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Original Article

Dysregulated expressions of PTEN, NF-κB, WWP2, p53 and c-Myc in different subtypes of B cell lymphoma and reactive follicular hyperplasia

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Abstract: This study aimed to investigate the value of PTEN, NF-κB, WWP2, p53 and c-Myc expressions in distinguishing B cell lymphomas from reactive follicular hyperplasia (RFH), and their abilities to discriminate different B cell lymphoma subtypes. Lymphoma tissue samples were obtained from 30 follicular lymphoma (FL) patients, 30 germinal center B-cell like (GCB) diffuse large B cell lymphoma (DLBCL) patients, 30 non-GCB DLBCL patients and 30 Burkitt's lymphoma (BL) patients. And hyperplasia tissue samples were obtained from and 30 RFH patients. Immunohistochemistry was used to quantify the expressions of PTEN, NF-κB, WWP2, P53 and c-Myc. PTEN expression was elevated in GCB DLBCL and BL compared with RFH, and in GCB DLBCL, non-GCB DLBCL and BL than that in FL; WWP2 expression was higher in FL, GCB DLBCL, non-GCB DLBCL and BL compared with RFH; p53 expression increased in non-GCB DLBCL compared with RFH, and in BL compared with RFH, FL or GCB DLBCL; c-Myc expression was higher in GCB DLBCL, non-GCB DLBCL and BL compared with RFH; c-Myc expression was elevated in GCB DLBCL, non-GCB DLBCL and BL compared with FL. Additionally, PTEN negatively correlated with p53 expression in FL and CGB DLBCL, whereas NF-κB negatively correlated with WWP2 in GCB DLBCL, but positively associated with PTEN in RFH and c-Myc in BL. PTEN, WWP2, p53 and c-Myc expressions might be served as biomarkers for identification of B cell lymphomas from RFH as well as distinguishing different B cell lymphoma subtypes.

Keywords: B cell lymphoma, biomarkers, reactive follicular hyperplasia, differentiation, expression

Introduction

Non-Hodgkin's lymphomas (non-HL), account for over 90% of lymphoma cases, are a class of hematologic malignancies arising from immune cells in lymph nodes with complex pathology and classification, which are divided into two major groups including B-cell lymphomas and T-cell/natural killer cell lymphomas according to the World Health Organization (WHO) guidance [1, 2]. As the most common class of non-HL, B-cell lymphoma encompasses diverse subtypes such as diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), Burkitt's lymphoma (BL) and lymphoblastic lymphoma that are characterized by high proliferation of cancerous lymphocytes, and those subtypes are distinguished by using invasive tissue biopsies in clinical practice [3, 4]. However, a great clinical challenge still exists in correct identification of B cell lymphomas from lymph nodes reactive hyperplasia as well as the identification among different B cell lymphoma subtypes due to shared histological characteristics (such as proliferation of lymphocytes) [5, 6]. Hence, exploring convincing biomarkers that differentiate the B cell lymphomas from reactive lymphocyte hyperplasia and distinguish different subtypes of B cell lymphoma is critical for accurate differential diagnosis of B cell lymphomas and the subtypes as well as optimizing individualized therapy.

Translational products consisting of phosphatase and tensin homolog deleted on chromosome ten (PTEN), proto-oncogene c-Myc (c-Myc), nuclear factor-kappa B (NF-κB), WW Domain Containing E3 Ubiquitin Protein Ligase

Table 1. Comparison of PTEN expression among varied groups

Items	RFH	FL	GCB DLBCL	non-GCB DLBCL	BL
RFH	-	0.593	**0.018**	0.100	**0.009**
FL	-	-	**0.006**	**0.032**	**0.003**
GCB DLBCL	-	-	-	0.393	0.807
non-GCB DLBCL	-	-	-	-	0.238
BL	-	-	-	-	-

Data was presented as *P* value. PTEN expression was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Comparison was determined by Wilcoxon rank sum test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; RFH, reactive follicular hyperplasia; FL, follicular lymphoma; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

2 (WWP2) and p53 are pivotal in regulating tumorigenesis and disease progression of lymphoma, and PTEN has been discovered to be involved in the regulation of c-Myc, NF-κB, WWP2 and p53 in various cancers including hematological cancers [7-10]. Moreover, these biomarkers have been revealed to potentially distinguish subtypes of B cell lymphoma, for instance, PTEN have been disclosed to be differentially expressed in a particular B cell lymphoma subtype GCB-DLBCL and potentially distinguish GCB-DLBCL from another subtype non-GCB DLBCL [11-13]. Although the above-mentioned proteins have been individually studied in regard to its ability to tell different B cell lymphoma subtypes, the value of these proteins in discriminating B cell lymphomas from hyperplasia is still obscure, and there is also a lack of studies comprehensively investigating a group of potential biomarkers for identification of B cell lymphoma subtypes from each other. Therefore, we assessed the expressions of PTEN, NF-κB, WWP2, p53 and c-Myc in four different subtypes of B cell lymphoma (including FL, GCB DLBCL, non-GCB DLBCL as well as BL) and RFH in this study, aiming to determine the value of PTEN, NF-κB, WWP2, p53 and c-Myc in distinguishing B cell lymphomas from reactive follicular hyperplasia (RFH), and their ability to discriminate different B cell lymphoma subtypes.

Methods

Patients

30 FL patients, 30 GCB DLBCL patients, 30 non-GCB DLBCL patients, 30 BL patients and 30 RFH patients, at Jiangxi Provincial People's Hospital between Jan 2014 and Dec 2016 were consecutively enrolled in this study. All patients were pathologically diagnosed as corresponding diseases with age above 18 years. This study was approved by the Ethics Committee of Jiangxi Provincial People's Hospital, and all patients provided the written informed consents.

Samples

Lymphoma tissue samples were obtained from FL, GCB DLBCL, non-GCB DLBCL and BL patients during biopsy, while hyperplasia tissue samples were obtained from RFH patients during biopsy as controls. Samples were then fixed by formaldehyde and embedded in paraffin for further detection.

Immunohistochemistry (IHC)

Formaldehyde-fixed, paraffin-embedded samples were deparaffinized and rehydrated after cutting into sections, and antigen was retrieved using ethylene diamine tetraacetic acid (EDTA). Subsequently, H_2O_2 was used to block the endogenous peroxidase, and sections were then immersed with 4% bovine serum albumin to block nonspecific endogenous antigens. Then the sections were incubated with rabbit PTEN antibody with dilution 1:1000 (Santa Cruz, USA), rabbit NF-κB antibody with dilution 1:1000 (Santa Cruz, USA), rabbit WWP2 antibody with dilution 1:500 (Santa Cruz, USA), rabbit P53 antibody with dilution 1:1000 (Santa Cruz, USA) and rabbit c-Myc antibody with dilution 1:1000 (Santa Cruz, USA) respectively at 4°C overnight, which was followed by being washed and incubated with horseradish peroxidase (HRP)-conjugated anti-IgG (rabbit) with dilution 1:500 (MXB Biotechnologies, China) as secondary antibody at room temperature for 30 minutes. Then the tissue sections were washed and treated with diaminobenzidine (DAB) followed by being counterstained with hematoxylin. Finally, sections were dehydrated and mounted.

Assessment of IHC result

Light microscopy (Nikon, Japan) was utilized to detect the results of IHC, and the expressions

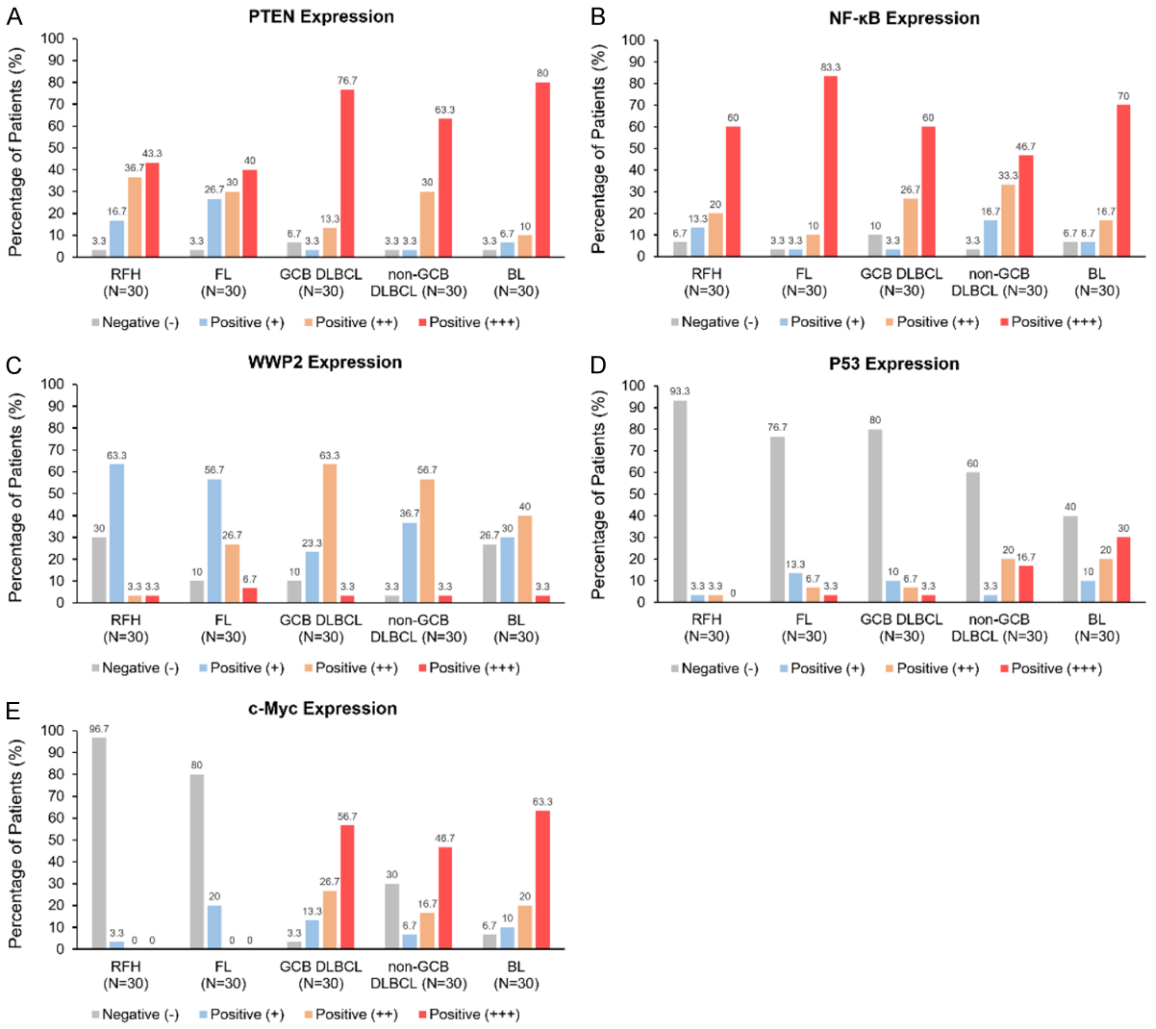

Figure 1. Expressions of PTEN, NF-κB, WWP2, P53 and c-Myc among different disease groups. A. The expression status of PTEN in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups. B. The expression status of NF-κB in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups. C. The expression status of WWP2 in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups. D. The expression status of P53 in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups. E. The expression status of c-Myc in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups. Data were presented as histogram, and expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; RFH, Reactive follicular hyperplasia; FL, follicular lymphoma; GCB-DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

of PTEN, NF-κB, WWP2, P53 as well as c-Myc were measured using the following methods: 100 cells in 5 high-power fields (HPF, ×400) were counted for evaluating the intensity of positive cells, which was scored as 0 (negative), 1 (weak), 2 (moderate), to 3 (strong), while labeling frequency was also graded as 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), and 4 (76%-100%) on the basis of the percentage of positively stained cells, then multiplying the score of staining intensity by the labeling frequency score was used to divide the expressions into 4 grades: 0-3, negative (-) expression; 4-6, positive (+) expression; 7-9, positive (++) expression; 10-12, positive (+++) expression. In the assessment processes, two specialists without knowing any information about patients measured the IHC results, and the average score was applied in the analysis. The representative IHC images of each candidate proteins were shown in Supplementary Figure 1.

Table 2. Comparison of NF-κB expression among varied groups

Items	RFH	FL	GCB DLBCL	non-GCB DLBCL	BL
RFH	-	0.054	0.927	0.489	0.449
FL	-	-	0.061	**0.005**	0.260
GCB DLBCL	-	-	-	0.356	0.497
non-GCB DLBCL	-	-	-	-	0.114
BL	-	-	-	-	-

Data was presented as *P* value. NF-κB expression was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Comparison was determined by Wilcoxon rank sum test. P < 0.05 was considered as significant and presented as bold font. NF-κB, nuclear factor-kappa B; RFH, reactive follicular hyperplasia; FL, follicular lymphoma; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

Table 3. Comparison of WWP2 expression among varied groups

Items	RFH	FL	GCB DLBCL	non-GCB DLBCL	BL
RFH	-	**0.007**	**< 0.001**	**< 0.001**	**0.050**
FL	-	-	0.055	0.066	0.785
GCB DLBCL	-	-	-	0.815	0.067
non-GCB DLBCL	-	-	-	-	0.073
BL	-	-	-	-	-

Data was presented as *P* value. WWP2 expression was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Comparison was determined by Wilcoxon rank sum test. P < 0.05 was considered as significant and presented as bold font. WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; RFH, reactive follicular hyperplasia; FL, follicular lymphoma; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

Table 4. Comparison of P53 expression among varied groups

Items	RFH	FL	GCB DLBCL	non-GCB DLBCL	BL
RFH	-	0.109	0.182	**0.001**	**< 0.001**
FL	-	-	0.864	0.085	**0.001**
GCB DLBCL	-	-	-	0.052	**< 0.001**
non-GCB DLBCL	-	-	-	-	0.142
BL	-	-	-	-	-

Data was presented as *P* value. P53 expression was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Comparison was determined by Wilcoxon rank sum test. P < 0.05 was considered as significant and presented as bold font. P53, tumor protein p53; RFH, reactive follicular hyperplasia; FL, follicular lymphoma; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

Statistics

Statistical analysis was performed using SPSS 21.0 Software (IBM, USA) and graphs were made using GraphPad 6.01 Software (GraphPad Int, USA). Comparison on expression of PTEN, NF-κB, WWP2, P53 and c-Myc between each two disease groups respectively was determined by Wilcoxon rank sum test, and the correlation between each two candidate biomarkers in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups respectively was determined by Spearman test. P < 0.05 was considered significant.

Results

Comparison of PTEN expression among different disease subtypes

Compared with RFH group, PTEN expression was elevated in GCB DLBCL group (P = 0.018) and BL group (P = 0.009), and higher PTEN expression was observed in GCB DLBCL group (P = 0.006), non-GCB DLBCL group (P = 0.032), as well as BL group (P = 0.003) than that in FL group (**Table 1**; **Figure 1A**).

Comparison of NF-κB expression among different disease subtypes

Lower NF-κB expression was presented in non-GCB DLBCL group compared with FL group (P = 0.005) (**Table 2**; **Figure 1B**), while no difference was shown between each two other disease subtypes (All P > 0.05).

Comparison of WWP2 expression among different disease subtypes

WWP2 expression was higher in FL group (P = 0.007), GCB DLBCL group (P < 0.001), non-GCB DLBCL group (P < 0.001) and BL group (P = 0.050) compared with RFH group (**Table 3**; **Figure 1C**).

Comparison of p53 expression among different disease subtypes

Increased p53 expression was found in non-GCB DLBCL group (P = 0.001) compared with

Table 5. Comparison of c-Myc expression among varied groups

Items	RFH	FL	GCB DLBCL	non-GCB DLBCL	BL
RFH	-	0.103	**< 0.001**	**< 0.001**	**< 0.001**
FL	-	-	**< 0.001**	**< 0.001**	**< 0.001**
GCB DLBCL	-	-	-	0.135	0.738
non-GCB DLBCL	-	-	-	-	0.082
BL	-	-	-	-	-

Data was presented as *P* value. c-Myc expression was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Comparison was determined by Wilcoxon rank sum test. P < 0.05 was considered as significant and presented as bold font. c-Myc, proto-oncogene c-Myc; RFH, reactive follicular hyperplasia; FL, follicular lymphoma; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma.

Table 6. Correlation of PTEN, NF-κB, WWP2, P53 and c-Myc in RFH patients

Items		PTEN	NF-κB	WWP2	P53	c-Myc
PTEN	r	-	**0.420**	-0.214	-0.044	0.206
	P value	-	**0.021**	0.257	0.818	0.275
NF-κB	r	-	-	0.274	-0.098	0.146
	P value	-	-	0.143	0.608	0.440
WWP2	r	-	-	-	0.127	-0.266
	P value	-	-	-	0.503	0.156
P53	r	-	-	-	-	-0.050
	P value	-	-	-	-	0.795
c-Myc	r	-	-	-	-	-
	P value	-	-	-	-	-

Data was presented as r and *P* value. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Correlation was determined by Spearman test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; RFH, reactive follicular hyperplasia.

RFH group, and it was also up-regulated in BL group compared with RFH group (P < 0.001), FL group (P = 0.001) or GCB DLBCL group (P < 0.001) (**Table 4**; **Figure 1D**).

Comparison of c-Myc expression among different disease subtypes

Compared with RFH group, c-Myc expression was raised in GCB DLBCL group (P < 0.001), non-GCB DLBCL group (P < 0.001) and BL group (P < 0.001) (**Table 5**; **Figure 1E**). In addition, elevated c-Myc expression was discovered in GCB DLBCL (P < 0.001), non-GCB DLBCL (P < 0.001) and BL (P < 0.001) groups compared with FL group.

Correlation between candidate biomarkers in RFH patients

In order to evaluate the association between each two candidate biomarkers in each disease subtype, Spearman tests were further performed. In RFH patients, PTEN expression was positively correlated with NF-κB expression (r = 0.420, P = 0.021), while no correlation between other candidate biomarkers was found (**Table 6**; **Figure 2A**).

Correlation between candidate biomarkers in FL patients

Negative correlation between PTEN and p53 levels (r = -0.407, P = 0.026) was observed in FL patients, whereas there was no correlation between other candidate biomarkers (**Table 7**; **Figure 2B**).

Correlation between candidate biomarkers in GCB DLBCL patients

In GCB DLBCL patients, PTEN expression was negatively associated with p53 expression (r = -0.368, P = 0.045), and NF-κB expression was negatively correlated with WWP2 expression (r = -0.381, P = 0.038) (**Table 8**; **Figure 2C**). There was no correlation between other candidate biomarkers.

Correlation of candidate biomarkers in non-GCB DLBCL patients

For non-GCB DLBCL patients, no correlation between candidate biomarkers (All P > 0.05) was discovered (**Table 9**; **Figure 2D**).

Correlation of candidate biomarkers in BL patients

NF-κB level was positively associated with c-Myc level (r = 0.379, P = 0.039), while no correlation was presented between other candi-

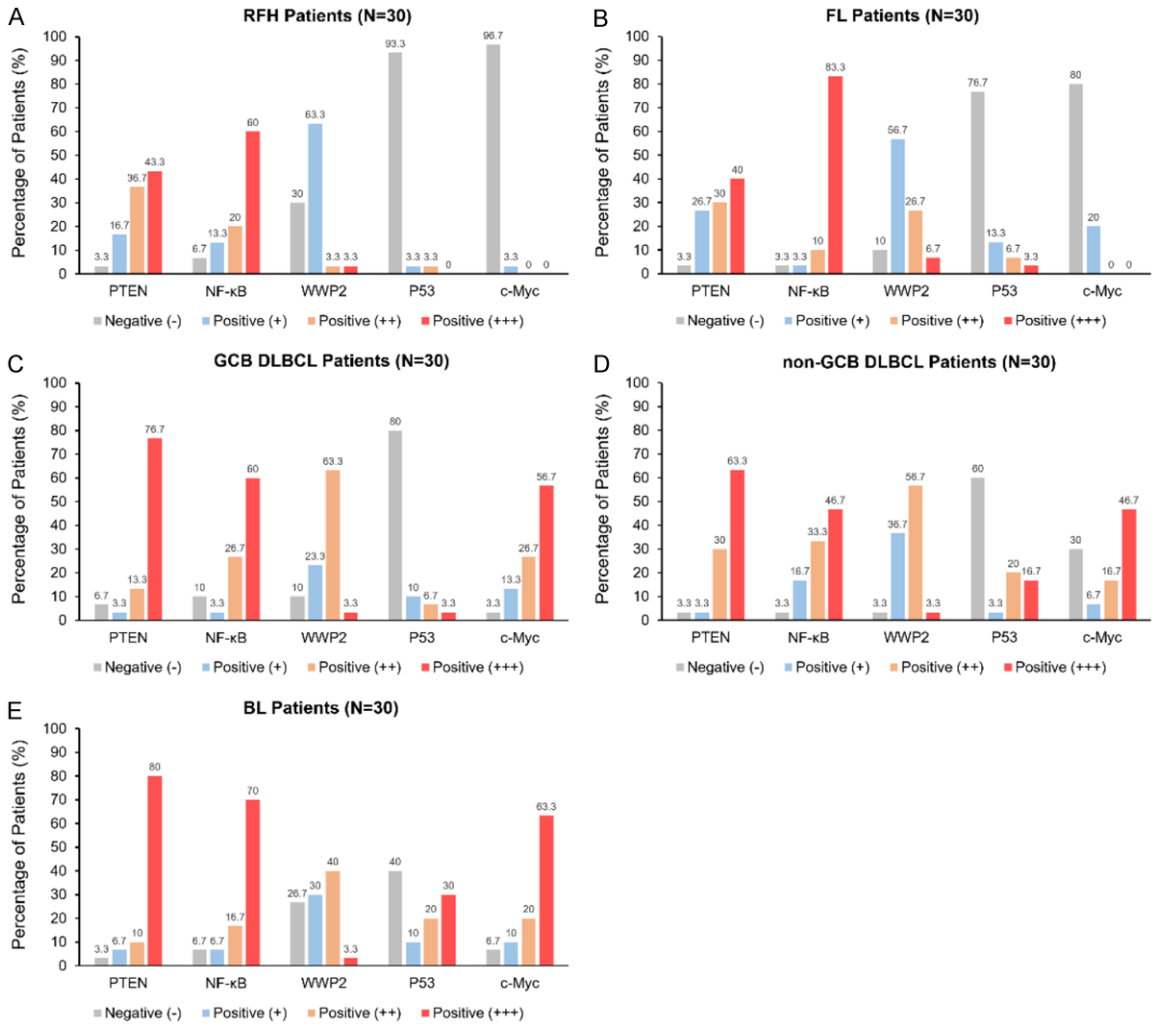

Figure 2. Expression status of PTEN, NF-κB, WWP2, P53 and c-Myc in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups respectively. A. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc in RFH patients. B. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc in FL patients. C. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc in GCB DLBCL patients. D. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc in non-GCB DLBCL patients. E. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc in BL patients. Data were presented as histogram, and expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). RFH, Reactive follicular hyperplasia; FL, follicular lymphoma; GCB-DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma; PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc.

date biomarkers in BL patients (**Table 10**; **Figure 2E**).

Discussion

In this study, we observed that: (1) PTEN, WWP2, p53 and c-Myc but not NF-κB were highly expressed in B cell lymphoma subgroups compared with RFH group. (2) PTEN and c-Myc expressions were reduced in FL group compared with other subgroups of B cell lymphoma, while p53 expression was increased in BL group compared with FL group or GCB DLBCL group. (3) PTEN was negatively associated with p53 in FL and GCB DLBCL groups, whereas NF-κB was negatively correlated with WWP2 in GCB DLBCL group, but positively associated with PTEN in RFH group and c-Myc in BL group.

B cell lymphomas are tumors of the immune system that originate from B-lymphocytes, including a wide range of subtypes. The distinct

Table 7. Correlation of PTEN, NF-κB, WWP2, P53 and c-Myc in FL patients

Items		PTEN	NF-κB	WWP2	P53	c-Myc
PTEN	r	-	0.255	-0.025	**-0.407**	-0.097
	P value	-	0.174	0.895	**0.026**	0.610
NF-κB	r	-	-	0.190	0.243	0.223
	P value	-	-	0.315	0.195	0.237
WWP2	r	-	-	-	0.066	-0.119
	P value	-	-	-	0.730	0.533
P53	r	-	-	-	-	0.293
	P value	-	-	-	-	0.116
c-Myc	r	-	-	-	-	-
	P value	-	-	-	-	-

Data was presented as r and *P* value. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Correlation was determined by Spearman test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; FL, follicular lymphoma.

Table 8. Correlation of PTEN, NF-κB, WWP2, P53 and c-Myc in GCB DLBCL patients

Items		PTEN	NF-κB	WWP2	P53	c-Myc
PTEN	r	-	0.205	-0.332	**-0.368**	-0.119
	P value	-	0.276	0.073	**0.045**	0.532
NF-κB	r	-	-	**-0.381**	-0.338	0.211
	P value	-	-	**0.038**	0.067	0.263
WWP2	r	-	-	-	0.171	-0.015
	P value	-	-	-	0.367	0.937
P53	r	-	-	-	-	-0.143
	P value	-	-	-	-	0.449
c-Myc	r	-	-	-	-	-
	P value	-	-	-	-	-

Data was presented as r and *P* value. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Correlation was determined by Spearman test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; GCB DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma.

features of different B cell lymphoma subtypes have been well described previously, for instance, DLBCL is the most common type of B cell lymphomas among adults and has been reported to be an aggressive tumor that is derived from in virtually any part of the body, which contains several subgroups according to cellular or gene properties [14]. As for BL, it is a typical representative of aggressive B cell lymphomas originated from GCB, which has been reported to present translocation and active expression of c-Myc gene [12]. In addition, FL, an indolent lymphoma, is characterized by carcinogenesis of follicle center B cells, and it is likely to histologically transform into DLBCL if enter an aggressive stage [15]. Although these B cell lymphoma subtypes are currently distinguished by biopsy according to their distinct histological features and symptoms, the discrimination of these subtypes from hyperplasia as well as the identification among subtypes remains confusing in clinical settings. Therefore, exploration of convincing markers that biologically assist with identification of B cell lymphomas from hyperplasia and discrimination of B cell lymphoma subtypes, which has been enabled by modern proteomic and immunologic approaches, may contribute to promising improvement in lymphoma treatment [16-18].

In the recent decades, researchers have dedicated in exploring biomarkers that distinguish different subtypes of lymphoma, and the ability of PTEN, NF-κB, WWP2, p53 and c-Myc to discriminate some B cell lymphoma subtypes has been discovered, which illustrates that: (1) deletion of PTEN is common in GCB DLBCL whereas less observed in non-GCB DLBCL [13]; (2) enhanced NF-κB activity is observed in non-GCB DLBCL rather than GCB DLBCL by a pervious study using high accuracy quantitative proteomics [11]; (3) there is report illuminating that WWP2 interacts with c-Myc in myeloma and is closely involved in regulation of PTEN in cancers [8, 19]; (4) p53 phosphorylation and the following cellular senescence is more common in DLBCL and FL compared with other subtypes [20]; (5) increased c-Myc expression has been presented in non-GCB DLBCL as well as in BL patients compared with other subtypes [12, 21]. Additionally, close interaction of NF-κB, WWP2, p53 and c-Myc with PTEN has been observed in several cancers including hematological cancers [7-10, 22]. Therefore, considering that these aforementioned biomarkers were distinctively expressed in different subtypes of B cell lymphoma individually, and close interaction was presented among their mediations in hematological cancers, we assumed

Table 9. Correlation of PTEN, NF-κB, WWP2, P53 and c-Myc in non-GCB DLBCL patients

Items		PTEN	NF-κB	WWP2	P53	c-Myc
PTEN	r	-	0.176	0.278	0.289	-0.128
	P value	-	0.353	0.137	0.122	0.499
NF-κB	r	-	-	0.036	-0.169	0.095
	P value	-	-	0.850	0.371	0.617
WWP2	r	-	-	-	-0.002	0.061
	P value	-	-	-	0.993	0.748
P53	r	-	-	-	-	
	P value	-	-	-	-	
c-Myc	r	-	-	-	-	-
	P value	-	-	-	-	-

Data was presented as r and *P* value. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Correlation was determined by Spearman test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma.

Table 10. Correlation of PTEN, NF-κB, WWP2, P53 and c-Myc in BL patients

Items		PTEN	NF-κB	WWP2	P53	c-Myc
PTEN	r	-	-0.320	0.213	0.061	-0.205
	P value	-	0.085	0.259	0.747	0.277
NF-κB	r	-	-	-0.157	-0.024	**0.379**
	P value	-	-	0.409	0.902	**0.039**
WWP2	r	-	-	-	-0.038	-0.023
	P value	-	-	-	0.844	0.905
P53	r	-	-	-	-	-0.113
	P value	-	-	-	-	0.552
c-Myc	r	-	-	-	-	-
	P value	-	-	-	-	-

Data was presented as r and *P* value. Expression of PTEN, NF-κB, WWP2, P53 and c-Myc was scored as 0-Negative (-), 1-Positive (+), 2-Positive (++), 3-Positive (+++). Correlation was determined by Spearman test. P < 0.05 was considered as significant and presented as bold font. PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc; BL, Burkitt's lymphoma.

that these proteins (including PTEN, NF-κB, WWP2, p53 and c-Myc) had the potential to tell B cell lymphomas from RFH as well as to distinguish different B cell lymphoma subtypes.

In the current study, 30 FL patients, 30 GCB DLBCL patients, 30 non-GCB DLBCL patients, 30 BL patients as well as 30 RFH patients were enrolled, and the expressions of PTEN, NF-κB, WWP2, P53 and c-Myc were detected by IHC in this study, which disclosed that: (1) PTEN, WWP2, p53 and c-Myc but not NF-κB were highly expressed in B cell lymphoma subgroups compared with RFH group. This is possibly due to that, for PTEN, WWP2, p53 and c-Myc, they were specifically associated with tumorigenesis but not reactive hyperplasia, thereby presenting distinct expression in malignant B cell lymphoma subtypes compared with RFH, while NF-κB was constitutively active in many lymphoma subtypes, and its expression was also reported to be elevated in B-lymphocyte hyperplasia in response to antigens, therefore its expression was parallel in all five disease subtypes [23, 24]. (2) PTEN and c-Myc expressions were reduced in FL group compared with other subgroups of B cell lymphoma, possibly resulting from that mutation and overexpression of c-Myc or PTEN changed the immunoglobin structure of B cell receptor (BCR) on B-lymphocytes and dysregulated BCR signaling, which led to aggressive proliferation of B-lymphocytes in B cell lymphoma subtypes (except FL that is indolent and less aggressive than other subtypes) [25]. (3) P53 expression was increased in BL group compared with FL or GCB DLBCL group, a probable explanation might be that BL presented with obviously high rates of cells apoptosis compared with other subtypes, which is related to the activation of p53 gene, suggesting that p53 was highly expressed in BL and had potential to tell BL from other subtypes of B cell lymphoma [26, 27]. These results suggested that proteins that include PTEN, WWP2, p53 and c-Myc were potential biomarkers for discrimination among B cell lymphoma subtypes and RFH, which provided valuable information for differentiative diagnosis and personalized treatment for B cell lymphoma patients. In addition, another interesting result in the present study revealed a negative correlation between PTEN expression and p53 expression in FL group as well as GCB DLBCL groups, also, NF-κB expression negatively correlated with WWP2 expression in GCB DLBCL group but positively associated with PTEN expression in RFH group as well as c-Myc expression in BL group. These findings provid-

ed additional and valuable information for researches on pathological discrimination of B cell lymphomas.

There still remained several limitations in our study: (1) The expression levels of proteins were only detected by IHC, thus, further study using other methods to validate the results was needed. (2) Detailed mechanisms about distinct expression of proteins as well as the protein-protein correlation in different disease subtypes were needed to be further explored in basic experiments. (3) Sample size for each disease subtype was only 30, which was relatively small to present a good statistic power.

In conclusion, PTEN, WWP2, p53 and c-Myc expressions might be served as biomarkers for identification of B cell lymphomas from RFH as well as distinguishing different B cell lymphoma subtypes.

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Disclosure of conflict of interest

None.

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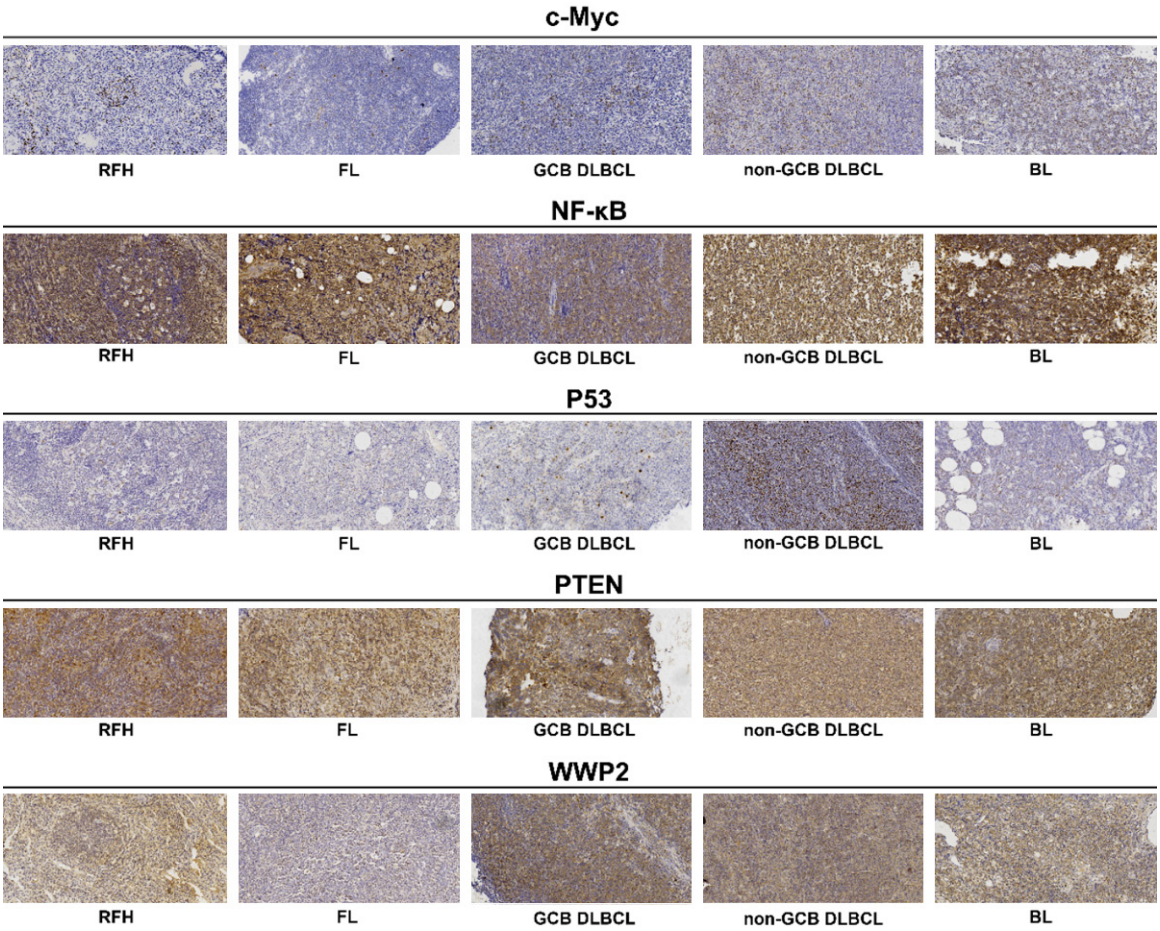

Supplementary Figure 1. Representative IHC images of PTEN, NF-κB, WWP2, P53 and c-Myc in RFH, FL, GCB DLBCL, non-GCB DLBCL and BL groups respectively. RFH, Reactive follicular hyperplasia; FL, follicular lymphoma; GCB-DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; non-GCB DLBCL, non-germinal center B-cell-like diffuse large B-cell lymphoma; BL, Burkitt's lymphoma; PTEN, phosphatase and tensin homolog deleted on chromosome ten; NF-κB, nuclear factor-kappa B; WWP2, WW Domain Containing E3 Ubiquitin Protein Ligase 2; P53, tumor protein p53; c-Myc, proto-oncogene c-Myc.