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ORIGINAL ARTICLE

Pattern of MEF2B expression in lymphoid tissues and in malignant lymphomas

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Abstract Myocyte enhancer binding factor 2 B (MEF2B) is a member of the evolutionary conserved transcription family MEF2. MEF2B has been shown to directly control biological activity of the B cell lymphoma 6 (*BCL6*) gene in germinal center (GC) B cells. To validate MEF2B as an immunohistochemical marker, we studied a large consecutive series of hyperplastic lymphoid tissues ($n = 38$) and malignant lymphoproliferative conditions ($n = 471$), including all major categories of B and T cell neoplasms. In hyperplastic lymphoid tissues, MEF2B staining revealed intense and crisp nuclear expression confined to GC B cells. Unlike BCL6, MEF2B was not detected in follicular T cells. In addition, weak nuclear staining of plasma cells was noted. MEF2B staining labeled neoplastic cells of follicular lymphoma both in common and variant cases as well as in bone marrow biopsies with high sensitivity, while it was almost consistently negative in marginal zone lymphoma. Consistent MEF2B expression was found in Burkitt lymphoma and nodular lymphocyte predominant Hodgkin lymphoma as well as in the large majority of cases of mantle cell lymphoma and diffuse large cell B cell lymphoma. MEF2B protein expression showed a statistically significant association with that of BCL6 in cases of diffuse large B cell lymphoma, not otherwise specified. We conclude that MEF2B is a valuable marker of normal GC B cells, potentially useful in differential diagnosis of small B cell lymphomas.

Keywords MEF2B protein expression · Germinal center B cells · Germinal center B cell-derived lymphomas

Introduction

Myocyte enhancer binding factor 2 (MEF2) proteins represent an evolutionary conserved transcription factor family, encoded by four different MEF2 genes: MEF2A, MEF2B, MEF2C, and MEF2D [1–4].

These factors share a high degree of homology in their amino(N)-terminal region characterized by two conserved motifs, the evolutionarily ancient MADS (MCM1-agamous-deficiens-serum response factor) box which mediates dimerization and DNA binding, and an adjacent MEF2 domain which also influences dimerization [1, 3, 4]. MEF2 proteins have been found to play a central role in the activation of genetic programs that control cell differentiation, proliferation, survival, apoptosis, and epigenetic regulatory mechanisms through interactions with various coactivators and corepressors, in response to a wide variety of extracellular signaling pathways of different cell types [4–8]. Several reports claimed that MEF2 proteins have important regulatory functions in essential physiological processes of T and B lymphocytes, including development, proliferation, and apoptosis [7, 9–12]. MEF2B is highly homologous to the other MEF2 factors within the MADS and MEF2 domains, whereas the region C-terminal to the MEF2 domain displays relatively little homology to the other vertebrate MEF2 factors [13]. Most recently, MEF2B was found to directly control biological activity of the B cell lymphoma 6 (*BCL6*) gene and have an

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expression pattern similar to that of BCL6 protein in normal germinal center (GC) B cells [14].

We undertook the present study to validate MEF2B as a possible diagnostic immunohistochemical (IHC) marker, on a large series of non-neoplastic and neoplastic lymphoid tissue, with particular emphasis on GC B cell-derived lymphomas.

Materials and methods

Tissue samples

Cases were selected from the Laboratory of Tumor Pathology and Molecular Diagnostics, Szeged, Hungary. To study MEF2B protein expression in non-neoplastic tissues, samples from hyperplastic lymph node (n = 15), chronic tonsillitis (n = 12), spleen (traumatic rupture) (n = 2), chronic gastritis (n = 3), bone marrow (BM) (n = 2), autoimmune sialadenitis (n = 2), and cutaneous B cell hyperplasia (n = 2) were included in this study. To characterize MEF2B protein expression in lymphoid neoplasms, 471 malignant lymphoma cases were evaluated including follicular lymphoma (FL), mantle cell lymphoma (MCL), nodal and extranodal marginal zone lymphoma (MZL), splenic marginal zone lymphoma (SMZL), plasma cell neoplasm, Waldenström's macroglobulinemia (WM), B cell chronic lymphoid leukemia (B-CLL), hairy cell leukemia (HCL), diffuse large B cell lymphoma (DLBCL), Burkitt lymphoma (BL), B lymphoblastic lymphoma/leukemia (B-LBL), lymphocyte predominant Hodgkin's lymphoma (LPHL), classical Hodgkin's lymphoma (cHL), and peripheral T cell lymphoma (PTCL) as listed in Table 1. Each case was diagnosed and classified according to the 2008 WHO classification for lymphomas [15]. Inclusion criteria were the availability of representative paraffin blocks or adequate unstained slides for additional IHC analysis.

BM trephine biopsies were fixed for 12–24 h in neutral buffered formalin supplemented with methanol and glucose (Schaffer's fixative), decalcified in 12.5 % (w/v) EDTA solution (adjusted to pH 7.0 by cc NaOH) at 60 °C for 16–24 h. The remaining samples were fixed for 24–72 h in 10 % (v/v) neutral buffered formalin. Each tissue specimen was routinely embedded in paraffin.

Table 1 Lymphoma subtypes and MEF2B protein expression

Lymphoma subtypes	Total positive	
B cell lymphoma; n = 393		
Follicular lymphoma	61/61	100 %
Grade 1	17/17	100 %
Grade 2	32/32	100 %
Grade 3	12/12	100 %
Mantle cell lymphoma	34/42	81 %
Marginal zone lymphoma	2/58	3 %
Nodal	0/13	0 %
Extranodal	2/27	7 %
Splenic	0/18	0 %
Plasma cell neoplasms	12/25	48 %
Extraosseal plasmacytoma	5/5	100 %
Multiple myeloma	7/20	35 %
Waldenström's macroglobulinemia	1/7	14 %
B cell chronic lymphoid leukemia	0/28	0 %
Hairy cell leukemia	0/15	0 %
Diffuse large B cell lymphoma	113/143	79 %
Diffuse large B cell lymphoma + follicular lymphoma grade 3	6/6	100 %
Primary mediastinal large B cell lymphoma	10/10	100 %
T cell/histiocyte-rich large B cell lymphoma	7/9	78 %
de novo CD5+ diffuse large B cell lymphoma	2/6	33 %
EBV+ diffuse large B cell lymphoma of the erderly	0/2	0 %
Plasmablastic lymphoma	3/3	100 %
Diffuse large B cell lymphoma, not otherwise specified	81/100	81 %
Gray zone of Burkitt/diffuse large B cell lymphoma	3/3	100 %
Gray zone of classical Hodgkin/diffuse large B cell lymphoma	1/4	25 %
Burkitt lymphoma	7/7	100 %
B lymphoblastic lymphoma/leukemia	0/7	0 %
Hodgkin lymphoma n = 37		
Lymphocyte predominant Hodgkin lymphoma	6/6	100 %
Classical Hodgkin lymhoma	1/31	0 %
T cell lymphoma; n = 41		
Angioimmunblastic T cell lymphoma	0/16	0 %
Enteropathy-associated T cell lymphoma	0/3	0 %
Anaplastic large cell lymphoma	0/4	0 %
Primary cutaneous CD4+ small/medium T cell lymphoma	0/8	0 %
Peripherial T cell lymphoma, not otherwise specified	0/9	0 %
Nasal T/NK cell lymphoma	0/1	0 %

Tissue microarray construction

We constructed tissue microarray (TMA) blocks with a Tissue Micro-Array Builder instrument (Histopathology Ltd., Pécs, Hungary), according to the manufacturer's instructions. Briefly, the recipient paraffin block with 24 holes arranged in four columns and six rows was formed. Cores of 2.0 mm from the donor paraffin blocks were punched out with the Paraffin-Punch-Extractor and were arrayed in the recipient paraffin block. Each sample was represented at least in duplicate to avoid inadequate sampling.

Immunohistochemistry and evaluation of the reactions

IHC reactions were executed either on whole tissue sections or on TMA slides. Briefly, 2-μm paraffin sections were routinely dewaxed, endogenous peroxidase activity was blocked in ethanol containing 1.5 % (*v*/*v*) H_2O_2, and antigen retrieval was performed by heating in appropriate buffer (Table 2) using a household electric pressure cooker. After protein blocking in 50 mM TRIS-buffered saline (TBS pH 7.4) containing 5 % (*w*/*v*) low-fat milk powder, the sections were incubated with primary antibody (Table 2) at room temperature for 70 min. Detection was performed using Novolink polymer kit (Leica Biosystems/Novocastra, Newcastle Upon Tyne, UK), and nuclear staining was carried out with Mayer's hematoxylin. For primary goat antibodies, a rabbit anti-goat linker antibody (DAKO, Glostrup, Denmark) was used. IHC staining was executed in a 4-channel TECAN Freedom Evo liquid handling platform (TECAN, Mannedorf, Switzerland).

A tumor sample was considered to be MEF2B-positive if at least 50 % of the apparent lesional/neoplastic cells showed nuclear staining of any intensity.

Samples were independently assessed by two of the authors (DK and LK). In cases with discordant result, consensus was reached by a second look evaluation made jointly.

MEF2B antibodies

In a pilot study, we tested two different anti-MEF2B antibodies: a polyclonal rabbit antibody raised against a 141aa recombinant fragment mapping to the internal region of the human MEF2B protein (HPA004734; Atlas Antibodies, Stockholm, Sweden, validated in the Human Protein Atlas http://www.proteinatlas.org/ENSG00000213999-MEF2B/antibody) and a polyclonal goat antibody specific for an epitope mapping near the N-terminus of human MEF2B (T-17, sc-30243; Santa Cruz Biotechnology, Dallas, TX, USA). Although the antibodies showed a similar staining pattern of GC B cells, the N-terminal-specific antibody demonstrated widespread nuclear staining of stromal cells, including follicular dendritic cells and epithelioid histiocytes as well as stronger staining of plasma cells. We therefore chose the internal region-specific antibody for the study.

Table 2 Antibodies and conditions used in the study

Antibody/Clone	Source	Origin	Dilution
MEF2B/polyclonal[a]	Sigma/Atlas	Rabbit	1:500
MEF2B/polyclonal[a]	Santa Cruz	Goat	1:2000
BCL2/100/D5[b]	Leica/Novocastra	Mouse	1:100
BCL6/LN22[a]	Leica/Novocastra	Mouse	1:100
BCL6/polyclonal[a]	Santa Cruz	Rabbit	1:500
CD10/56C6[a]	Leica/Novocastra	Mouse	1:300
CD20/polyclonal[b]	Thremo Scientific	Rabbit	1:1500
CD21/2G9[a]	Leica/Novocastra	Mouse	1:100
CD23/1B12[b]	Leica/Novocastra	Mouse	1:100
CD38/SPC32[a]	Leica/Novocastra	Mouse	1:2000
Cyclin D1/EPR241[a]	BioGenex	Rabbit	1:200
MUM1/polyclonal[b]	Santa Cruz	Goat	1:1000
PD1/NAT105C[a]	G. Roncador[c]	Mouse	1:10

[a] Antigen retrieval in 10 mM TRIS buffer (0.05 % Tween-20, pH 10.0)

[b] Antigen retrieval in 10 mM Sodium citrate buffer (0.05 % Tween-20, pH 6.0)

[c] Monoclonal antibodies Core Unit, Spanish National Cancer Centre, Madrid, Spain

Sequential double immunostaining

Sequential double immunostaining was performed on TMA slides containing hyperplastic tonsils. Briefly, paraffin sections were cut, pretreated, and immunostained with the internal region-specific MEF2B antibody or with a polyclonal rabbit BCL6 antibody (N-3, sc-858, Santa Cruz), as outlined above. Subsequently, the slides were incubated with monoclonal mouse anti-PD1 antibody (NAT105C, kindly provided by Giovanna Roncador, Monoclonal antibodies Core Unit, Spanish National Cancer Centre, Madrid, Spain) which was detected with an anti-mouse alkaline phosphatase micropolymer and AP-Red chromogen (both from Histopathology Ltd., Pécs, Hungary).

Statistical analysis

Mann-Whitney *U* test was used to correlate the prevalence of expression of the MEF2B protein in the phenotypic subgroups of DLBCL not otherwise specified (NOS) subtype, according to the algorithm used by Hans et al. (i.e., GCB or non-GCB type) [16]. Spearman's Rho test was used to determine the ranked correlation of the expression of MEF2B and BCL6 in DLBCL NOS cases.

Results

MEF2B in reactive lymphoid hyperplasia

In hyperplastic nodal and extranodal lymphoid tissues, intense and crisp nuclear staining of GC B cells for MEF2B was observed, whereas mantle zone, marginal zone, and monocytoid B cells consistently lacked MEF2B expression (Fig. 1a, b). MEF2B-positive lymphocytes were not observed in paracortical, interfollicular, and medullary areas, with the exception of plasma cells which showed weak nuclear staining (Fig. 1b). In addition, weak to moderate staining of endothelial cells, pericytes, and some stromal cells was observed. The anti-BCL6 antibody stained reactive GC B cells in a

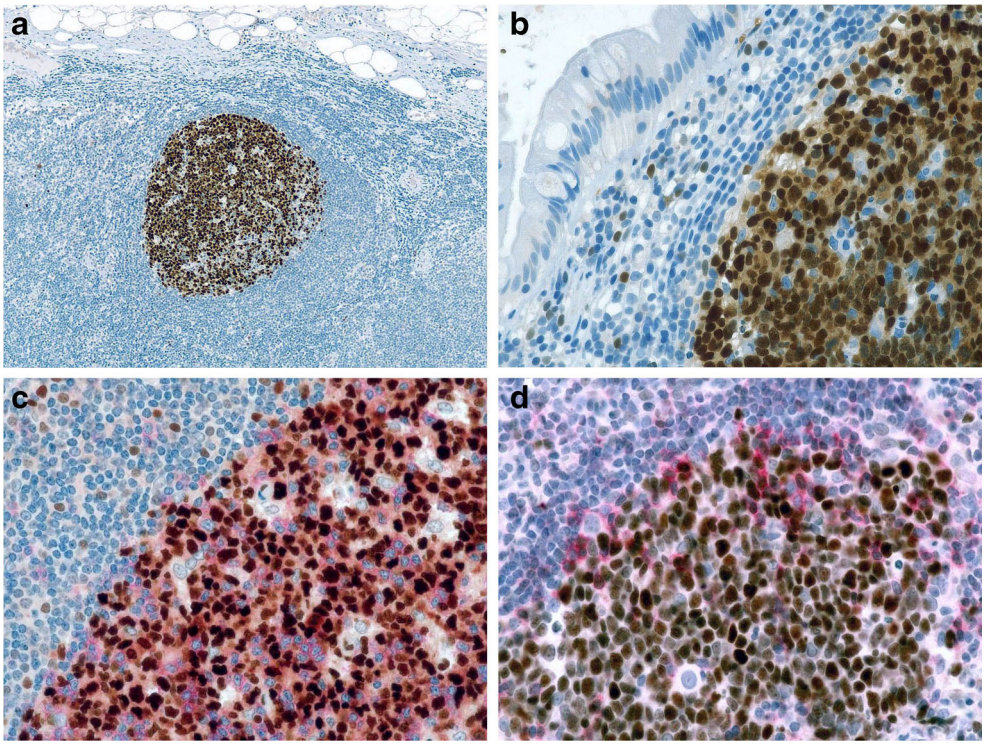

Fig. 1 Immunohistochemical staining pattern of MEF2B in hyperplastic lymphoid tissues. **a** Germinal center (GC) B cells are highlighted by MEF2B staining in a hyperplastic lymph node. Mantle zone and interfollicular cells lack staining. **b** Strongly MEF2B-positive GC B cells and scattered weakly stained subepithelial plasma cells are demonstrated in the normal ileal mucosa. **c** Double immunohistochemical labeling for MEF2B (*brown nuclear stain*) and PD1 (*red membrane stain*) in normal tonsil shows that PD1-positive follicular T (Tfh) cells are MEF2B negative. **d** In contrast, double staining for BCL6 (*brown nuclear stain*) and PD1 (*red membrane stain*) shows that PD1-positive Tfh cells are BCL6 positive. Original magnifications: **a** ×100; **b**–**d** ×400

similar pattern, but also a proportion of mantle zone cells and scattered interfollicular lymphoid cells.

In BM trephine biopsies, weak nuclear MEF2B staining of plasma cells as well as cytoplasmic staining of erythropoietic precursors and megakaryocytes was noted. In hyperplastic tonsils, double staining for BCL6/PD1 and MEF2B/PD1 revealed expression of BCL6 in both GC B cells and follicular T cells (Tfh), while MEF2B was expressed selectively in GC B cells but not in Tfh cells (Fig. 1c, d).

MEF2B in B cell neoplasms

Of the 61 FL cases, 49 were low grade (17 grade 1; 32 grade 2) and 12 were grade 3 (9 grade 3A and 3 grade 3B). All FL were MEF2B-positive, including all CD10-negative (17/61) and BCL2-negative cases (7/61), with centrocytes and centroblasts in the follicular compartment invariably showing crisp and intense nuclear staining, while MEF2B staining in the interfollicular compartment was weaker and more heterogeneous (Fig. 2). All FL cases with either predominantly diffuse or diffuse growth pattern (Table 3) displayed moderate to strong MEF2B staining (Fig. 3). No difference in MEF2B staining pattern was noticed between nodal and extranodal FL cases. The staining pattern for MEF2B and BCL6 showed overlapping features in samples other than BM trephines (Figs. 2, 3).

Twenty-two BM trephine biopsies with FL involvement were studied for MEF2B expression (Table 4). All but four trephines (18/22, 81 %) were distinctly MEF2B positive, while a notably lower proportion of cases stained for BCL6 (12/22, 55 %) and CD10 (8/22, 37 %) (Fig. 4).

In MCL, 81 % (34/42) of the cases expressed MEF2B but with variable staining intensity (Fig. 5a, b). Of the 7 cases of classical gastric MCL (43 %) and of 8 cases of blastoid nodal MCL (50 %), 3 and 4 were negative, respectively.

In the setting of MZL (i.e., nodal, extranodal, and splenic) cases, in the large majority of cases (55 of 58, 97 %), neoplastic cells did not stain for MEF2B, while plasma cells and residual GC B cells were positive. Staining of plasma cells was usually rather faint and heterogeneous, while GC B cells in residual follicles or intermingled with MZL cells in colonized follicles showed distinct strong MEF2B expression. In two MZL cases (one from parotid and one from thyroid), neoplastic cells with plasmacytic differentiation were weakly MEF2B positive in numbers exceeding the cutoff value (Fig. 5c, d). Two additional MZL cases with plasmacytic differentiation (30–80 % of the neoplastic cells) were entirely MEF2B negative.

All 5 extraosseal plasmacytomas (EP) and 7 of 20 (35 %) multiple myelomas (MM) were MEF2B positive. Expression in EP was strong and mostly homogenous, while staining in MM tended to be weak and heterogeneous. The plasmacytic component of 1 of the 7 (14 %) WM cases was weakly MEF2B positive.

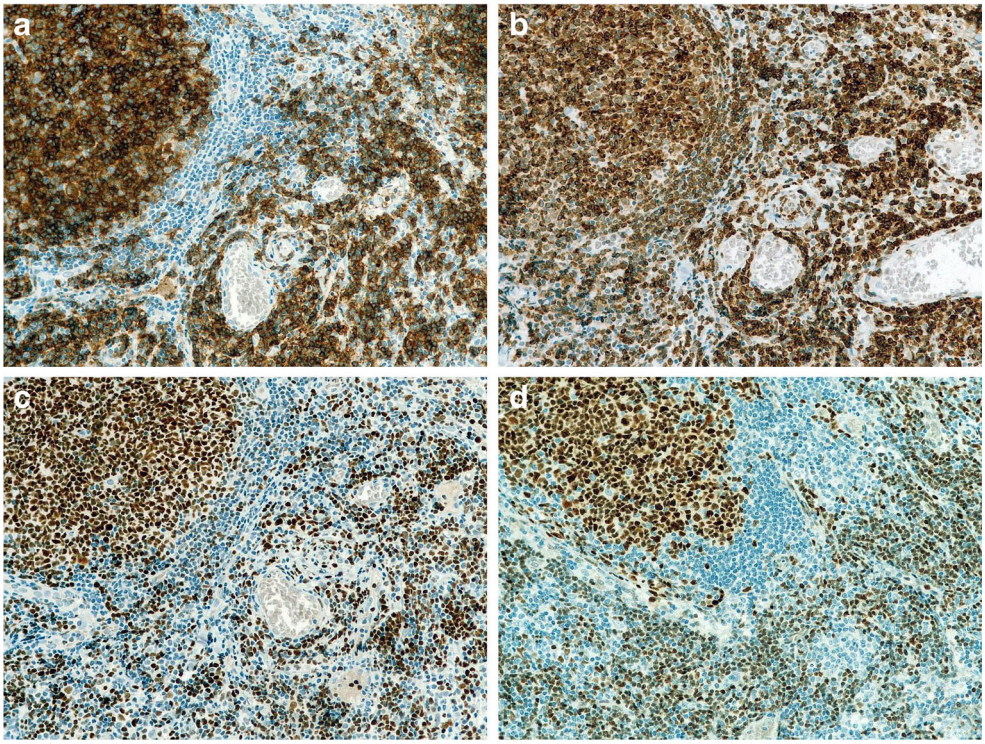

Fig. 2 MEF2B expression in classical follicular lymphoma. A neoplastic follicle (*upper left*) with partly preserved mantle zone shows coexpression of CD10 (**a**), BCL2 (**b**), BCL6 (**c**), and MEF2B (**d**). Each marker, including MEF2B, stains interfollicular neoplastic cells (*lower left* and *right*). Original magnifications: **a**–**d** ×200

MEF2B staining was positive in 113 of 143 (79 %) of DLBCL cases (Table 1), in the majority (90 of 113, 80 %) strongly and homogenously. Of 100 DLBCL NOS cases, 81 (81 %) were MEF2B positive. Of DLBCL NOS cases, 77 could be classified according to Hans et al. [16], with MEF2B expression in 24 of 28 cases (86 %) classified as GCB and in 37 of 49 (76 %) cases classified as non-GCB (Fig. 6). The correlation between MEF2B expression and DLBCL phenotype was not statistically significant (Mann-Whitney *U* test: *Z* score −0.736, $P = 0.4593$). MEF2B and BCL6 expression in DLBCL NOS was statistically significantly correlated (Spearman's Rho *R* value 0.39684; *P* value 0.00035). In 6 cases, FL grade 3 was found in combination with DLBCL and both components showed strong homogenous MEF2B staining. All 10 cases of primary mediastinal DLBCL, 7 of 9 (78 %) T cell/histiocyte rich large B cell lymphomas, 2 of 6 (33 %) de novo CD5-positive DLBCL, 3 of 3 (100 %) plasmablastic lymphomas, none of 2 EBV-positive diffuse large B cell lymphomas of the elderly, 1 of 4 (25 %) cHL/DLBCL, and all 3 BL/DLBCL gray zone lymphomas were MEF2B positive.

All 7 classical BL showed moderate to marked nuclear MEF2B staining (Fig. 7a, b). No other B cell neoplasm, including 28 B-CLL, 15 HCL/v, and 7 B-LBL, expressed MEF2B.

MEF2B in Hodgkin lymphoma

Expression of MEF2B was marked in all 6 NLPHL cases, confined to popcorn cells (Fig. 7c). BCL6-stained neoplastic

Table 3 MEF2B in follicular lymphoma cases with diffuse growth pattern

No.	Age/gender	Localization	Pattern	FDC[a]	Grade	BCL6	CD10	BCL2	CD23	MEF2B
1.	63/F	Inguinal LN	DF> > Fo	Focal	1	+	+	+	0	+
2.	75/F	Inguinal LN	DF	No	2	0	+	+	+/−	+
3.	91/F	Skin	DF	No	2	+	+	+	−	+
4.	94/F	Inguinal LN	DF	No	2	+	+	+	+/−	+
5.	70/F	Cervical LN	DF> > Fo	Focal	2	+	−	+	+	+

+ positive; +/− focally positive; − negative; 0 not done

DF diffuse, *F* female, *FDC* follicular dendritic cell meshwork, *Fo* follicular, *LN* lymph node, *M* male

[a] CD21 or CD23

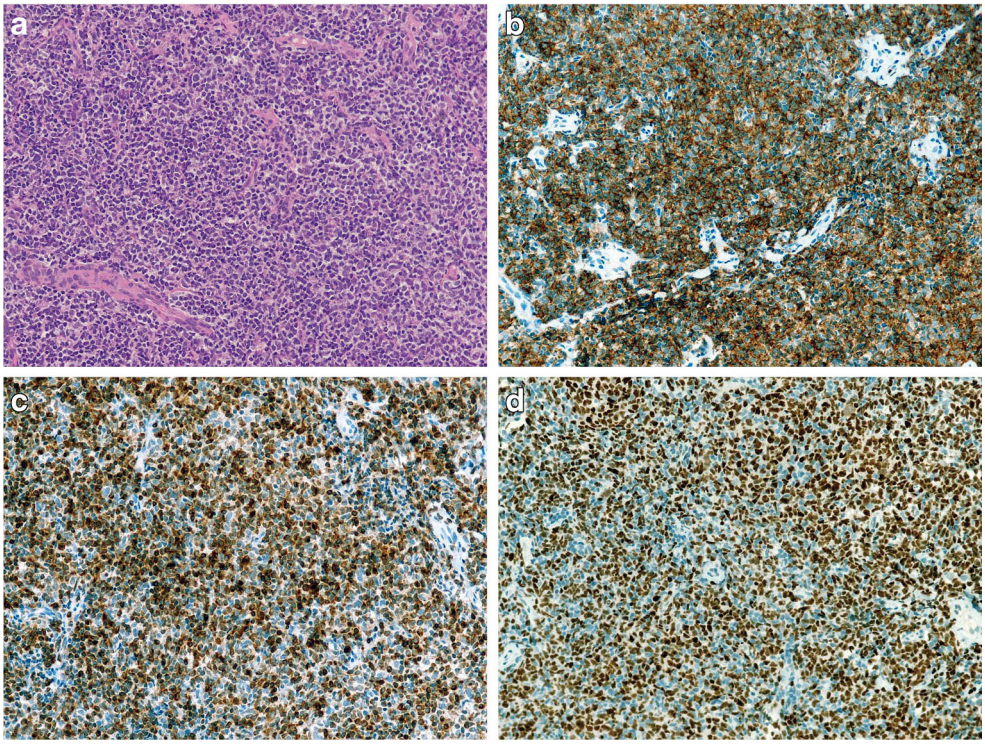

Fig. 3 MEF2B expression in diffuse follicular lymphoma. **a** Diffuse infiltration pattern predominantly composed of centrocytic cells. Neoplastic cells show coexpression of CD10 (**b**) and BCL2 (**c**). MEF2B is strongly expressed in the infiltrate and is confined to the neoplastic B cells. Original magnifications: **a**–**d** ×200

Table 4 Comparison of CD10, BCL6, BCL2, and MEF2B in follicular lymphoma infiltrates in bone marrow trephine biopsies

No.	Age/gender	CD10	BCL6	BCL2	MEF2B	Pattern
1.	60/F	+	+	+	+	PT + IS
2.	58/M	+	+	+	+	DF
3.	64/M	+	+	+	+	PT + ND
4.	67/F	+	+	+	+	PT
5.	59/F	+	+	+	+	ND
6.	74/M	+	+	+	+	DF
7.	86/M	−	+	+	+	PT
8.	58/M	−	+	+	+	DF
9.	48/M	−	+	+	+	ND + IS
10.	40/F	−	+	+	+	PT
11.	48/F	+	−	+	+	PT
12.	60/F	−	−	+	+	PT
13.	53/M	−	−	+	+	PT
14.	62/M	−	−	+	+	PT
15.	60/M	−	−	+	+	PT
16.	65/M	−	−	+	+	PT
17.	56/M	−	−	+	+	PT + IS
18.	44/M	−	−	+	+	PT + IS
19.	70/F	+	+	+	−	PT
20.	68/F	−	+	+	−	PT
21.	81/F	−	−	+	−	IS
22.	53/M	−	−	+	−	PT + IS

+ positive; − negative

DF diffuse, *F* female, *IS* interstitial, *M* male, *ND* nodular, *PT* paratrabecular

cells showed a similar staining pattern against a background of numerous immunoreactive lymphocytes (Fig. 7d).

Only one of 31 (3 %) cHL showed faint MEF2B staining of Hodgkin- and Reed-Sternberg cells. In the majority of cases, aggregates of strongly positive residual GC B cells and faintly stained plasma cells, endothelial cells, and other stromal cells were noted.

MEF2B in T cell lymphomas

PTCL ($n = 41$) including 16 angioimmunoblastic T cell lymphomas, 3 enteropathy-associated T cell lymphomas, 4 anaplastic large cell lymphomas, 8 primary cutaneous CD4-positive small/medium cell T cell lymphomas, 9 peripheral T cell lymphomas NOS, and 1 nasal T/NK-cell lymphoma were consistently MEF2B negative. In many of these, residual GC B cells and plasma cells were MEF2B positive, while characteristic Hodgkin- and Reed-Sternberg-like large B cells in nodal PTCLs were MEF2B negative.

Discussion

MEF2B is a transcriptional activator directly controlling biological activity of the *BCL6* gene and shows an expression pattern similar to that of BCL6 protein in normal GC B cells [14]. Since expression of MEF2B has not yet been systematically characterized, we undertook the present study to

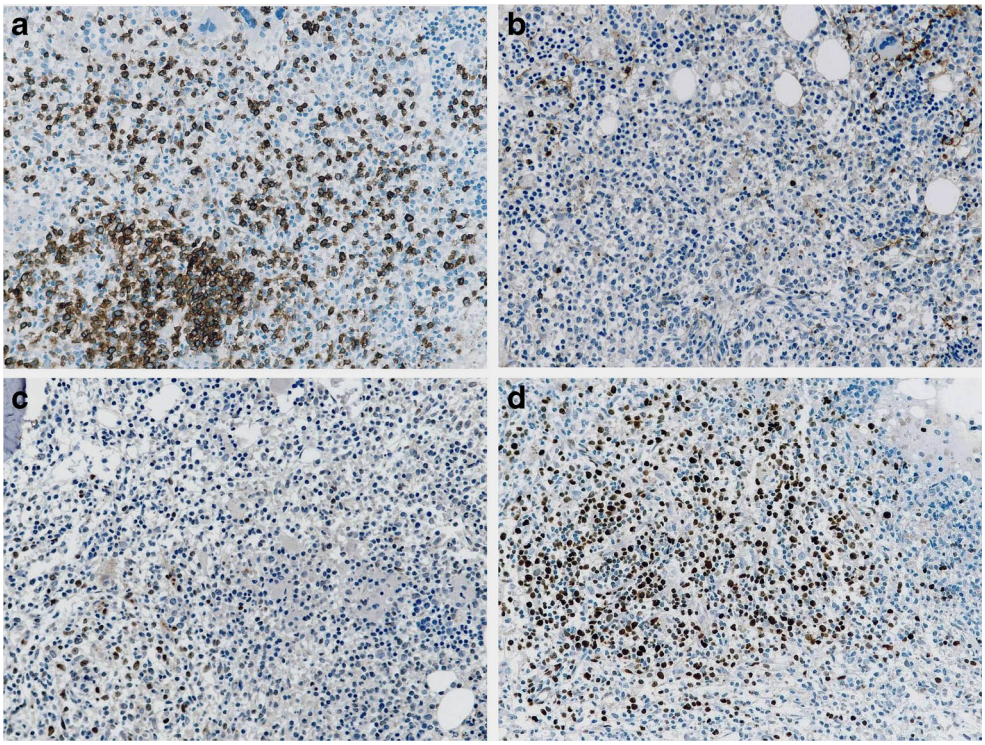

Fig. 4 Immunohistological features of MEF2B expression in bone marrow (BM) infiltrate of follicular lymphoma (FL). **a** BM infiltrate of a known FL case with nodular and interstitial growth pattern is highlighted by CD79a staining. **b** Neoplastic cells demonstrate lack of CD10 (**b**), weak focal staining for BCL6 (**c**), and widespread MEF2B expression (**d**). Original magnifications: **a**–**d** ×200

validate MEF2B as an IHC marker of GC B cells and GC B cell-derived malignant lymphomas.

In non-neoplastic tissues, we found crisp nuclear staining for MEF2B confined to centrocytes and centroblasts, but not in Tfh cells, which is in contrast with the pattern of expression of BCL6. Plasma cells and some stromal cells stained weakly for MEF2B with a pattern clearly distinct from that of GC B cells. Our observations confirm that MEF2B is a robust GC B cell marker.

FL, which arises from GC B cells, is one of the most common subtypes of indolent lymphoma [17]. The diagnosis of FL is based on morphology and immunohistological

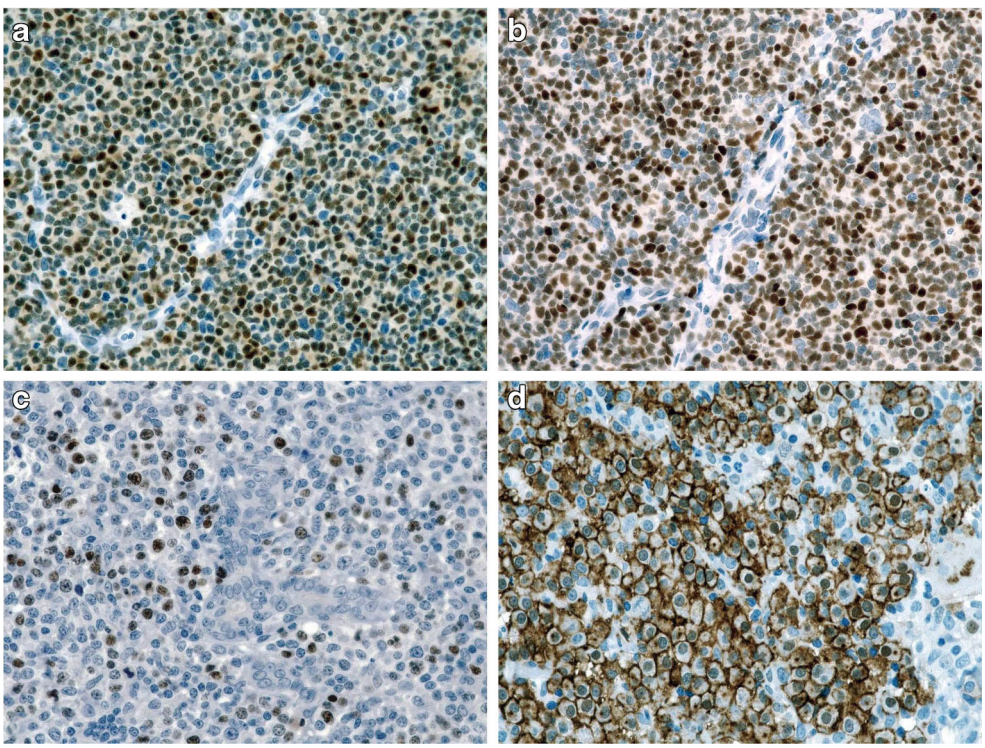

Fig. 5 MEF2B expression in mantle cell lymphoma (MCL) and in marginal zone lymphoma (MZL) with plasmacytic differentiation. A classical MCL case shows cyclin D1 overexpression (**a**) and MEF2B staining (**b**) with a similar staining pattern. **c** Plasmacytic neoplastic cells in a parotid MZL demonstrate variable MEF2B staining. **d** Thyroid MZL with prominent plasmacytic differentiation concurrently stained for MEF2B and CD38 is shown. Lesional plasmacytic cells display strong membranous staining for CD38 and variable nuclear staining for MEF2B. Original magnifications: **a**–**d** ×400

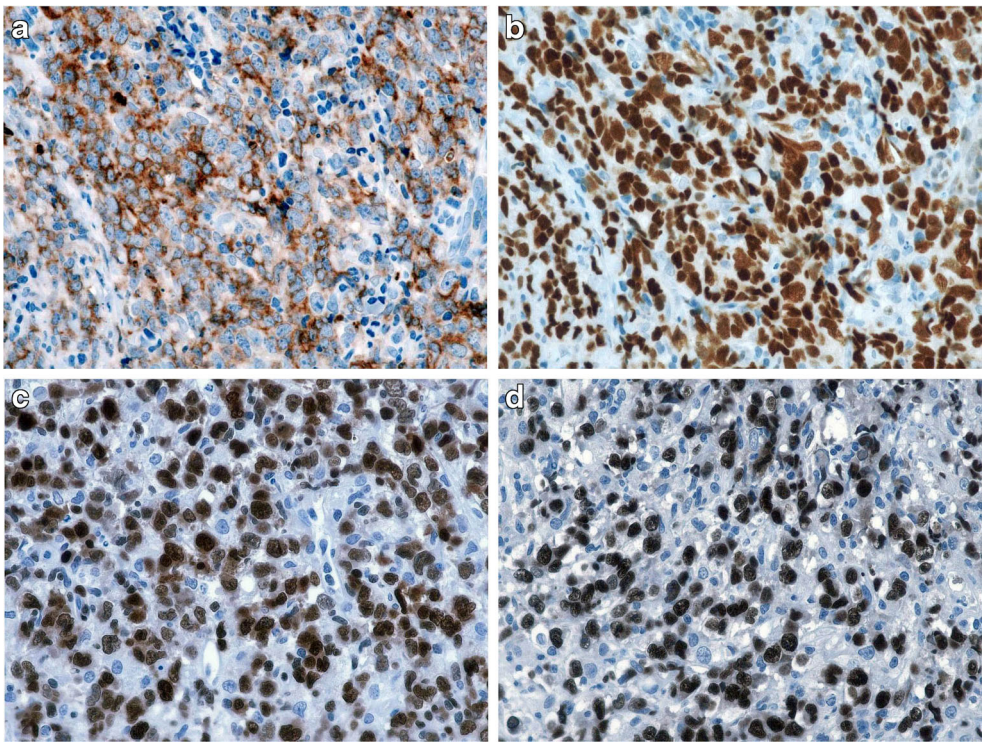

Fig. 6 MEF2B expression in diffuse large B cell lymphoma (DLBCL), not otherwise specified. A germinal center B cell (GCB) type DLBCL shows CD10 staining (**a**) and strong homogenous MEF2B expression (**b**). A non-GCB type DLBCL shows marked MUM1 (**c**) and MEF2B staining (**d**). Original magnifications: **a**–**d** ×400

detection of expression by pathological B cells of GC B cell antigens, especially BCL6 and CD10, and overexpression of BCL2 [17]. This can be complemented with molecular detection of the t(14;18)(q32;q21) translocation, which is responsible for deregulated BCL2 expression and is observed in 85–90 % of cases [17]. Some FL cases are atypical, do not express CD10 or BCL2 protein, or lack the t(14;18) translocation or show a diffuse growth pattern [17–20]. FL shows a characteristic follicular growth pattern, but in almost all cases, neoplastic cells also spread between follicles [21]. Interfollicular neoplastic cells are small- to medium-sized cells resembling centrocytes with

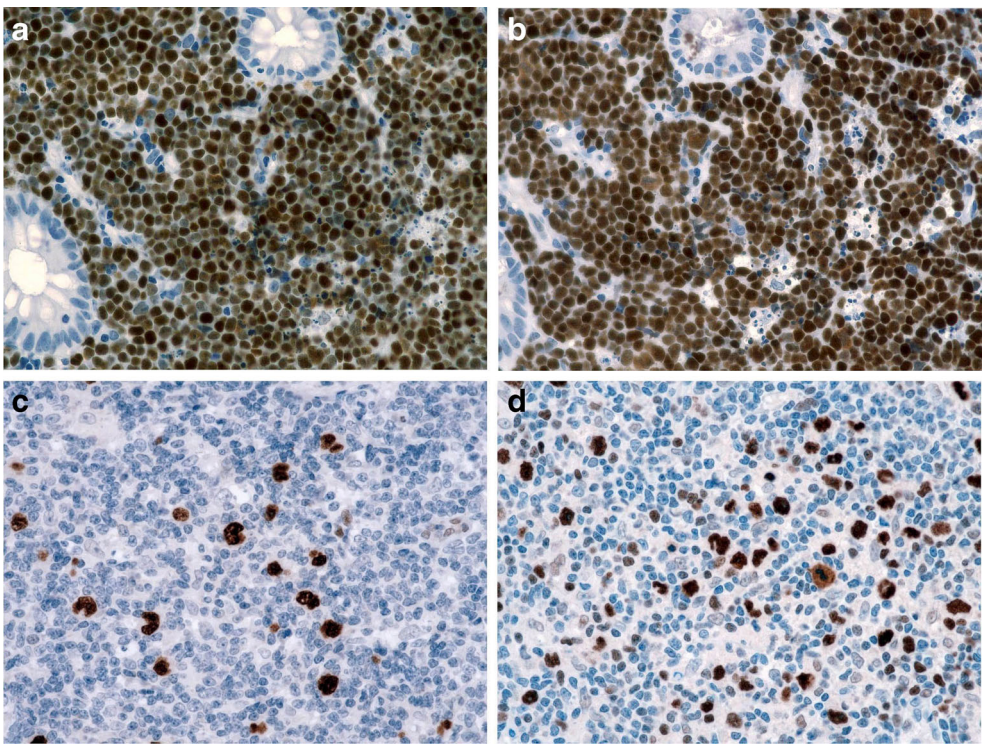

Fig. 7 Immunohistochemical features of MEF2B expression in Burkitt lymphoma (BL) and in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). A classical BL case shows intense BCL6 (**a**) and MEF2B (**b**) staining. In NLPHL, MEF2B expression (**c**) is confined to neoplastic cells, while BCL6 is expressed both in large popcorn cells and smaller (follicular T helper) lymphocytes in the background. Original magnifications: **a**–**d** ×400

lower expression of GC B cell markers [17, 21, 22]. All our FL cases, including all grades and variants, expressed MEF2B which supports its value as GC B cell marker. Nuclear staining for MEF2B in follicular neoplastic cells was marked and homogeneous, while interfollicular expression was weaker and heterogeneous. While expression of CD10 or BCL6 can be lower in diffuse or predominantly diffuse FL [17, 21–23], all our cases of diffuse or predominantly diffuse FL showed marked MEF2B expression, which underscores its usefulness.

When diagnosed, most FLs are disseminated including BM involvement [17]. Identification of BM involvement in staging of FL in patients with established disease, or separation of FL from other small B cell lymphomas in primary BM biopsies, is of great importance. BM involvement in FL is predominantly paratrabecular but this pattern is not entirely specific as interstitial, diffuse, and nodular infiltrates, which are characteristic in other small B cell lymphomas, can also occur in FL. Consequently, the differential diagnosis of small B cell lymphomas in BM biopsies may rely on IHC analysis. In BM trephines, the efficacy of currently available GC B cell markers, including CD10, BCL6, HGAL, and LMO2, is limited [22, 24, 25]. In a large majority of the trephine biopsies in our series MEF2B staining of neoplastic cells was distinctly positive, supporting the reliability of MEF2B as a marker of FL involvement of BM.

Somatic *MEF2B* mutations have been reported in 13.4 % of FL cases [26]. These apparently do not interfere with the expression of MEF2B protein in FL, as we found consistent immunoreactivity using an internal region-specific antibody.

FL can present with variant immunoarchitectural features that can be confused with that of other small B cell lymphomas, particularly MCL and MZL. MCL is a mature B cell neoplasm with aggressive behavior and typically poor outcome, characteristically composed of small- to medium-sized lymphocytes with irregular nuclear contour [27, 28]. MCL is characterized by a t(11;14)(q13;q32) translocation resulting in overexpression of cyclin D1 [28, 29], the diagnostic hallmark of the disease. We found MEF2B expression in a large majority of MCL cases. This is apparently not correlated with *MEF2B* mutation, which has been reported in 3–5 % of cases [29, 30]. The number and clinicopathological characteristics of MEF2B-negative cases (mostly cases of classical gastric and blastic MCL) suggest that *MEF2B* might be mutated in a larger proportion of MCL, but this needs to be further investigated. The high frequency of MEF2B expression in MCL might be taken as an indication of limited efficacy in distinguishing between MCL and FL. Expression of other currently used GC B cell markers, including CD10, BCL6, and LMO2, has also been found in MCL [31–33], which may further complicate the differential diagnosis of MCL. However, while expression of cyclin D1 establishes the diagnosis, heterogeneous MEF2B staining, as in the interfollicular compartment of FL, can still be a useful feature in the diagnosis of MCL.

A common differential diagnostic problem in small B cell lymphomas is to distinguish between FL and MZL. MZL is a group of indolent B cell lymphomas, with heterogeneous clinical course but overlapping morphological characteristics, including nodal MZL, extranodal MZL, and SMZL [34–36]. The term MZL refers to the growth pattern, which resembles the marginal zone in hyperplastic extranodal lymphoid tissue [34–36]. Morphologic features of MZL can vary according to the site of involvement with extranodal MZL frequently showing marginal zone expansion with or without monocytoid B cells or plasmacytic differentiation [34–36]. In SMZL, a nodular and perifollicular/marginal zone pattern predominates [34]. In nodal MZL, diffuse, nodular, interfollicular, and perifollicular patterns are distinguished [36]. In typical cases, MZL can be readily distinguished from FL based on morphologic and immunophenotypic features. However, MZL can be nodular and colonize lymphoid follicles, which raises FL as differential diagnosis [34–36]. To differentiate between MZL and low-grade CD10-negative FL, particularly in case of a diffuse growth pattern, can be even more challenging. We found MEF2B staining to be mostly negative in the non-plasmacytic component of MZL, irrespective of lymphoma site and architectural pattern. In our two MZL MEF2B-positive cases, only lesional plasma cells showed weak staining in a number exceeding the cutoff value. These results support MEF2B as a reliable marker to distinguish between MZL and FL.

Non-neoplastic plasma cells expressed MEF2B but at a significantly lower intensity than GC B cells. MEF2B expression in EP was marked and homogenous, suggesting upregulation of its expression in EP. In contrast, MM and WM tended to show focal weak or negative MEF2B staining, suggestive of downregulation of its expression in certain plasma cell neoplasms. These findings suggest that MEF2B might play a role in plasma cell neoplasms, but this needs to be further studied.

MEF2B encodes a transcriptional activator mutated in 8.3–18 % of DLBCL [26, 37–39]. Somatic mutations of *MEF2B* may contribute to lymphomagenesis by deregulating BCL6 [14], a zinc-finger protein acting as a transcriptional repressor in GC B cells [40]. DLBCL, a diverse group of aggressive mature B cell neoplasms, is the most common lymphoma category accounting for 30–40 % of all lymphoid malignancies [41]. Gene expression profiling studies have identified two pathogenetically and prognostically distinct subtypes: GC B cell like (GCB) and activated B cell like (ABC) [42]. Subsequently, IHC algorithms have been developed to recognize the molecular subtypes [16, 43]. We detected expression of MEF2B in a large majority of DLBCL cases. The expression of MEF2B was not significantly different in GCB and

non-GCB IHC subtypes, but statistically associated with BCL6 expression, in support of a relationship between these two transcription factors. A relationship between MEF2B protein expression and *MEF2B* mutation in DLBCL is yet to be investigated.

MEF2B was unequivocally positive in all NLPHL cases, while negative in all but one (97 %) cHL. In NLPHL, MEF2B staining labels popcorn cells more selectively than any other known marker, which offers easier interpretation and simplifies assessment of tumor cell characteristics. Our findings support the assumption that the popcorn cells are derived from GC B cells [44].

In conclusion, we show that MEF2B is a robust marker of normal GC B cells and a sensitive marker for the diagnosis of common and variant forms of FL in lymphoid tissues as well as in BM biopsies. MEF2B expression is almost without exception negative in MZL, which can be helpful to differentiate between FL and MZL. Other GC B cell-derived neoplasms, including DLBCL GCB subtype, BL, and NLPHL, almost without exception also express MEF2B. MEF2B is also frequently expressed in MCL and DLBCL of non-GCB subtype.

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