitope of TCL1 that spans amino acid residues 71–78 and is recognized by cytotoxic T lymphocytes. The epitope represents a lymphoma-associated antigen that can be used to generate new immunotherapeutic agents against leukemia and lymphomas. Weng J et al also revealed that TCL1 can serve as a shared tumor-associated antigen for immunotherapy against B-cell lymphomas [33]. Therefore, immunotherapy by targeting TCL1 could be considered against B-cell lymphomas in the future for patients with DLBCL who do not respond well to R−CHOP and other treatments but exhibit high TCL1. TCL1 may be a biomarker of poor prognosis in DLBCL and may be used as a target drug for the clinical treatment of DLBCL in the future. TCL1 immunodetection is an independent marker of adverse outcomes that could be used in routine settings for the management of patients with DLBCL.

AKT comprises a multiprotein family, including AKT1, AKT2 and AKT3, which have idiosyncratic but largely overlapping functions [34]. AKT protein kinase regulates cell proliferation, survival, and apoptosis while its mediated phosphorylation can alter BCL-2 family members, NF-κB, and other transcription factors that initiate and inhibit

Table 5
Univariable and multivariable analysis for overall survival and progression-free survival.

Clinicopathologic variables	Overall survival			Progression-free survival		
	Univariate	Multivariate		Univariate	Multivariate	
	p-value	p-value	HR (95% CI)	p-value	p-value	HR (95% CI)
Age						
> 60 vs. ≤ 60	0.001	0.021	2.426(1.140-5.162)	0.002	0.541	
Sex						
Male vs. female	0.397			0.610		
Primary site						
Extranodal vs. nodal	0.007	0.534		0.120		
B symptoms						
Present vs. absent	0.484			0.237		
the Eastern Cooperative Group Performance Status (ECOG PS)						
≥ 2 vs. < 2	0.001	0.623		0.005	0.767	
Serum lactate dehydrogenase (LDH)						
Elevated vs. normal	< 0.001	0.113		< 0.001	0.083	
Ann Arbor stage						
III–IV vs. I–II	< 0.001	0.014	4.475(1.360-14.726)	< 0.001	0.185	
International prognostic index (IPI)						
3–5 vs. 0–2	< 0.001	0.623		< 0.001	0.939	
Bulky mass (cm)						
≥ 10 cm vs. < 10 cm	0.808			0.979		
Hans classification						
Non-GCB vs. GCB	0.296			0.368		
Different kinds of peripheral blood cells counts at diagnose(10^9/L)						
Lymphocyte count						
Decreased vs. normal	0.002	0.605		0.001	0.838	
Monocyte count						
Elevated vs. normal	0.889			0.619		
Neutrophil count						
Elevated vs. normal	0.414			0.478		
Hb						
Anemia vs. normal	0.023	0.016	2.194(1.156-4.165)	0.050	0.029	1.973(1.071-3.635)
β2-microglobulin						
Elevated vs. normal	0.003	0.885		< 0.001	0.042	3.498(1.044-11.713)
Treatment regimen						
R-CHOP vs. others	< 0.001	0.001	0.239(0.113-0.505)	< 0.001	< 0.001	0.254(0.131-0.492)
BCL2 expression						
Expression ≥70% vs. Expression < 70%	0.004	0.348		0.007	0.069	
C-myc expression						
Expression ≥40% vs. Expression < 40%	0.090			0.100		
P53 expression						
Positive vs. negative	0.351			0.558		
Ki-67 expression						
Expression ≥50% vs. Expression < 50%	< 0.001	0.026	2.556(1.120-5.833)	< 0.001	0.008	3.062(1.341-6.990)
CD30 expression						
Positive vs. negative	0.007	0.588		0.023	0.968	
CD5 expression						
Positive vs. negative	0.020	0.484		0.005	0.115	
TCL1 expression						
Positive vs. negative	0.003	0.003	3.804(1.553-9.361)	0.002	0.035	2.648(1.071-6.545)
AKT1 + 2+3 expression						
Positive vs. negative	0.029	0.071		0.077		
IKKβ expression						
Positive vs. negative	0.150			0.125		

apoptosis. The maladjustment of PI3K/AKT may lead to tumor formation [6], translocation and resistance to chemotherapy in DLBCL [35]. Wang J et al showed that the clinical significance of AKT activation in DLBCL is not well analyzed. They assessed the expression of phosphorylated AKT (p-AKT) in 522 DLBCL patients and found that the high expression observed in 24.3% of the study cohort was associated with significantly poor PFS and c-myc and Bcl-2 overexpression. However, multivariate analysis indicated that p-AKT hyperactivation is not an independent factor [35]. Our findings demonstrated that AKT1 + 2+3 was highly positively expressed (81.0%) in DLBCL and that the non-GCB subtype tended to be associated with a high expression (69.4%) relative to GCB- DLBCL (30.6%) ($p = 0.131$), but the difference was not statistically significant. AKT1 + 2+3 was related to age ($p = 0.045$), and we hypothesized that age is associated with the activity of AKT1 + 2+3. AKT1 + 2+3 was associated with primary site ($p = 0.010$) and was positively expressed in extranodal DLBCL. AKT1 + 2+3 expression was associated with the BCL2 expression (BCL2 expression ≥70%, $p < 0.001$) and Ki-67 expression (Ki-67 expression ≥50%, $p = 0.003$), thus, AKT1 + 2+3 may be associated with cell proliferation and apoptosis in DLBCL. AKT1 + 2+3 expression was not associated with c-myc expression (c-myc expression ≥40%, $p = 0.069$). Notably, we found that AKT1 + 2+3 expression was significantly associated with P53 ($p = 0.005$); however, the functions in DLBCL are unclear and should be further studied. We found that AKT1 + 2+3 positive expression was associated with significantly worse OS ($p = 0.029$), but the multivariate analysis indicated that AKT1 + 2+3 positive expression was not an independent factor this result was similar to our previous finding. AKT has been studied in drug research in DLBCL. Erdmann T et al. revealed that the PI3Kα/δ inhibitor AZD8835 showed marked potency in non-GCB DLBCL models, whereas the AKT inhibitor

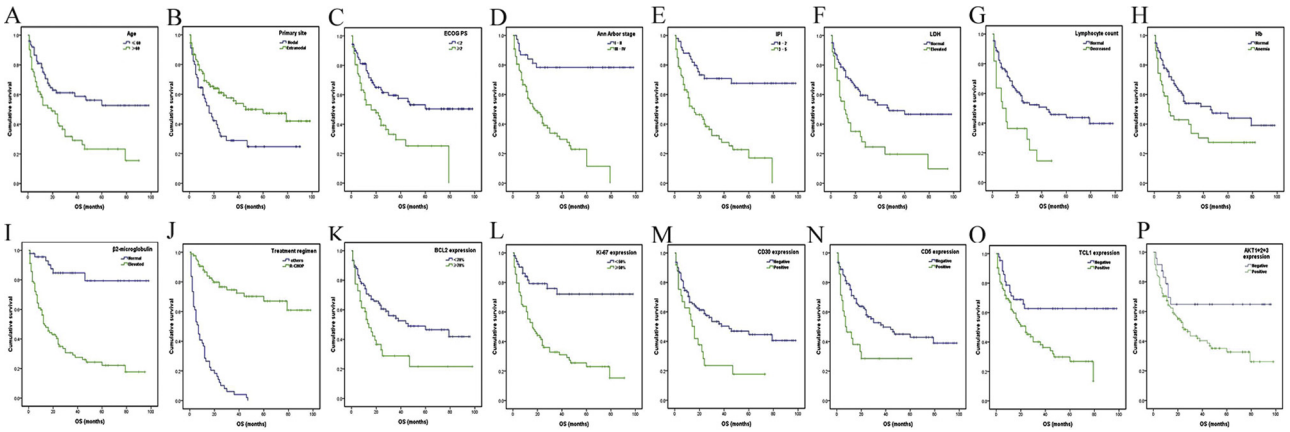

Fig. 2. Results of univariate survival analysis through Kaplan–Meier method on OS.

AZD5363 induced apoptosis in PTEN-deficient DLBCLs regardless of their molecular subtype. These in vitro results were confirmed in various cell line xenograft and patient-derived xenograft mouse models in vivo. Treatment with AZD8835 inhibited nuclear factor κB signaling, prompting us to combine AZD8835 with the Bruton's tyrosine kinase inhibitor ibrutinib. The combination was synergistic and effective in vitro and in vivo. The AKT inhibitor AZD5363 was effective in PTEN-deficient DLBCLs by downregulating the oncogenic transcription factor MYC. Thus, patients should be stratified in accordance with their oncogenic dependencies when treated with PI3K and AKT inhibitors [36]. Wang J et al demonstrated AKT hyperactivation and the potential of AKT-targeted therapy in DLBCL; they suggested that AKT inhibitors should be combined with other targeted agents for DLBCL to achieve optimal clinical efficacy [35].

Activating the transcription factor NF-κB induced by extracellular stimuli requires IKKα and IKKβ kinase activity. However, the mechanism through which IKKα and IKKβ are activated by various upstream signaling molecules is not fully understood [37]. Inhibiting IKKβ has been investigated as a therapeutic option for treating cancer [38].Our results indicated that IKKβ was negatively expressed in 21.2% (29/137) of the patients and positively expressed in 78.8% (108/137) of the patients with DLBCL. The positive expression of IKKβ was 34.3% (37/137) in GCB and 65.6% (71/137) in non-GCB, thus suggesting that the positive expression of IKKβ mostly observed in non-GCB DLBCL. Ishak et al indicated that cancer is the ability to reprogram cellular metabolism to increase the uptake of necessary nutrients such as glucose and glutamine. The results of the study offer an insight into the metabolic reprogramming in cancer cells that depends on a previously unidentified IKKβ-p53 signaling axis in response to glutamine depletion [39]. Thus, in the current study, IKKβ was correlated with P53 ($p < 0.001$), but this finding should be further verified to explore the function of IKKβ in DLBCL development. Our study revealed the correlation between AKT1 + 2+3 and IKKβ ($p < 0.001$; $r = 0.342$), but this result should be further verified. Dan HC et al demonstrated that IKKα, a component of the IKK complex that controls NF-κB activation, participates in the AKT-dependent regulation of mTORC1. IKKα also serves as a feedforward regulator of mTORC2 and could serve as a key therapeutic target for blocking mTORC2 and AKT activation in some cancers [40]. OS and PFS analysis did not reveal significant results in relation to IKKβ. Our findings might provide a clue to lymphomagenesis and lead to further investigations into IKKβ expression in DLBCL given that existing studies on IKKβ in DLBCL and cancers are few.

5. Conclusions

In summary, the present study revealed via immunohistochemistry analysis that TCL1, AKT1 + 2+3 and IKKβ as differential proteins were associated with the PI3K/AKT signaling pathway. TCL1, AKT1 + 2+3 and IKKβ were all highly positively expressed in DLBCL. TCL1 expression was associated with AKT1 + 2+3 ($p < 0.001$; $r = 0.475$). Survival analysis revealed that not receiving R−CHOP, positively expressed TCL1, Ki-67 ≥ 50%, and anemia were independent factors that predicted short OS and PFS in the total DLBCL cohort. However, the present study had limited sample size, which may lead to a high false-positive rate; thus, further studies are needed. We suggest that TCL1 immunodetection is an independent marker of adverse outcomes that

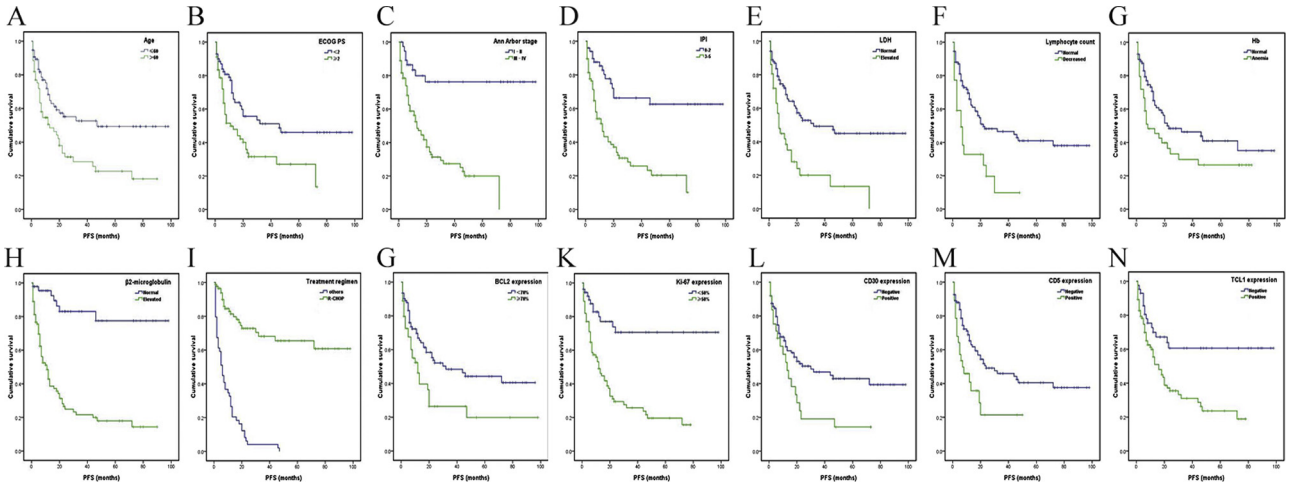

Fig. 3. Results of univariate survival analysis through Kaplan–Meier method on PFS.

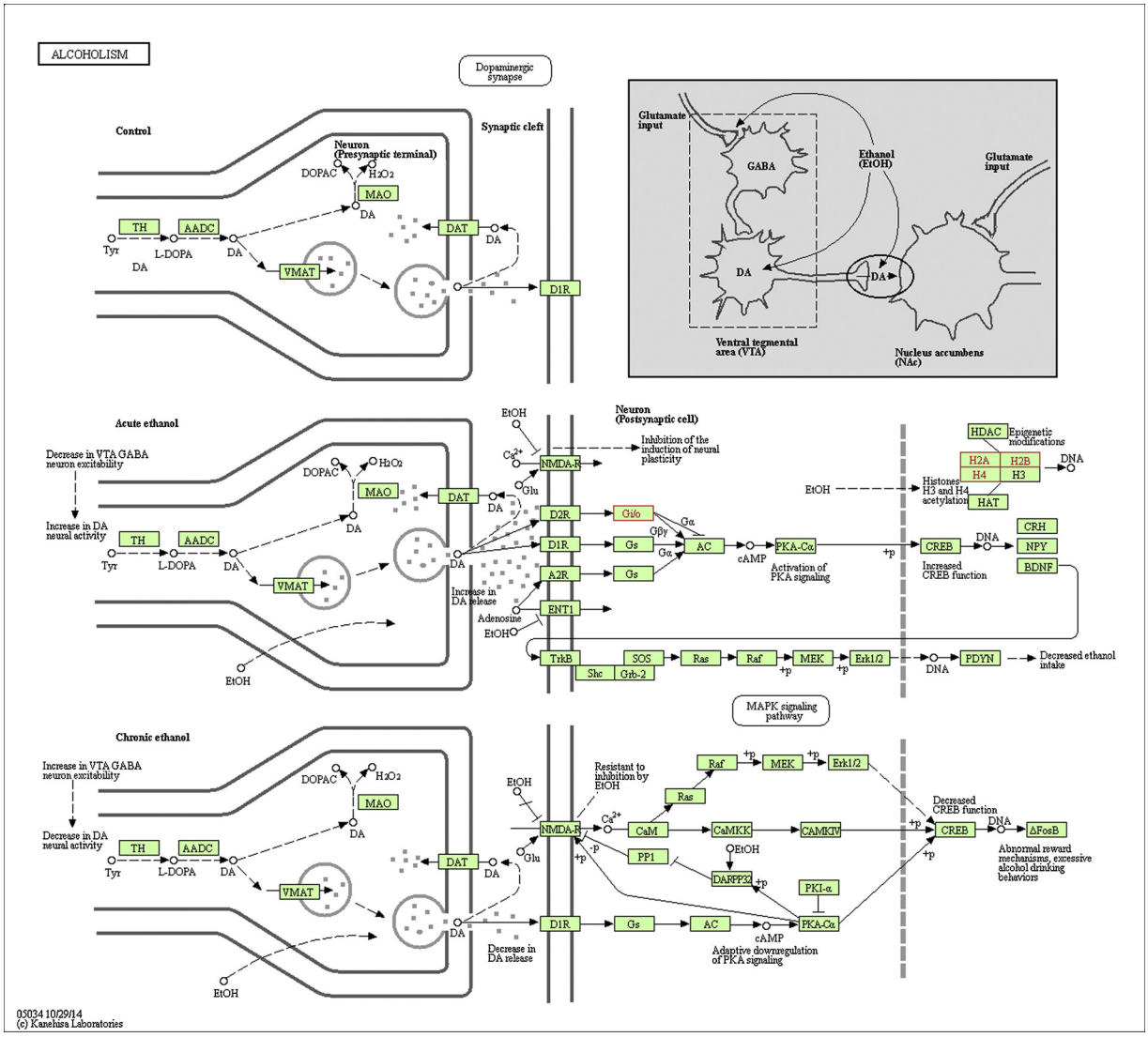

Fig. 4. Alcoholism signaling pathway.

could be used in routine settings for the management of DLBCL patients. These findings may open potential immunotherapeutic strategies for the treatment of DLBCL.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed 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 Foundation of China under Grant No. 81660036.

Authors contributions

Hai-Xia Gao wrote the paper and performed the experiments. Xin-Xia Li and Wei

Zhang evaluated all slides of the immunostained sections and scored the percentage of cells. Other authors collected paraffin-embedded tissue samples and clinicopathological information.

Author statement

We would like to submit the enclosed manuscript entitled “TCL1 as a hub protein associated with the PI3K/AKT signaling pathway in diffuse large B-cell lymphoma based on proteomics methods” which we wish to be considered for publication as a research article in your journal.

Declaration of Competing Interest

All authors have seen and approved the manuscript for submission to your journal. This paper has not been published or accepted elsewhere, and the authors declare no conflicts of interest.

Acknowledgement

Not applicable.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152799.

References

[1] S. Li, K.H. Young, L.J. Medeiros, Diffuse large B-cell lymphoma, Pathology 50 (1) (2018) 74–87, https://doi.org/10.1016/j.pathol.2017.09.006.
[2] M. Roschewski, L.M. Staudt, W.H. Wilson, Diffuse large B-cell lymphoma-treatment approaches in the molecular era, Nat. Rev. Clin. Oncol. 11 (1) (2014) 12–23, https://doi.org/10.1038/nrclinonc.2013.197.
[3] C.P. Hans, D.D. Weisenburger, T.C. Greiner, R.D. Gascoyne, J. Delabie, G. Ott, H.K. Müller-Hermelink, E. Campo, R.M. Braziel, E.S. Jaffe, Z. Pan, P. Farinha, L.M. Smith, B. Falini, A.H. Banham, A. Rosenwald, L.M. Staudt, J.M. Connors, J.O. Armitage, W.C. Chan, Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray, Blood 103 (1) (2004) 275–282, https://doi.org/10.1182/blood-2003-05-1545.
[4] A. Rosenwald, G. Wright, W.C. Chan, J.M. Connors, E. Campo, R.I. Fisher, R.D. Gascoyne, H.K. Muller-Hermelink, E.B. Smeland, J.M. Giltnane, E.M. Hurt, H. Zhao, L. Averett, L. Yang, W.H. Wilson, E.S. Jaffe, R. Simon, R.D. Klausner, J. Powell, P.L. Duffey, D.L. Longo, T.C. Greiner, D.D. Weisenburger, W.G. Sanger, B.J. Dave, J.C. Lynch, J. Vose, J.O. Armitage, E. Montserrat, A. López-Guillermo, T.M. Grogan, T.P. Miller, M. LeBlanc, G. Ott, S. Kvaloy, J. Delabie, H. Holte, P. Krajci, T. Stokke, L.M. Staudt, The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma, N. Engl. J. Med. 346 (25) (2002) 1937–1947, https://doi.org/10.1056/NEJMoa012914.
[5] B. Coiffier, C. Sarkozy, Diffuse large B-cell lymphoma: R-CHOP failure–what to do? Hematology Am. Soc. Hematol. Educ. Program 2016 (1) (2016) 366–378, https://doi.org/10.1182/asheducation-2016.1.366.
[6] A. Majchrzak, M. Witkowska, P. Smolewski, Inhibition of the PI3K/Akt/mTOR signaling pathway in diffuse large B-cell lymphoma: current knowledge and clinical significance, Molecules 19 (9) (2014) 14304–14315, https://doi.org/10.3390/molecules190914304.
[7] D. Wang, P. Liu, Y. Zhang, H.Y. Liu, D. Shen, Y.Q. Che, Bone marrow molecular markers associated with Relapsed/Refractory activated B-Cell-Like diffuse large B-Cell lymphoma, Biomed Res. Int. 2018 (2018) 1042597.
[8] S.A. Alsagaby, F.A. Alhumaydhi, Proteomics insights into the pathology and prognosis of chronic lymphocytic leukemia, Saudi Med. J. 40 (4) (2019) 317–327, https://doi.org/10.15537/smj.2019.4.23598.
[9] M.R. Chance, J. Chang, S. Liu, G. Gokulrangan, D.H. Chen, A. Lindsay, R. Geng, Q.Y. Zheng, K. Alagramam, Proteomics, bioinformatics and targeted gene expression analysis reveals up-regulation of cochlin and identifies other potential biomarkers in the mouse model for deafness in Usher syndrome type 1F, Hum. Mol. Genet. 19 (8) (2010) 1515–1527, https://doi.org/10.1093/hmg/ddq025.
[10] J.D. Bortner, J.P. Richie, A. Das, J. Liao, T.M. Umstead, A. Stanley, B.A. Stanley, C.P. Belani, K. El-Bayoumy, Proteomic profiling of human plasma by iTRAQ reveals down-regulation of ITI-HC3 and VDBP by cigarette smoking, J. Proteome Res. 10 (3) (2011) 1151–1159, https://doi.org/10.1021/pr100925p.
[11] S.A. Alsagaby, S. Khanna, K.W. Hart, G. Pratt, C. Fegan, C. Pepper, I.A. Brewis, P. Brennan, Proteomics-based strategies to identify proteins relevant to chronic lymphocytic leukemia, J. Proteome Res. 13 (11) (2014) 5051–5062, https://doi.org/10.1021/pr5002803.
[12] N.K. El-Mallawany, N. Day, J. Ayello, C. Van de Ven, K. Conlon, D. Fermin, V. Basrur, K. Elenitoba-Johnson, M. Lim, M.S. Cairo, Differential proteomic analysis of endemic and sporadic Epstein-Barr virus-positive and negative Burkitt lymphoma, Eur. J. Cancer 51 (1) (2015) 92–100, https://doi.org/10.1016/j.ejca.2014.10.017.
[13] H.X. Gao, A. Nuerlan, G. Abulajiang, W.L. Cui, J. Xue, W. Sang, S.J. Li, J. Niu, Z.P. Ma, W. Zhang, X.X. Li, Quantitative proteomics analysis of differentially expressed proteins in activated B-cell-like diffuse large B-cell lymphoma using quantitative proteomics, Pathology, research and practice 215 (9) (2019) 152528, , https://doi.org/10.1016/j.prp.2019.152528.
[14] I. Nakayama, S. Murao, S. Kitazawa, A. Azumi, S. Maeda, Activation of the TCL1 protein in B cell lymphomas, Pathol. Int. 50 (3) (2000) 191–199, https://doi.org/10.1046/j.1440-1827.2000.01023.
[15] D. Chihara, H. Ito, T. Matsuda, A. Shibata, A. Katsumi, S. Nakamura, S. Tomotaka, L.M. Morton, D.D. Weisenburger, K. Matsuo, Differences in incidence and trends of haematological malignancies in Japan and the United States, Br. J. Haematol. 164 (4) (2014) 536–545, https://doi.org/10.1111/bjh.12659.
[16] J.C. Wight, G. Chong, A.P. Grigg, E.A. Hawkes, Prognostication of diffuse large B-cell lymphoma in the molecular era: moving beyond the IPI, Blood Rev. 32 (5) (2018) 400–415, https://doi.org/10.1016/j.blre.2018.03.005.
[17] K. Miyazaki, Treatment of diffuse large B-Cell lymphoma, Journal of Clinical & Experimental Hematopathology Jceh 56 (2) (2016) 79–88, https://doi.org/10.3960/jslrt.56.79.
[18] I.A. Mayer, C.L. Arteaga, The PI3K/AKT Pathway as a Target for Cancer Treatment, Annu. Rev. Med. 67 (2016) 11–28, https://doi.org/10.1146/annurev-med-062913-051343 Epub 2015 Oct 14.
[19] X. Han, T. Zheng, F.M. Foss, S. Ma, T.R. Holford, P. Boyle, B. Leaderer, P. Zhao, M. Dai, Y. Zhang, Alcohol consumption and non-Hodgkin lymphoma survival, J. Cancer Surviv. 4 (2) (2010) 101–109, https://doi.org/10.1007/s11764-009-0111-4.
[20] K.E. Smedby, J.N. Sampson, J.J. Turner, S.L. Slager, M. Maynadie, E. Roman, T.M. Habermann, C.R. Flowers, S.I. Berndt, P.M. Bracci, Medical History, Lifestyle, Family History, and Occupational Risk Factors for Mantle Cell Lymphoma: The InterLymph Non-Hodgkin Lymphoma Subtypes Project, J. Natl. Cancer Inst. Monographs 2014 (2014) 76–86, https://doi.org/10.1093/jncimonographs/lgu007.
[21] A. Teitell Michael, The TCL1 family of oncoproteins: co-activators of transformation, Nature reviews, Cancer 5 (2005) 640–648, https://doi.org/10.1038/nrc1672.
[22] R. Virginie, D. Gilles, S. Marc-Henri, B. Serge, R. Christian, T. Arold Stefan, K. Bostjan, The TCL1A oncoprotein interacts directly with the NF-κB inhibitor IκB, PLoS One 4 (2009), https://doi.org/10.1371/journal.pone.0006567 e6567.
[23] E. Gaudio, R. Spizzo, F. Paduano, Z. Luo, C.M. Croce, Tcl1 interacts with Atm and enhances NF-kB activation in hematologic malignancies, Blood 119 (1) (2011) 180–187, https://doi.org/10.1182/blood-2011-08-374561.
[24] E. Gaudio, F. Paduano, A. Ngankeu, F. Lovat, M. Fabbri, H.-L. Sun, P. Gasparini, A. Efanov, Y. Peng, N. Zanesi, Heat shock protein 70 regulates Tcl1 expression in leukemia and lymphomas, Blood 121 (2) (2013) 351–359, https://doi.org/10.1182/blood-2012-09-457374.
[25] E. Gaudio, F. Paduano, S. Pinton, S. D'Agostino, R. Rocca, G. Costa, A. Ngankeu, R.I. Aqeilan, C.M. Croce, F. Bertoni, S. Alcaro, F. Trapasso, TCL1A interacts with TP63 and enhances the survival of Raji Burkitt lymphoma cell line, Br. J. Haematol. 183 (2018) 509–512, https://doi.org/10.1111/bjh.14989.
[26] G.F. Widhopf, B. Cui, E.M. Ghia, L. Chen, K. Messer, Z. Shen, S.P. Briggs, C.M. Croce, T.J. Kipps, ROR1 can interact with TCL1 and enhance leukemogenesis in Eμ-TCL1 transgenic mice, Proc. Natl. Acad. Sci. U.S.A. 111 (2) (2014) 793–798, https://doi.org/10.1073/pnas.1308374111.
[27] F. Paduano, E. Gaudio, A.A. Mensah, S. Pinton, F. Bertoni, F. Trapasso, T-cell Leukemia/Lymphoma 1 (TCL1): an oncogene regulating multiple signaling pathways, Front. Oncol. 8 (2018) 317, https://doi.org/10.3389/fonc.2018.00317.
[28] C. Kiss, J. Nishikawa, K. Takada, P. Trivedi, G. Klein, L. Szekely, T cell leukemia I oncogene expression depends on the presence of Epstein-Barr virus in the virus-carrying Burkitt lymphoma lines, Proc. Natl. Acad. Sci. U.S.A. 100 (8) (2003) 4813–4818, https://doi.org/10.1073/pnas.0730710100.
[29] S.W. French, C.S. Malone, R.R. Shen, M. Renard, S.E. Henson, M.D. Miner, R. Wall, M.A. Teitell, Sp1 transactivation of the TCL1 oncogene, J. Biol. Chem. 278 (2) (2003) 948–955, https://doi.org/10.1074/jbc.M207166200.
[30] J. Laine, G. Künstle, T. Obata, M. Sha, M. Noguchi, The protooncogene TCL1 is an Akt kinase coactivator, Mol. Cell 6 (2) (2000) 395–407, https://doi.org/10.1016/S1097-2765(00)00039-3.
[31] S.J. Shin, J. Roh, H.J. Cha, Y.D. Choi, J.M. Kim, S.K. Min, J.E. Kim, D.W. Eom, H. Lee, H.J. Kim, D.H. Yoon, C. Suh, J. Huh, TCL1 expression predicts overall survival in patients with mantle cell lymphoma, Eur. J. Haematol. 95 (6) (2015) 583–594, https://doi.org/10.1111/ejh.12539.
[32] O. Ramuz, R. Bouabdallah, E. Devilard, N. Borie, A. Groulet-Martinec, V.J. Bardou, P. Brousset, F. Bertucci, F. Birg, D. Birnbaum, L. Xerri, Identification of TCL1A as an immunohistochemical marker of adverse outcome in diffuse large B-cell lymphomas, Int. J. Oncol. 26 (1) (2005) 151–157, https://doi.org/10.3892/ijo.26.1.151.
[33] J. Weng, S. Rawal, F. Chu, H.J. Park, R. Sharma, D.A. Delgado, L. Fayad, M. Fanale, J. Romaguera, A. Luong, TCL1: a shared tumor-associated antigen for immunotherapy against B-cell lymphomas, Blood 120 (8) (2012) 1613–1623, https://doi.org/10.1182/blood-2011-09-382838.
[34] S.R. Datta, A. Brunet, M.E. Greenberg, Cellular survival: a play in three Akts, Genes Dev. 13 (22) (1999) 2905–2927, https://doi.org/10.1101/gad.13.22.2905.
[35] J. Wang, Z.Y. Xu-Monette, K.J. Jabbar, Q. Shen, G.C. Manyam, A. Tzankov, C. Visco, J. Wang, S. Montes-Moreno, K. Dybkær, AKT hyperactivation and the potential of AKT-Targeted therapy in diffuse large B-Cell lymphoma, Am. J. Pathol. 187 (8) (2017) 1700–1716, https://doi.org/10.1016/j.ajpath.2017.04.009.
[36] Tabea Erdmann, Pavel Klener, James T. Lynch, Michael Grau, Petra Vočková, Jan Molinsky, Diana Tuskova, Kevin Hudson, Urszula M. Polanska, Michael Grondine, Sensitivity to PI3K and AKT inhibitors is mediated by divergent molecular mechanisms in subtypes of DLBCL, Blood 130 (2017) 310–322, https://doi.org/10.1182/blood-2016-12-758599.
[37] S.W. Kim, M. Schifano, D. Oleksyn, C. Jordan, D. Ryan, R. Insel, J. Zhao, L. Chen, Protein kinase C-associated kinase regulates NF-κB activation through inducing IKK activation, Int. J. Oncol. 45 (2014) 1707–1714, https://doi.org/10.3892/ijo.2014.2578.
[38] J.A. Prescott, S.J. Cook, Targeting IKKβ in Cancer: challenges and opportunities for the therapeutic utilisation of IKKβ inhibitors, Cells 7 (9) (2018), https://doi.org/10.3390/cells7090115.
[39] M.B. Ishak Gabra, Y. Yang, X.H. Lowman, M.A. Reid, T.Q. Tran, M. Kong, IKKβ activates p53 to promote cancer cell adaptation to glutamine deprivation, Oncogenesis 7 (11) (2018) 93, https://doi.org/10.1038/s41389-018-0104-0.
[40] H.C. Dan, R.J. Antonia, A.S. Baldwin, PI3K/Akt promotes feedforward mTORC2 activation through IKKα, Oncotarget 7 (16) (2016) 21064–21075, https://doi.org/10.18632/oncotarget.8383.