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Original Article

CD21 and CD23 expression differences in small B-cell lymphomas: comparative analysis in follicular dendritic cells and tumor cells

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Abstract: Objective: Different follicular dendritic cells (FDCs) meshwork patterns were observed in small B-cell lymphomas (SBL) specimens. However, the accurate CD21 and CD23 expression differences in both follicular dendritic cells (FDCs) and tumor cells in SBLs were not sufficiently investigated. Methods: CD21 and CD23 immunostainings were performed on our surgically resected SBL samples to compare their differences in detecting of FDC meshwork immunoarchitectural patterns (FDC patterns), three parameters were introduced for accurate description and comparison of FDC patterns: including the ratio of FDC meshwork outlined area to total tumor area (ROT), staining intensity, and major patterns type (I, expanded follicles with sharp margin; II, contracted follicles with moth-eaten margin; III, ill-defined expanded nodules; IV, absent). The exact expression frequencies of CD21 and CD23 in all SBL groups was also evaluated and compared using double immunohistochemistry (D-IHC). Results: 103 nodal SBL cases, including 32 follicular lymphoma (FL), 28 marginal zone lymphoma (MZL), 23 mantle cell lymphoma (MCL), and 20 chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) cases were examined for more accurate data of FDC pattern parameters, including the mean of ROTs, the percentages of cases exhibiting strongly positive staining and the most common distribution patterns. For CD21 and CD23 they were (46.3% and 39.2%), (81.3% and 78.1%) and (type I, accounting for 93.8% and 81.3%) of all cases respectively in the FL group, (21.4% and 16.8%), (56.5% and 82.6%) and (type III, accounting for 47.8% and 52.2%) in the MCL group, (23.7% and 28.6%), (71.4% and 92.8%) and (type II, accounting for 50% and 67.9%) respectively in the MZL group, and (2.2% and 0.3%), (20% and 5%) and (type IV, accounting for 60% and 95% cases) respectively in the CLL/SLL group. No significant difference was seen in CD21 and CD23 labeling of the FDC pattern parameters. Based on the results of D-IHC, CD21 was detected in 28.1%, 60.9%, 60.7% and 55% of the tumor cells from the FL, MCL, MZL and CLL/SLL samples, respectively, and CD23 was detected in 34.4%, 21.7%, 28.6% and 95% of the respective samples. Conclusion: The most common distribution pattern of FDC patterns was identical between these two markers. Both displayed pattern I in FL, III in MCL, II in MZL and IV in CLL/SLL. The D-IHC results showed that both CD21 and CD23 were expressed in partial cases from each SBL subtype. CD21 expression was much less abundant in FL tumor cells, and CD23 expression was much more abundant in CLL/SLL tumor cells.

Keywords: B cell lymphoma, follicular dendritic cell, biomarker, comparative study, double immunohistochemical staining

Introduction

Small B-cell lymphomas (SBL) is not a specific type of B cell non-Hodgkin lymphoma [1, 2], they are often small in cell size, grow in a diffuse/nodular pattern and all express CD20 [1]. In this study, we focused on the four most common subtypes of SBL: low-grade follicular lymphoma (FL, grade 1/2), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). There are differences in their clinical presentation, pathobiology, prognosis, as well as specific treatment options, in accordance with their heterogeneous origin of neoplastic cells, thus, their accurate classification is important.

Although the classic forms of these entities can be diagnosed by routine histopathologic and

immunophenotypic examination, fairly specific markers existed in differential diagnosis of SBL subtypes, for example, Cyclin D1 in MCL [3, 4], LEF1 in CLL/SLL [5] and germinal center markers in follicular lymphoma [1]. However, accurate diagnosis for several instances of SBLs with borderline histopathology/immuno-phenotypes might still be difficult. Resent works revealed reliable microenvironmental findings including follicular dendritic cells (FDC) patterns could be used as additional markers of differential diagnosis [6-10].

MCL, MZL and FL are follicle-derived lymphomas (FDLs) [9], excluding CLL/SLL. Fakan and our previous study [11, 12] also confirmed that there were significant differences in the distribution of follicular dendritic cell immunoarchitectural patterns (FDC patterns) among types of SBLs on formalin fixed paraffin embedded (FFPE) tissue, which also demonstrated that alterations in FDC patterns provided supplemental assistance in the identification of specific SBL subtypes.

CD3d receptor (CD21) and low affinity IgE receptor (CD23) were two most frequently makers used to reveal FDC patterns with immunohistochemistry [3, 13, 14], for FFPE lymphoid tissues. Troxell's group [15] reported that CD21 was more sensitive than CD23 for labeling FDCs in AITL, and Jin et al. [16] reported that CD23 expression by FDCs was decreased in FL, MCL and AITL relative to healthy controls, but the alterations in CD21 expression, comparison with CD23 expression and CD21 and CD23 expression frequencies on SBL tumor cells were not fully studied, and traditional IHC method would not be sufficient because expression of FDC patterns would be mixed with tumor cells, for example, CD23 expression was seen in 94% of the CLL/SLL [17].

This study would like to present a distinctive new characteristic: it was carried out on SBL samples collected from our department, in an attempt to accurately identify the differences in FDC patterns labeled by CD21 and CD23 in all SBL subtypes. Double immunohistochemistry of CD21/PAX5 and CD23/PAX5 were also performed to determine whether the PAX5-positive SBL tumor cells truly express CD21 and/or CD23 and the exact frequencies. The value to evaluate these staining results in differential diagnosis was further discussed.

Materials and methods

Specimen collection

Excisional specimens diagnosed as SBL were collected at our department from November 2008 to August 2014. The pathological diagnosis was reviewed by at least two hematopathologists according to the WHO 2008 lymphoma classification.

Hematoxylin & eosin staining

Specimens were fixed in 10% neutralized formalin, embedded in paraffin, and cut into 4-μm thick tissue sections. Morphological characteristics were observed via hematoxylin & eosin staining under an Olympus BX43 microscope.

Immunohistochemical staining

IHC for CD21 (EP3093, Abcam, Cambridge, UK) and CD23 (SP23, Thermo Fisher, Rockford, USA) was performed using a BenchMark ULTRA automated IHC/ISH staining instrument (Tucson, VENTANA-Roche, USA) and a ultraVIEW kit (Tucson, VENTANA-Roche, USA). Dilution of CD21 and CD23 in IHC were 1:100.

Double immunohistochemical staining

Double immunohistochemistry (D-IHC) for CD21/PAX5 and CD23/PAX5 was performed according to a method described in the literature [18, 19]. Antibodies against CD21 (EP30-93, Abcam, Cambridge, UK), CD23 (SP23, Thermo Fisher, Rockford, USA) and Pax5 (ZP007, Thermo Fisher, Rockford, USA) were used for D-IHC, the two antibodies were from mouse (PAX5) and rabbit (CD21&CD23), the dilutions of these 3 antibodies were 1:50. The polymer D-IHC chromogen kit (DS201, GBI labs, Bothell, USA) was used to detect two different sources of antibodies in the same tissue sections; the kit contains a goat anti-mouse secondary antibody labeled with horseradish peroxidase or a goat anti-rabbit secondary antibody labeled with alkaline phosphatase. For CD21 and CD23, the labeling was Fast Red (red), and DAB was used for the colorimetric detection of Pax5 (brown). All procedures were performed strictly according to the manufacturers' instructions.

Briefly, heat-induced epitope retrieval was performed by heating slides in EDTA buffer at pH 8.0 using a stainless pressure cooker for 90 seconds, and all antibodies applied were incubated overnight in a moist chamber at 4-8°C.

Settings for experimental controls

Reactive lymph nodes were used as positive controls: In reactive germinal centers (GC), IHC for FDC markers showed sharply dense FDC meshwork by both CD21 and CD23, meshed FDCs of the light zone were much denser than those in the dark zone, which was in accordance with polarization of GC. Signals were located on the cell membrane, in the cytoplasm and dendritic processes at high magnification. The positive D-IHC signals of CD21 and CD23 on FDCs were red, whereas those of PAX5 were brown, as detected in mature B lymphocytes. A negative control was also established for IHC or D-IHC staining (primary antibodies omitted).

Pathological interpretation

The following parameters were assessed for accurate comparisons between those two makers: (1) ratio of FDC meshwork outlined area to total tumor area(ROT): observed the entire slide at low magnification (20 ×); (2) FDC staining intensity, which was categorized as strongly positive (uniform and complete surface staining of FDC as strong as the 'positive control' sample), weakly positive (uneven or moderate surface staining, appeared light brown) or negative (faintly expressed or no reaction); and (3) FDC staining distribution pattern types were classified according to the most evident (>80%) distribution pattern in each sample, they were recorded as: I (abnormal expanded follicles, sharp margin), type II (contracted follicular meshwork, moth-eaten margin), type III (loosely arranged, ill-defined, and irregularly expanded nodules;) or type IV (absent FDC meshwork),

D-IHC interpretation

CD21 and CD23 antibodies were used to examine the membrane and cytoplasm expression in FDCs and tumor cells (Fast Red); PAX5 exhibited nuclear staining in tumor cells (DAB). This approach can assist in confirming that malignant B cells (PAX5-positive cells) also expressed CD21 and/or CD23. CD21 or CD23 was considered positive by the PAX-positive tumor if more than 10% of the cells are positive.

Statistical analysis

SPSS 17.0 statistical software was used to perform χ^2 tests for comparisons of measurement data between two antibodies staining and correlation analysis and to perform *t* tests for comparisons of the mean values of measurement data.

Results

Ultimately, 103 patients were selected. There were 32 cases of FL, 23 cases of MCL, 28 cases of MZL and 20 cases of CLL/SLL. Given that differential pathological diagnosis is usually difficult for SBL located in lymph nodes (here after referred to as "nodal cases"), only nodal cases were included to increase the consistency of the results.

Histological features and tumor growth pattern of the various SBL subtypes

FL: Most FL (Low-grade) samples exhibited follicular distribution pattern (90.6%, 29/32), while 3 of them had additional diffuse areas (10.3%, 3/29); other FL cases showed diffuse tumor growth (9.4%, 3/32). Most of the tumor cells were small centrocytes, which were accompanied by varying amounts of larger, scattered centroblasts.

MCL: These samples typically displayed a diffuse tumor cell distribution (12/23, 52.2%), but a subset of cases exhibited vaguely nodular patterns (9/23, 39.1%), and 1 with mantle zone pattern (nodular growth pattern with residual GCs, 1/23, 4.3%), 1 with diffuse patterns (1/23, 4.3%). Tumor cells were often observed to contain dispersed chromatin, inconspicuous nucleoli, thickened nuclear membrane, and hyalinized small vessels were commonly detected.

Nodal MZL: 39.3% (11/28) cases exhibited nodular pattern, 1 of them was admixed with interfollicular pattern and 1 were partially diffuse pattern; 35.7% (10/28) were found with interfollicular pattern, of which 4 cases were mixed nodular and 2 cases were mixed with diffuse, and "follicular colonization" was observed obviously in 1 case; 7 cases (7/28, 25.0%) showed diffuse growth pattern (2 of which with partial nodular); the tumor cells were also small in size including variable centrocyte-like and monocytoid B cells.

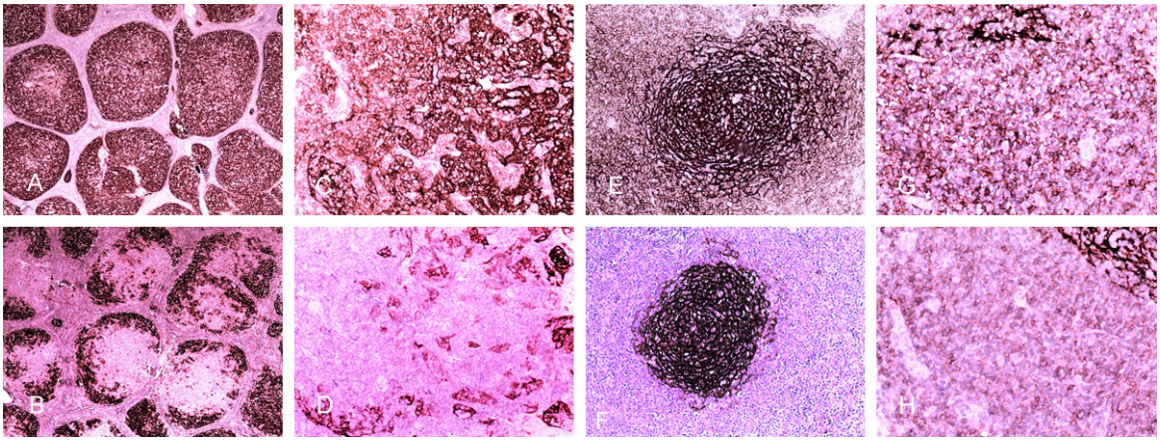

Figure 1. Immunohistochemical staining for follicular dendritic cell immunoarchitectural patterns (FDC patterns) in small B cell lymphoma (SBL) specimens; differences in staining intensity between CD21 and CD23 are shown in slides within a similar region from the same cases. Notes: The same case of follicular lymphoma with a type I distribution pattern (follicular hyperplasia of FDCs) is shown in (A and B), but labeling of the FDC meshwork for CD21 (A) was clearly more abundant than that for CD23 (B). The same case of mantle cell lymphoma with a type III distribution (patchy and clustered FDCs exhibiting irregular hyperplasia) is shown in (C and D), but labeling of the FDC meshwork for CD23 (D) was significantly less abundant than that for CD21 (C). A case of marginal zone lymphoma mainly displaying a type II distribution (erosion of the FDCs) is shown in (E and F). More CD21-positive cells (E) than CD23-positive cells were observed (F), and CD21-positive/CD23-negative expression by MZL tumor cells could be predicted based on morphological analysis. A case of chronic lymphocytic leukemia/small lymphocytic lymphoma is shown in (G and H). This sample displayed a type IV distribution pattern (disappearance of FDCs), and only a few scattered FDCs expressing CD21 (G) and CD23 (H) were detected. Neoplastic cells displaying moderate expression of CD21 (G) and CD23 (H) were identified based on morphological analysis using the *ultra* VIEW staining method at moderate magnification.

CLL/SLL: 50.0% (10/20) cases typically exhibited diffuse infiltration, others (10/20, 50.0%) grew in pseudo-follicular pattern (regularly distributed pale areas corresponding to "proliferation centers"). The small tumor B-cells exhibited round nuclei with small nucleoli. The "proliferation center" usually contained larger cells in a dark background of small cells.

In summary, the tumor morphology of all four SBL subtypes exhibited many common features: Their tumor cells were small in size, 52.4% (54/103) of them could grow in follicle-like pattern (that is, nodular/follicular/pseudo-follicular, and 29 FL, 9 MCL, 9 MZL, 10 CLL/SLL). 29.1% (30/103) of them would have diffuse areas (3 FL, 12 MCL, 5 MZL, 10 CLL/SLL), thus, the differential diagnosis based solely on morphology would be difficult.

Analysis of the IHC staining results in different SBL subtypes

Multi-parameter analysis of FDC patterns by CD21 and CD23 IHC staining in different SBL subtypes

FL: The ROT for CD21 (**Figure 1A**) ranged from 10% to 70%, with a mean value of 46.3%. Approximately 81.3% (26/32) of the samples showed strongly positive CD21 staining, and 93.8% (30/32) of all samples were classified as type I. For CD23 (**Figure 1B**), the ROT ranged from 5% to 80%, with a mean value of 39.2%. Approximately 78.1% (25/32) of the samples showed strongly positive staining; whereas the most common distribution pattern was also type I, which accounted for 81.3% (26/32) of all cases. Compared with CD23, CD21 showed significantly higher staining intensity and greater proportion of type I ($P=0.003$ and $P<0.001$, respectively). Although the ROT was higher for CD21 than for CD23, this difference was not significant ($P=0.051$).

MCL: The ROT for CD21 ranged from 0% to 60% (**Figure 1C**), with a mean value of 21.4%. Approximately 56.5% (13/23) of all cases showed strongly positive CD21 staining. The type III distribution of CD21 was the most common, accounting for 52.2% (12/23) of all cases. The ROT for CD23 ranged from 0% to 60% (**Figure 1D**), with a mean value of 16.8%. Additionally, 82.6% (19/23) of the cases showed strongly positive staining, and CD23 staining primarily exhibited a type III distribution (47.8% of all cases, 11/23). The staining intensity was sig-

Table 1. Main parameters for FDC-IP stained by CD21 and CD23 in small B-cell lymphoma

		Ratio of FDC-IP covered area to tumor area			FDC-IP staining intensity		FDC-IP distribution pattern	
		Range (%)	Mean (%)	*P* value	Strongly Positive (No.)	Strongly positive ratio (%)	Most common type	Number (%)
FL (n=32)	CD21	10%-70%	46.3%	0.051	26	81.3	I	30 (93.8%)
	CD23	5%-80%	39.2%		25	78.1	I	26 (81.3%)
MCL (n=23)	CD21	0%-60%	21.4%	0.128	13	56.5	III	12 (52.2%)
	CD23	0%-60%	16.8%		19	82.6	III	11 (47.8%)
MZL (n=28)	CD21	0%-70%	23.7%	0.213	20	71.4	II	14 (50%)
	CD23	0%-80%	28.6%		26	92.8	II	19 (67.9%)
CLL/SLL (n=20)	CD21	0%-10%	2.5%	0.029	4	20.0	IV	12 (60%)
	CD23	0%-5%	0.3%		1	5.0	IV	19 (95%)

Abbreviations: FDC-IP: follicular dendritic cell immunoarchitectural patterns; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone B-cell lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma; Type I: FDC-IP follicular hyperplasia; Type II: residual FDC-IP eroded peripherally; Type III: FDC-IP with patches and clusters of irregular shape; Type IV FDC-IP disappeared.

Table 2. The relationship between the SBL histopathologic type and the intensity of FDC-IP stained by CD21 or CD23

			FDC-IP staining intensity			
			CD21			
			Negative (%)	Weakly positive (%)	Strongly positive (%)	*P*-value
CD23	FL (n=32)	Negative	0	0	0	0.003
		Weakly positive	0	4 (12.5%)	3 (9.4%)	
		Strongly Positive	0	2 (6.3%)	23 (71.9%)	
	MCL (n=23)	Negative	4 (17.4%)	0	0	0.011
		Weakly positive	0	0	0	
		Strongly Positive	4 (17.4%)	2 (8.7%)	13 (56.5%)	
	MZL (n=28)	Negative	1 (3.6%)	0	1 (3.6%)	0.001
		Weakly positive	0	0	0	
		Strongly Positive	0	7 (25.0%)	19 (67.8%)	
	CLL/SLL (n=20)	Negative	13 (65.0%)	3 (15.0%)	3 (15.0%)	0.122
		Weakly positive	0	0	0	
		Strongly Positive	0	0	1 (5.0%)	

Abbreviations: FDC-IP: follicular dendritic cell immunoarchitectural patterns; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone B cell lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma.

nificantly higher for CD23 than for CD21 (P= 0.011) based on statistical analysis. The ROT and the proportion of samples displaying a type III distribution were higher for CD21 than for CD23, but these differences were not significant (P=0.128 and P=0.182, respectively).

MZL: The ROT for CD21 ranged from 0% to 70% (**Figure 1E**), with a mean value of 23.7%. Approximately 71.4% (20/28) of all samples showed strongly positive staining. The most common distribution of CD21 staining was type II (50% of all cases, 14/28). The ROT for CD23 ranged from 0% to 80% (**Figure 1F**), with a mean value of 28.6%. Strongly positive staining was observed in 92.8% (26/28) of all cases, and the type II distribution of CD23 labeling accounted for 67.9% (16/28) of all cases. Based on statistical analysis, CD23 staining intensity was significantly higher than CD21 (P=0.001). Additionally, the ROT and the proportion of samples displaying a type II distribution were higher for CD23 than for CD21, but these differences were not significant (P=0.213 and P=0.054, respectively).

Table 3. The relationship between the SBL histopathologic type and the FDC distribution pattern labeled by CD21 and CD23

			FDC-IP distribution pattern				
			CD21				
			Type I (%)	Type II (%)	Type III (%)	Type IV (%)	*P*-value
CD23	FL (n=32)	Type I	26 (81.3%)	0	0	0	0.000
		Type II	0	1 (3.1%)	0	0	
		Type III	4 (12.5%)	0	1 (3.1%)	0	
		Type IV	0	0	0	0	
	MCL (n=23)	Type I	0	0	0	0	0.182
		Type II	1 (4.3%)	1 (4.3%)	3 (13.0%)	2 (8.7%)	
		Type III	1 (4.3%)	0	8 (34.8%)	2 (8.7%)	
		Type IV	0	0	1 (4.3%)	4 (17.4%)	
	MZL (n=28)	Type I	0	1 (3.6%)	0	0	0.054
		Type II	5 (17.9%)	11 (39.3%)	2 (7.1%)	1 (3.6%)	
		Type III	0	2 (7.1%)	4 (14.3%)	0	
		Type IV	0	0	1 (3.6%)	1 (3.6%)	
	CLL/SLL (n=20)	Type I	0	0	0	0	0.051
		Type II	0	0	0	0	
		Type III	0	0	1 (5.0%)	0	
		Type IV	4 (20.0%)	0	1 (5.0%)	13 (65.0%)	

Abbreviations: FDC-IP: follicular dendritic cell immunoarchitectural patterns; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone B-cell lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma; Type I: FDC-IP follicular hyperplasia; Type II: residual FDC-IP eroded peripherally; Type III: FDC-IP with patches and clusters of irregular shape; Type IV: FDC-IP disappeared.

CLL/SLL: The ROT for CD21 (**Figure 1G**) ranged from 0% to 10% (mean 2.2%). Strongly positive CD21 staining was detected in 20% (4/20) of all cases. The most common staining distribution was type IV, which accounted for 60% (12/20) of all cases. The ROT for CD23 ranged from 0% to 5% (**Figure 1H**), with a mean value of 0.3%. Strongly positive staining was detected in 5% (1/20) of all cases. The most common distribution of CD23 staining was type IV, accounting for 95% (19/20) of all cases. Based on statistical analysis, the ROT was significantly higher for CD21 than for CD23 (P=0.029).A higher staining intensity and fewer cases displaying a type IV distribution (disappearance of FDC patterns) were observed for CD21 than for CD23, although these differences were not significant (P=0.122 and P=0.051, respectively). All details are presented in **Tables 1**-**3**.

Grading of ROT and Comparison of all parameters in different types of SBL

ROT was further graded as low (<5%), moderate (5-30%) or high (>30%) to facilitate statistical analysis and application to pathological diagnosis. The percentages of ROT scored as "high" were as follows: for CD21 and CD23, 90.6% (29/32) and 68.8% (22/32) in FL, 28.1% (9/23) and 28.1% (9/23) in MCL, 39.3% (11/28) and 46.4% (13/28) in MZL, and 5% (1/20) and 5% (1/20) in CLL/SLL, respectively.

Further statistical analysis revealed significant differences (all $P<0.001$) for all examined parameters of CD21 and CD23 staining, including the ROT classification, the staining intensity grade, and the distribution pattern classification, among all four pathological types of SBL (all with *P* value <0.01, **Table 4**).

Comparison of CD21 and CD23 expression in SBL tumor cells based on D-IHC

D-IHC staining for CD21/PAX5 and CD23/PAX5 was successfully performed, and the analyses of these results were combined with the morphological and classical IHC staining results. The percentages of CD21-positive tumor cells were as follows: FL (28.1%), MCL (60.9%, see **Figure 2**), MZL (60.7%), and CLL/SLL (55%, **Figure 2A** and **2B**). The percentages of CD23-

Table 4. Differences among the different pathological types of small B-cell lymphomas with respect to the characteristic parameters of the FDC-IP revealed by immunohistochemical staining with CD21 and CD23

		FDC-IPcovered area to tumor area				FDC-IP staining intensity				FDC-IP distribution pattern				
		Low (≤5%)	Moderate (5%-30%)	High (≥30%)	*P*-value	Negative	Weakly positive	Strongly positive	*P*-value	Type I	Type II	Type III	Type IV	*P*-value
CD21	FL (n=32)	0	3 (9.4%)	29 (90.6%)	<0.001	0	6 (18.8%)	26 (81.3%)	<0.001	30 (93.8%)	1 (3.1%)	1 (3.1%)	0 (%)	<0.001
	MCL (n=23)	10 (43.5%)	4 (17.4%)	9 (39.1%)		8 (34.8%)	2 (8.7%)	13 (56.5%)		2 (8.7%)	1 (8.7%)	12 (52.2%)	8 (34.8%)	
	MZL (n=28)	3 (10.7%)	14 (50.0%)	11 (39.3%)		1 (3.6%)	7 (25.0%)	20 (71.4%)		5 (17.9%)	14 (50.0%)	7 (25.0%)	2 (7.1%)	
	CLL/SLL (n=20)	17 (85.0%)	3 (15.0%)	0		13 (65.0%)	3 (15.0%)	4 (20.0%)		4 (20.0%)	0	3 (15.0%)	12 (60.0%)	
CD23	FL (n=32)	1 (3.1%)	9 (28.1%)	22 (68.8%)	<0.001	0	7 (21.9%)	25 (78.1%)	<0.001	26 (81.3%)	1 (3.1%)	5 (15.6%)	0	<0.001
	MCL (n=23)	10 (43.5%)	4 (17.4%)	9 (39.1%)		4 (17.4%)	0	19 (82.6%)		0	7 (30.4%)	11 (47.8%)	5 (21.7%)	
	MZL (n=28)	3 (10.7%)	12 (42.9%)	13 (46.4%)		2 (7.1%)	0	26 (92.9%)		1 (3.6%)	19 (67.9%)	6 (21.4%)	2 (7.1%)	
	CLL/SLL (n=20)	20 (100.0%)	0	0		19 (95.0%)	0	1 (5.0%)		0	0	1 (5.0%)	19 (95.0%)	

Abbreviations: FDC-IP: follicular dendritic cell immunoarchitectural patterns; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone B-cell lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma; Type I: FDC-IP follicular hyperplasia); Type II: residual FDC-IP eroded peripherally; Type III: FDC-IP with patches and clusters of irregular shape; Type IV: FDC-IP disappeared.

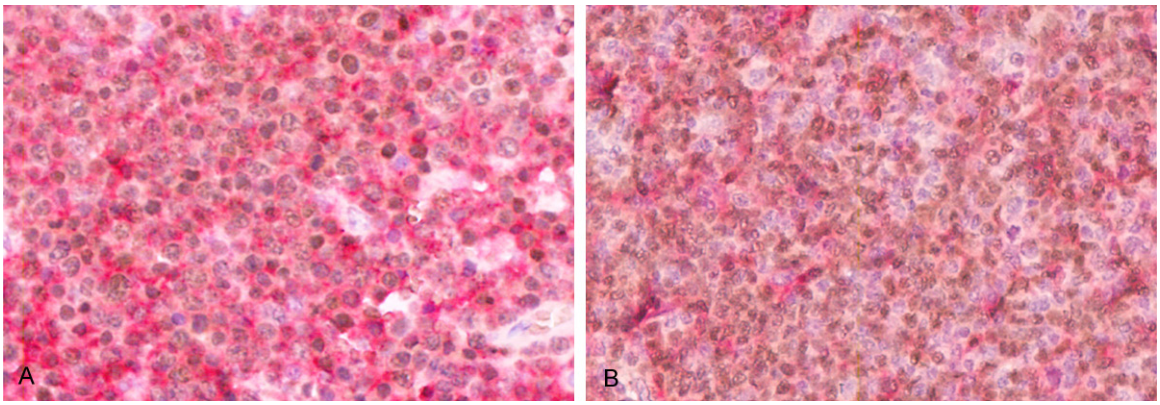

Figure 2. The evaluation of CD21 expression by tumor cells via double immunohistochemical staining of SBL samples. Nuclear expression of PAX5 (brown) indicates in mantle cell lymphoma cells in A. CD21 (red) expression in tumor cell membrane cytoplasm was also detected. While in B, nuclear expression of PAX5 (brown) highlights tumor cells in a sample from one follicular lymphoma without CD21 expression, however memberous and cytoplasmic CD21 (red) expressed in the PAX5-negative follicular dendritic cells, signals were detected using an insitu polymer double-staining method at high magnification.

positive tumor cells were as follows: FL (34.4%), MCL (21.7%), MZL (28.6%), and CLL/SLL (95%). Statistical analysis showed that the percentages of SBL tumor cells expressing CD21 and CD23 were significantly different between all pathological subtypes ($P=0.034$ and $P<0.001$, respectively, **Table 5**).

Discussion

FDCs are physiologically confined to the GC of lymphoid follicles and they were generally believed to be derived from mesenchymal stem cells in bone marrow [20-22]. FDCs are important antigen-presenting cells that stimulate the activation of “T helper cells” and “B effector cells” to mediate humoral immune responses [21, 22]. In addition, FDCs directly influence the selection, differentiation and proliferation of B cells [10, 15, 23].

The interaction of FDCs with GC-B cells were mediated by Fc fragment-binding proteins (CD23, CD32), complement receptors (CD21 and CD35) and binding factors (CD54, CD106 and CD44) that form an immune complex [23]. Due to the interaction between FDCs and B cells, the FDC meshwork in the GC could also be divided into a “light zone” and a “dark zone”. FDCs in the “light zone” primarily involved in presenting immune complexes and stimulating B cell-selective differentiation, which induce the B cells to produce a high-affinity immunoglobulin directed against the antigen presented by FDCs. Therefore, in the FDC light zone, strong expression of Fc fragment-binding proteins (such as CD23 and CD32) and complement receptors (CD21 and CD35) can be observed. The FDCs in the “dark zone” express CD21 and CD23 at low levels, and it was suggested that the main function of FDCs in the dark zone was not to present immune complexes but rather to promote the selection and differentiation of B cells, which may be related to the stimulation of B cells [8, 15, 23].

FDC meshwork can be detected in reactive and neoplastic lymphoid tissues [3, 6, 10], including certain types of presumed FDLs, including nodular lymphocyte predominant Hodgkin lymphoma and several B cell lymphomas, such as FL, MCL, and MZL, as well as angioimmunoblastic T cell lymphoma (AITL) [7, 9, 10]. The degree of proliferation and the distribution pattern of FDCs vary among these lymphomas based on IHC staining for FDC-related markers [7, 10, 15]. The most common distribution of FDC patterns in FL, MCL, MZL, and CLL/SLL in our study were type I, type III, type II, and type IV, respectively. This FDC patterns was reproducible and were consistent with by previous findings [10-12].

Our characteristic results were the systemic comparison between CD21 staining and CD23 staining, which revealed that in FL, the percentage of samples displaying “strongly positive” staining and a “type I” pattern were significant-

Table 5. Comparison of the expression of CD21 and CD23 on SBL tumor cells and their relationships with histopathologic types (confirmed by CD21/PAX5 and CD23/PAX5 double immunohistochemical staining)

Expression in tumor cells				CD21			*P*-value
		CD23 total positive rate		Negative (No.)	Positive (No.)	CD21 Total positive rate	
CD23	FL (n=32)	34.4%	Negative (No.)	17 (53.1%)	4 (12.5%)	28.1%	0.115
			Positive (No.)	6 (18.8%)	5 (15.6%)		
	MCL (n=23)	21.7%	Negative (No.)	7 (30.4%)	11 (47.8%)	60.9%	0.964
			Positive (No.)	2 (8.7%)	3 (13.0%)		
	MZL (n=28)	28.6%	Negative (No.)	1 (3.6%)	10 (35.7%)	60.7%	0.066
			Positive (No.)	1 (3.6%)	7 (25.0%)		
	CLL/SLL (n=20)	95%	Negative (No.)	1 (5.0%)	0	55.0%	0.257
			Positive (No.)	8 (40.0%)	11 (55.0%)		

Abbreviations: FDC-IP: Follicular dendritic cell immunoarchitectural patterns; FL: follicular lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone B cell lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma.

ly higher for CD21 than for CD23. According to the literature, CD23 antigen expression by FDCs is believed to decrease in FL [16], so when FDC pattern was applied as an implement differentiating method for FL, CD21 staining might be more helpful. Similar to AITL, FL induces FDC hyperplasia in a follicular pattern because of the activities of malignant B cells from the GC [15, 16], and FL was associated with a clear decrease in the intensity of CD23 staining. In contrast to the reactive GC, centrocytes and centroblasts in FLs were randomly distributed, resulting in the loss of the "polarity" of the type I "follicular" meshwork.

In MCL, The staining intensity for CD23 by FDC meshwork was significantly stronger, and it might be more helpful in immunostaining for FDCs in the differential diagnosis. In MCL samples, Type III (nodular meshwork with irregular margin) meshwork was often observed. The characteristic hyalinized vascular proliferation commonly detected may stimulate FDC hyperplasia in MCL, although further study is needed to corroborate this hypothesis. In MZL, The staining intensity for FDC meshwork by CD23 staining was also significantly stronger, so it might be more helpful to utilize CD23 staining FDC pattern (contracted follicles with less evident margin) in assisting MZL differential diagnosis [11, 12, 24].

In CLL/SLL, the ROT for CD21 was significantly higher than for CD23, but the mean ROT were both <5%. The above results further support the inhibition and atrophy of FDCs in CLL/SLL, this finding was consistent with recent report that CLL/SLL was not follicle-derived B cell, and this characteristic "absence" pattern could also be used for SBL differential diagnosis.

Overall, there were sensitivity differences between CD21 and CD23 staining in FDCs in each type SBLs, however, no definitive advantage was identified. It seemed that CD21 was a more suitable marker of FDCs in FL, and CD23 appeared to be a more sensitive in MZL and MCL. The decrease in expression of CD21/CD23 might be similar to the function of FDCs in the dark area of the GC, which might involve the stimulation of malignant B cell proliferation [23].

The differential diagnostic value of FDC patterns in SBL subtypes would also be confirmed by analysis of more cases and by further investigations, in the hope of our previous conclusions would be enriched. The ROT and the staining intensity of FDC markers maybe useful as new indices in a helpful way in differential diagnosis of SBL subtypes, especially when other key makers on tumors were atypical or ambiguous.

For both CD21 and CD23 staining, the graded ROT of FDCs significantly decreased in the following sequence: FL>MZL>MCL>CLL/SLL. In FL, FDC hyperplasia was most evident and the tumor cells were most closely associated with the follicles. In CLL/SLL, the proliferation of

FDCs was suppressed or even lost, and these results confirmed that CLL/SLL is generally not a form of FDL [9].

The percentage of samples displaying strongly positive for CD21 labeling in FDCs significantly differed in the following order: FL>MZL>MCL> CLL/SLL; but for CD23, the order was MZL> MCL>FL>CLL/SLL. This discrepancy may be related to differences in the levels of CD21 and CD23 expression between the SBL subtypes. Moreover, these results confirmed that CD21 for FDCs was much more sensitive in the FL group (ROT and staining intensity).

Although due to variant histologic patterns as stated in results part 1, the utility in quantifying ROT metric or staining intensity of the meshwork alone seemed not really helpful in diagnosis of small B-cell lymphomas, and it would be much better to make the diagnosis of a lymphoma with FDC meshwork pattern based on other specific tumor makers into making a diagnosis.

It was reported that both CD21 and CD23 can also be expressed in mature B cells and mature B cell lymphomas [6, 14], and CD23 expression in CLL/SLL has been used as an important diagnostic tumoral indicator [3, 13]. D-IHC staining for CD21/PAX5 and CD23/PAX5 were successfully performed to confirm and systemically study the CD21 and CD23 expression in SBL tumor cells. The frequency of positive CD21 expression in tumor specimens in each pathological SBL subtype was as follows: MCL> MZL>CLL/SLL>FL; for CD23 expression, this order was CLL/SLL>FL>MZL>MCL. Significant differences in the expression of CD21 and CD23 were observed among these histological SBL subtypes. However, only CD23 expression frequency in CLL/SLL was obviously higher (>90%) as reported recently [3, 17], the CD23 positivity frequency was 21-34% in the other SBL tumor types. The frequency of CD21 expression in FL (approximately 28%) was much lower than the other types (ranged from 50 to 61%).

The finding that the FDC markers CD21 and CD23 can be expressed in all types of SBL tumor cells is important, which could avoid mistakenly assuming that a tumor cell was non-specifically stained or that the antibodies were non-specific and may avoid the confusion of tumor-associated FDC marker expression with FDC cell proliferation, leading to misdiagnosis. In addition, more than two-thirds of FL tumor cells do not express CD21, and FL tumors often proliferate mainly in the central of neoplastic follicles. This occupying effect made the FDC patterns appear loosely arranged, partially explaining the common phenomenon of "central destruction" as we had observed in FDC immunostaining via CD21 antibodies [12].

Conclusion

The most common distribution patterns of FDC patterns in our groups for FL, MCL MZL and CLL/SLL were type I, type III, type II and type IV, respectively. Our characteristic findings were: differences existed between CD21 and CD23 labeling of FDC pattern in all four SBL types. CD21 is likely to be more suitable for marking FDC in FL, but CD23 might be more sensitive for meshwork staining in MCL and MZL. CD21 and CD23 can be expressed in tumor cells of all SBL subtypes with significant difference among each type. The expression of CD21 in FL tumor cells was especially low, and the expression of CD23 in CLL/SLL tumor cells was extraordinarily high. The results of this study would provide supplementary information on using FDC patterns as one of the SBL differential diagnosis tools. However, further investigation of FDCs on its latent mechanism in promoting SBL and its prognostic role would still be needed.

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Disclosure of conflict of interest

None.

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