Modern Pathology (2007) 20, 1245–1252

www.modernpathology.org

Patterns of spectrin expression in B-cell lymphomas: loss of spectrin isoforms is associated with nodule-forming and germinal center-related lymphomas

Eric B Gorman, Lugen Chen, Joseph Albanese and Howard Ratech

Department of Pathology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, USA

Spectrins are a family of cytoskeletal proteins that organize and link membranes to subcellular motors and filaments. Although traditionally divided into erythroid and non-erythroid forms, the discovery of new spectrin isoforms in various tissues indicates that their distribution is not yet fully characterized. To our knowledge, there is no comprehensive analysis of spectrins in lymphoid malignancies. Using tumor microarrays of paraffin blocks, we immunohistochemically studied 10 lymph nodes with reactive lymphoid hyperplasia and 94 lymph nodes involved by B-cell malignant lymphoma. Expression of spectrins αI, αII, βI, βII, and βIII was scored using a 20% cutoff for positive immunoperoxidase staining. All spectrin isoforms, except erythroid-specific αI spectrin, were detected in lymph nodes with reactive lymphoid hyperplasia. In contrast, various spectrins were lost in particular B-cell malignant lymphomas. Based on the absence of staining for one or more spectrin isoforms in at least 50% of cases, we identified three patterns: (1) loss of αII and βII in follicular lymphoma, grades 2/3 and 3/3; nodular lymphocyte predominance Hodgkin's lymphoma; nodular sclerosis Hodgkin's lymphoma; (2) loss of βI only in Burkitt lymphoma; and (3) loss of αII and βI in mixed cellularity Hodgkin's lymphoma. In contrast, follicular lymphoma, grade 1/3 and diffuse large B-cell lymphoma retained spectrin in 67–100% of cases. The other lymphoma subtypes retained spectrin in greater than 50% of cases. We identified the loss of particular spectrin isoforms in B-cell malignant lymphomas that have a nodular growth pattern and/or are believed to arise from germinal center B-cells, that is follicular lymphoma, grades 2/3 and 3/3; Burkitt lymphoma; nodular sclerosis Hodgkin's lymphoma; mixed cellularity Hodgkin's lymphoma; and nodular lymphocyte predominance Hodgkin's lymphoma. The absence of particular spectrin isoforms may correlate with transformation or aggressive biologic behavior for some lymphoma subtypes.
Modern Pathology (2007) **20,** 1245–1252; doi:10.1038/modpathol.3800962; published online 21 September 2007

Keywords: spectrin; malignant lymphoma; Hodgkin's lymphoma

Spectrin genes have been found in the genomes of metazoans (*Drosophila melanogaster*, *Caenorhabditis elegans*, and vertebrates), but not in yeast (*Saccharomyces cerevisiae*).[1] As unicellular organisms developed into multicellular organisms, a short ancestral α-actinin gene transformed via duplications and rearrangements into the spectrin superfamily.[2] As metazoans evolved specialized cell types, spectrin molecules adapted to the challenges of mechanical stress, subcellular partitioning, cell-to-cell communication, and cellular orientation in complex tissue architecture.[1]

Correspondence: Dr H Ratech, MD, Department of Pathology, Montefiore Medical Center/Albert Einstein College of Medicine, Silver Zone, 4th Floor, Bronx, NY 10467, USA.
E-mail: hratech@montefiore.org
Received 26 April 2007; revised 23 July 2007; accepted 31 July 2007; published online 21 September 2007

Spectrins are large, rod-like, multifunctional proteins composed of α- and β-subunits. In humans, two α-subunits (αI, αII) and five β-subunits (βI, βII, βIII, βIV, βV) form various heterodimers. Although αI spectrin is restricted to erythrocytes, the other spectrin isoforms are widespread.[3] Actin-binding domains connect spectrin to the cytoskeleton, forming a bridge between the membrane and the subcellular motors, the filaments, and the rest of the cellular microenvironment.[1]

The spectrin isoforms localize to subplasma membrane (αI, αII, βI, and βII), cytoplasmic vesicle (βI and βIII), Golgi apparatus (βI and βIII),[4] and sarcolemma (αII).[5,6] Mutations in spectrins αI and βI cause hereditary elliptocytosis, poikilocytosis, and spherocytosis. As spectrins are scaffolding proteins, they could play a role in lymphomagenesis either by interfering with signal transmission from the external environment to the cytoskeleton or by

aberrantly localizing various kinases or phosphatases that dock to spectrin.[4,7] To our knowledge, the various spectrin isoforms have not been previously investigated in a comprehensive panel of B-cell malignant lymphomas.

Materials and methods

Lymphoma Samples and Clinical Data

We retrospectively studied spectrin expression in 10 lymph nodes with reactive lymphoid hyperplasia and in 94 lymph nodes with B-cell malignant lymphoma, including follicular lymphoma, grades 1/3 ($N=10$), 2/3 ($N=7$), and 3/3 ($N=7$); Burkitt lymphoma ($N=5$); diffuse large B-cell lymphoma ($N=6$); B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma ($N=15$); mantle cell lymphoma ($N=6$); marginal zone B-cell lymphoma ($N=11$); nodular sclerosis Hodgkin's lymphoma ($N=10$); mixed cellularity Hodgkin's lymphoma ($N=9$); and nodular lymphocyte predominance Hodgkin's lymphoma ($N=8$). The paraffin blocks were retrieved from the files of the Department of Pathology at Montefiore Medical Center (Bronx, NY, USA). Clinical data were obtained from the medical record or the patient's physician. The Institutional Review Board of Montefiore Medical Center (Bronx, NY, USA) has granted permission for us to use clinical information and archival patient tissue samples for research purposes.

Tissue Microarrays

Tissue microarrays were constructed from formalin-fixed, paraffin-embedded tissue blocks using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD, USA). To ensure adequate sampling, each case was represented in triplicate using 1.0 mm cores.

Immunohistochemistry

Conditions for immunoperoxidase staining of spectrins αI, αII, βI, βII, and βIII in paraffin tissue sections are listed in Table 1. Staining for a particular spectrin isoform was considered positive if it was expressed by at least 20% of the neoplastic cells. The subcellular localization was recorded as submembranous, cytoplasmic, or perinuclear dot-like. Positive control tissues included reactive tonsil, kidney, liver, and the Jurkat T-cell leukemia cell line. The spectrin isoforms αII and βII were generally easy to score because of their distinct submembranous staining pattern, which could be detected even at low-power magnification. On the other hand, spectrin isoforms βI and βIII often required either high-power magnification or a $\times 100$ oil immersion objective, particularly for reactive background lymphocytes. In addition, detection of βI and βIII spectrins sometimes required focusing up and down at different subcellular planes so as not to miss small aggregates of cytoplasmic vesicular or perinuclear dot-like staining.

Results

Spectrin Expression in Non-Neoplastic Cells

In 10 lymph nodes with reactive lymphoid hyperplasia (Figure 1a), both T cells and B cells expressed αII, βI, βII, and βIII spectrins (Tables 2 and 3). The αII and βII spectrins stained more intensely in the paracortical T-zone than in the B-cell follicle (Figure 1b). Tingible-body macrophages in the germinal center expressed βI and βIII spectrin isoforms, but lacked αII and βII; the absence of αII- and βII-staining in the histiocytes sharply contrasted with the surrounding uniformly positive lymphocytes (Figure 1b and c). Although impossible to identify in reactive lymph nodes (Figure 1b and c) or in B-cell malignant lymphomas that expressed spectrin (Figure 1d), the elongated cellular processes of residual reactive follicular dendritic cells could be easily seen against a background of spectrin-negative lymphoma cells (Figure 1e and f); the follicular dendritic cells expressed αII, βI, βII, and βIII spectrin isoforms. As expected, all lymphoid cells, whether

Table 1 Immunohistochemical reagents used for spectrin staining

Spectrin antibody	*Clone*	*Source*	*Antigen retrieval method*	*Primary antibody dilution*	*Second step reagents*
αI mouse monoclonal	17C7	1	Dako target retrieval solution 0.01 M citrate, pH 6.0	1:20	Envision+ anti-mouse
αII mouse monoclonal	35	2	1 mM EDTA, pH 8.0	1:50	Envision+ anti-mouse
βI mouse monoclonal	4C3	3	Dako target retrieval solution 0.01 M citrate, pH 6.0	1:100	Envision+ anti-mouse
βII mouse monoclonal	42	2	Dako target retrieval solution 0.01 M citrate, pH 6.0	1:2000	Envision+ anti-mouse
βIII goat polyclonal	Not applicable	4	Dako target retrieval solution 0.01 M citrate, pH 6.0	1:50	LSAB+ System HRP

All spectrin antibodies are anti-human. 1 = Abcam, Cambridge, MA, USA; 2 = Becton-Dickinson Biosciences, San Jose, CA, USA; 3 = Affinity Bioreagents, Golden, CO, USA; 4 = Santa Cruz Biotech, Santa Cruz, CA, USA. All antigen target retrieval solutions (except for 1 mM EDTA, pH 8.0) and second-step reagents were obtained from Dako, Carpinteria, CA, USA.

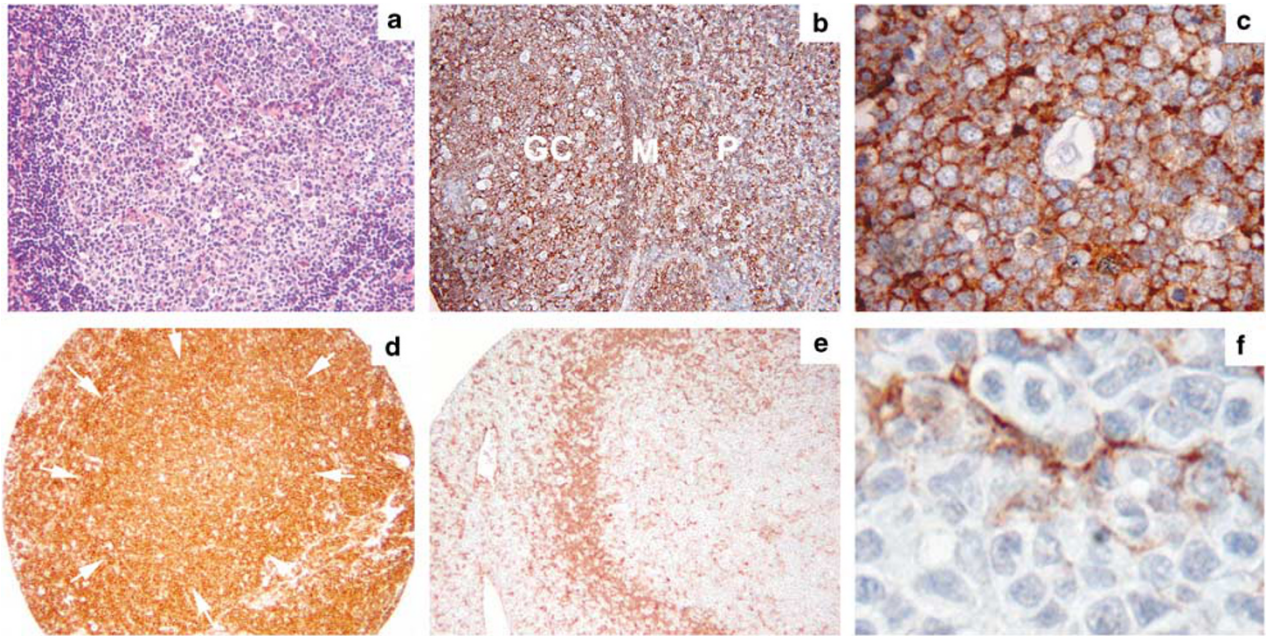

Figure 1 Comparison of βII spectrin staining in reactive lymphoid hyperplasia *vs* follicular lymphoma, grades 1/3 and 3/3. (**a**) Reactive lymphoid hyperplasia, low power magnification, hematoxylin and eosin (HE) stain. (**b**) Reactive lymphoid hyperplasia, low-power magnification. The B-cell follicle, including germinal center (GC) and mantle zone (M), and the surrounding paracortical T-zone (P) strongly express βII spectrin. Numerous tingible-body macrophages in the germinal center appear negative. (**c**) Reactive lymphoid hyperplasia, high-power magnification. The macrophage at the center of the image does not express βII spectrin. (**d**) Follicular lymphoma, grade 1/3, low-power magnification. Positive βII spectrin staining in neoplastic B-cell nodule (outlined by arrowheads) and in surrounding reactive lymphocytes. (**e**) Follicular lymphoma, grade 3/3. Absence of βII spectrin in the neoplastic B-cell nodule contrasts sharply with positive staining in the surrounding reactive lymphocytes. (**f**) Follicular lymphoma, grade 3/3, high-power magnification. βII spectrin-positive follicular dendritic cell processes are clearly visible against a background of negative neoplastic B-cells.

Table 2 Expression of spectrin isoforms in reactive lymphoid hyperplasia and in B-cell malignant lymphomas

Diagnosis	*Spectrin isoforms*				
	α*I*	α*II*	β*I*	β*II*	β*III*
Reactive lymphoid hyperplasia	0/10 (0)	10/10 (100)	10/10 (100)	10/10 (100)	10/10 (100)
Chronic lymphocytic leukemia/small lymphocytic lymphoma	0/15 (0)	12/15 (80)	8/15 (53)	15/15 (100)	10/15 (67)
Mantle cell lymphoma	0/6 (0)	6/6 (100)	5/6 (83)	6/6 (100)	4/6 (67)
Marginal zone B-cell lymphoma	0/11 (0)	6/11 (55)	9/11 (82)	11/11 (100)	9/11 (82)
Follicular lymphoma, grade 1/3	0/10 (0)	7/10 (70)	9/10 (90)	7/10 (70)	10/10 (100)
Follicular lymphoma, grade 2/3	0/7 (0)	1/7 (14)	6/7 (86)	0/7 (0)	7/7 (100)
Follicular lymphoma, grade 3/3	0/7 (0)	0/7 (0)	7/7 (100)	3/7 (43)	7/7 (100)
Diffuse large B-cell lymphoma	0/6 (0)	4/6 (67)	4/6 (67)	5/6 (83)	6/6 (100)
Burkitt lymphoma	0/5 (0)	4/5 (80)	1/5 (20)	5/5 (100)	5/5 (100)
Mixed cellularity Hodgkin's lymphoma	0/9 (0)	4/9 (44)	2/9 (22)	5/9 (56)	9/9 (100)
Nodular sclerosis Hodgkin's lymphoma	0/10 (0)	5/10 (50)	7/10 (70)	3/10 (30)	10/10 (100)
Nodular lymphocyte predominance Hodgkin's lymphoma	0/8 (0)	1/8 (13)	5/8 (63)	1/8 (13)	8/8 (100)

Number of positive cases/number of total cases (percent). Values for mixed cellularity Hodgkin's lymphoma and for nodular sclerosis Hodgkin's lymphoma refer to Reed–Sternberg cells. Values for nodular lymphocyte predominance Hodgkin's lymphoma refer to lymphocytic and/or histiocytic variant cells.

reactive or neoplastic, lacked erythroid-specific αI spectrin (Table 2).

Spectrin Expression in Neoplastic B-Cells

We immunohistochemically evaluated the expression of αI, αII, βI, βII, and βIII spectrin isoforms in 94 cases of B-cell malignant lymphomas representing 11 different diagnostic categories (Table 2; Figures 1–3). Cases were classified as positive if at least 20% of the lymphoma cells expressed a particular spectrin isoform. Six types of B-cell malignant lymphoma (Figure 3) lost at least one spectrin isoform in 50% or more of cases: follicular lymphoma, grades 2/3 and 3/3; Burkitt lymphoma; mixed cellularity Hodgkin's lymphoma; nodular sclerosis Hodgkin's lymphoma; nodular lymphocyte predominance Hodgkin's lymphoma

Table 3 Expression of spectrin isoforms in reactive lymphoid hyperplasia *vs* the background lymphocytes of classic and lymphocyte predominance Hodgkin's lymphoma

Diagnosis	*<20%*	*20–50%*	*>50%*	*≥20%*
		αII spectrin		
Reactive lymphoid hyperplasia	0	0	10	10/10 (100)
Mixed cellularity Hodgkin's lymphoma	3	2	4	6/9 (67)
Nodular sclerosis Hodgkin's lymphoma	0	0	10	10/10 (100)
Nodular lymphocyte predominance Hodgkin's lymphoma	0	0	8	8/8 (100)
		βI spectrin		
Reactive lymphoid hyperplasia	0	10	0	10/10 (100)
Mixed cellularity Hodgkin's lymphoma	5	1	3	4/9 (45)
Nodular sclerosis Hodgkin's lymphoma	10	0	0	0/10 (0)
Nodular lymphocyte predominance Hodgkin's lymphoma	8	0	0	0/8 (0)
		βII spectrin		
Reactive lymphoid hyperplasia	0	0	10	10/10 (100)
Mixed cellularity Hodgkin's lymphoma	0	0	9	9/9 (100)
Nodular sclerosis Hodgkin's lymphoma	10	0	0	0/10 (0)
Nodular lymphocyte predominance Hodgkin's lymphoma	0	0	8	8/8 (100)
		βIII spectrin		
Reactive lymphoid hyperplasia	0	10	0	10/10 (100)
Mixed cellularity Hodgkin's lymphoma	0	0	9	9/9 (100)
Nodular sclerosis Hodgkin's lymphoma	10	0	0	0/10 (0)
Nodular lymphocyte predominance Hodgkin's lymphoma	0	0	8	8/8 (100)

Number of cases with positive staining for <20%, 20–50%, and >50% of background lymphocytes are listed in columns 1–3. Number of cases with ≥20% positive background lymphocytes/number of total cases (percent) is listed in column 4.

(Table 2; Figure 3). The affected cells included both centrocytes and centroblasts in follicular lymphoma, grades 2/3 and 3/3; all neoplastic B-cells in Burkitt lymphoma; Reed–Sternberg cells in mixed cellularity Hodgkin's lymphoma and in nodular sclerosis Hodgkin's lymphoma; and lymphocytic and/or histiocytic variant cells (so-called 'popcorn' cells) in nodular lymphocyte predominance Hodgkin's lymphoma. Thus, lymphoma cells with medium to large size or bizarre shape tended to lose expression of various spectrin isoforms. An interesting exception to this observation was diffuse large B-cell lymphoma, which generally preserved spectrin expression, possibly because these were *de novo* cases that did not arise by transformation from pre-existing follicular lymphoma. In contrast, none of the B-cell malignant lymphomas composed of small lymphocytes, which varied minimally from the cytologic features of normal lymphocytes, had significant spectrin deficits, that is B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma; mantle cell lymphoma; marginal zone B-cell lymphoma; or follicular lymphoma, grade 1/3.

Subcellular Distribution of Spectrin Isoforms

In all spectrin-positive cases, we confirmed the subcellular distribution of the various spectrin isoforms.[4] αII and βII spectrins localized to the submembranous region, which was best seen in reactive interfollicular T cells and in reactive mantle zone B cells (Figure 1), but also in a variety of B-cell malignant lymphomas (Figure 3). βI and βIII spectrins occurred in diffuse cytoplasmic and in perinuclear dot-like patterns, which were especially well seen in mixed cellularity Hodgkin's lymphoma (Figure 3p) and in nodular sclerosis Hodgkin's lymphoma (Figure 3r and t).

Spectrin Expression in Non-Neoplastic Background Lymphocytes

We semiquantitatively compared the expression of spectrin isoforms αII, βI, βII, and βIII in the lymphocytes of reactive lymphoid hyperplasia *vs* the background lymphocytes of mixed cellularity Hodgkin's lymphoma, nodular sclerosis Hodgkin's lymphoma, and nodular lymphocyte predominance Hodgkin's lymphoma (Table 3). In reactive lymphoid hyperplasia, ≥20% of the lymphocytes stained positively for spectrin isoforms βI and βIII, and >50% stained positively for αII and βII. In 67–100% of the cases of mixed cellularity Hodgkin's lymphoma, nodular sclerosis Hodgkin's lymphoma, and nodular lymphocyte predominance Hodgkin's lymphoma, ≥20% of the background lymphocytes

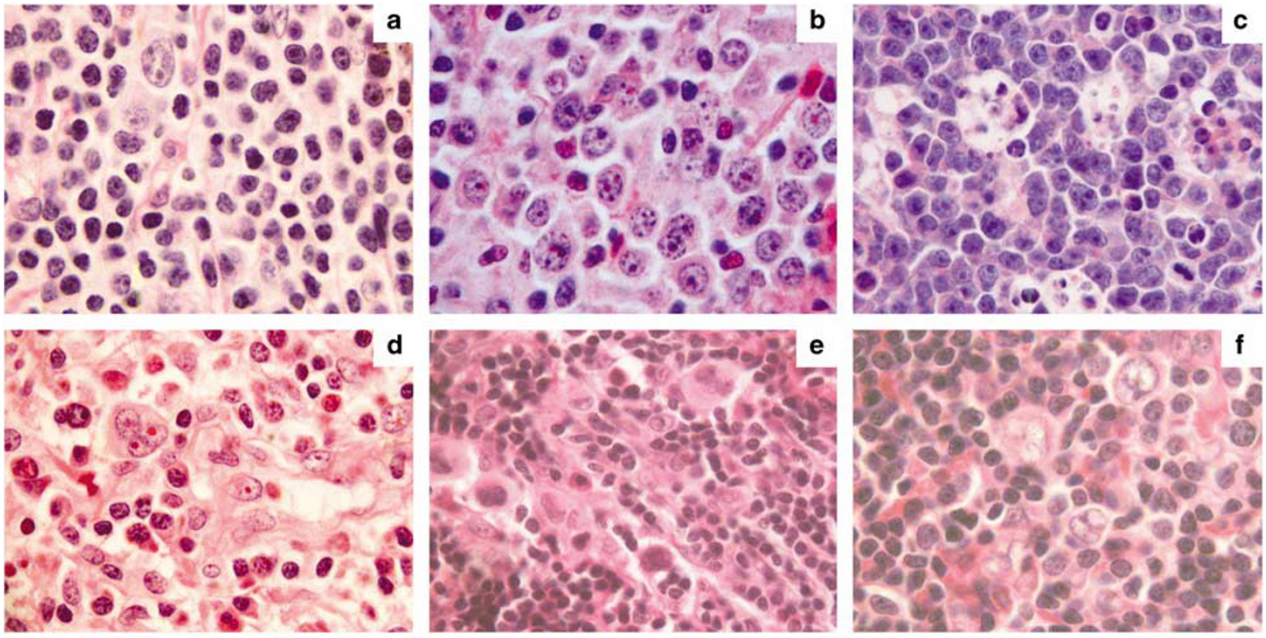

Figure 2 Hematoxylin and eosin stained images of representative B-cell malignant lymphomas (see Figure 3 for spectrin staining). (**a**) Follicular lymphoma, grade 1/3. (**b**) Follicular lymphoma, grade 3/3. (**c**) Burkitt lymphoma. (**d**) Nodular sclerosis Hodgkin's lymphoma. (**e**) Mixed cellularity Hodgkin's lymphoma. (**f**) Nodular lymphocyte predominance Hodgkin's lymphoma.

stained positively for αII spectrin. In contrast, the background lymphocytes expressed βI spectrin at this level in only 45% of the mixed cellularity Hodgkin's lymphoma cases, and in none of the nodular sclerosis Hodgkin's lymphoma or nodular lymphocyte predominance Hodgkin's lymphoma cases. For βII and βIII spectrins, ≥20% of the background lymphocytes stained positively in all mixed cellularity Hodgkin's lymphoma and nodular lymphocyte predominance Hodgkin's lymphoma cases, but in no nodular sclerosis Hodgkin's lymphoma cases. As can be seen in Table 3, the background lymphocytes of mixed cellularity Hodgkin's lymphoma, nodular sclerosis Hodgkin's lymphoma, and nodular lymphocyte predominance Hodgkin's lymphoma tended to be either predominantly positive (>50%) or predominantly negative (<20%) for the expression of a particular spectrin isoform. And, in contrast to the lymphocytes of reactive lymphoid hyperplasia, the background lymphocytes of Hodgkin's lymphoma rarely expressed an intermediate range of spectrin (20–50%; Table 3).

Discussion

Spectrin isoforms have been extensively characterized in peripheral blood leukocytes,[4] in the nervous system,[8] and in cardiac[6,9] and skeletal muscle.[5] However, little is known about their distribution in B-cell malignant lymphomas, other than for a few limited studies in chronic lymphocytic leukemia and hairy cell leukemia,[10,11] which employed xenoantisera raised against chicken erythrocyte spectrin. Alterations in spectrin organization following chemotherapy for leukemia, plus the many biochemical connections between spectrin and other molecules throughout the cell, suggest that a wide variety of influences could affect spectrin distribution in normal and neoplastic lymphoid cells.[10,12,13]

In the current immunohistochemical study, we have used antihuman monoclonal antibodies wherever available to analyze spectrin expression in reactive lymphoid hyperplasia and in a comprehensive panel of B-cell malignant lymphomas. We report the expression of αII, βI, βII, and βIII spectrin isoforms in non-neoplastic T cells, B cells, and follicular dendritic cells. Also, we detected βI and βIII, but neither αII nor βII, spectrin isoforms in tingible-body macrophages. As expected, both reactive lymphoid hyperplasia and B-cell malignant lymphoma lacked erythroid-specific αI spectrin.

We identified frequent absence of particular spectrin isoforms in 6 out of 11 B-cell malignant lymphoma subtypes. Spectrin loss occurred in three unique combinations: βI only (Burkitt lymphoma); αII and βI (mixed cellularity Hodgkin's lymphoma); and αII and βII (follicular lymphoma, grades 2/3 and 3/3; nodular sclerosis Hodgkin's lymphoma; nodular lymphocyte predominance Hodgkin's lymphoma). In the cases of either classic or lymphocyte predominance Hodgkin's lymphoma, it was either the Reed–Sternberg cells or the lymphocytic and/or histiocytic variant cells that were affected. In contrast, all B-cell malignant lymphoma subtypes maintained expression of βIII spectrin. Although several lymphoma subtypes failed to express two

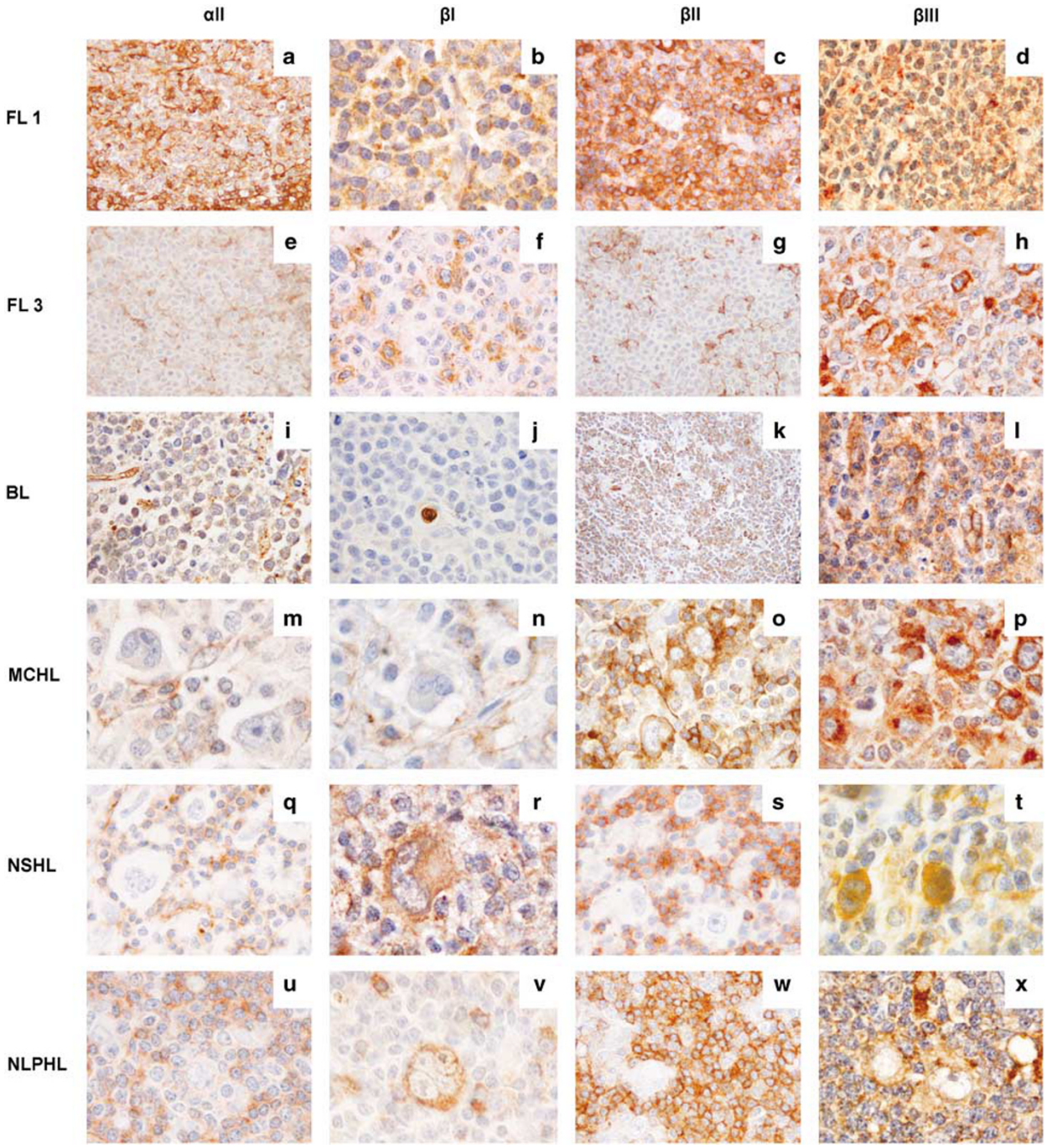

Figure 3 Staining patterns of αII, βI, βII and βIII spectrins in B-cell malignant lymphomas. (**a–d**) Follicular lymphoma, grade 1/3. There is expression of αII, βI, βII and βIII spectrin isoforms in the lymphoma cells. (**e–h**) Follicular lymphoma, grade 3/3. In contrast to follicular lymphoma, grade 1/3, the neoplastic B-cells of follicular lymphoma, grade 3/3 expressed neither αII- nor βII-spectrins (**e**, **g**). Spectrin-positive non-neoplastic follicular dendritic cell processes can be seen highlighted against spectrin-negative neoplastic B-cells (**g**). βI and βIII spectrins (**f**, **h**) appear similar in both percent of positive neoplastic B cells and in staining intensity. βIII spectrin is localized in strongly positive peri-nuclear dot-like aggregates (**h**). (**i–l**) Burkitt lymphoma. The Burkitt lymphoma cells are positive for αII and βII spectrins (**i**, **k**) βIII spectrin (**l**) is positive in most cells, with peri-nuclear dot-like aggregates. In contrast, the Burkitt lymphoma cells do not express βI spectrin (**j**); note the internal positive control staining by red blood cells inside a capillary. (**m–p**) Mixed cellularity Hodgkin's lymphoma. The Reed–Sternberg cells of mixed cellularity Hodgkin's lymphoma express βII and βIII (**o**, **p**), but neither αII nor βI (**m**, **n**) spectrin isoforms. (**q–t**) Nodular sclerosis Hodgkin's lymphoma. The Reed–Sternberg cells of nodular sclerosis Hodgkin's lymphoma, which do not express αII and βII spectrins (**q**, **s**), are highlighted against a spectrin-positive background of non-neoplastic T cells. The majority of Reed–Sternberg cells of nodular sclerosis Hodgkin's lymphoma express βI and βIII spectrins (**r**, **t**) in peri-nuclear dot-like aggregates. (**u–x**) Nodular lymphocyte predominance Hodgkin's lymphoma. The lymphocytic and/or histiocytic variant cells of nodular lymphocyte predominance Hodgkin's lymphoma express βI and βIII (**v**, **x**) but neither αII nor βII (**u**, **w**) spectrin isoforms.

different spectrins, no single case lost three isoforms. To our knowledge, these findings have not been previously reported.

We also noted the curious finding that, compared to the lymphocytes in reactive lymphoid hyperplasia, the background lymphocytes in some cases of classic Hodgkin's lymphoma and in nodular lymphocyte predominance Hodgkin's lymphoma appeared to downregulate spectrin. As α-interferon is known to affect spectrin in lymphocytes,[10] it is possible that other cytokines and chemokines, which are abundant in the Hodgkin's lymphoma environment,[14,15] might regulate spectrin. Furthermore, there are numerous CD4+/FOXP3+ regulatory T cells in classic HL, which secrete interleukin-10,[16] and many CD4+/CD57+ T cells in nodular lymphocyte predominance Hodgkin's lymphoma that are unlike the vast majority of lymphocytes found in reactive lymphoid hyperplasia with respect to their suppressive immune function[17,18] and unique cytokine profile.[19]

The presence or absence of spectrin isoforms could be helpful in solving several common problems in lymph node pathology such as confirming the identity of Reed–Sternberg cells in classic Hodgkin's lymphoma,[20] particularly in cases with a dense lymphocytic infiltrate; confirming the identity of lymphocytic and/or histiocytic variant cells in nodular lymphocyte predominance Hodgkin's lymphoma,[21] based on the loss of various spectrin isoforms in classic Hodgkin's lymphoma or in nodular lymphocyte predominance Hodgkin's lymphoma, but not in diffuse large B-cell lymphoma; distinguishing Burkitt lymphoma from diffuse large B-cell lymphoma,[22,23] based on the frequent loss of βI spectrin in Burkitt lymphoma, but not in diffuse large B-cell lymphoma; and grading follicular lymphomas,[24] which tended to lose αII and βII spectrin isoforms in grades 2 and 3 more often than in grade 1. To establish the value of spectrin isoforms in differential diagnosis would require more cases than in the current survey.

We wondered, could the loss of spectrin isoforms in various B-cell malignant lymphomas be due to a generalized, non-targeted effect of genome-wide instability? The SPTAN1, SPTB, SPTBN1, and SPTBN2 genes, which code for the αII, βI, βII, and βIII spectrin isoforms, map to chromosomal loci 9q34.11, 14q24.1-q24.2, 2p21, and 11q13.2.[2] A literature search for recurrent deletions at these sites did not reveal any.[25,26] Furthermore, genomic instability is considered rare in B-cell malignant lymphomas. Even microsatellite instability, a type of genomic instability secondary to defective DNA mismatch repair, has only occasionally been detected during progression or histologic transformation of B-cell malignant lymphomas.[27–29] And evidence for a mutator mechanism of genomic instability in B-cell malignant lymphomas seems to be limited to a small group of genes that are also the sites of recurrent chromosomal translocations.[30] None of these included the spectrin genes. Although recurrent deletions in spectrin genes have not been identified in lymphomas, other gene silencing mechanisms such as DNA hypermethylation, point mutations, transcriptional repression, or RNA interference could result in altered spectrin expression.

Alternatively, we asked, how could the loss of spectrin isoforms possibly contribute to lymphomagenesis? Several pathogenetic mechanisms involving spectrin can be theorized by interfering with spectrin nuclear scaffolding that binds gene regulatory machinery[31] or enhances repair of DNA interstrand crosslinks;[32] interfering with signal transduction that passes through spectrin,[33] which can function as an adaptor molecule,[2,7] from the external environment to the nucleus and causing abnormalities in the cell cycle;[34] or permitting various kinases or phosphatases[4] that dock to spectrin to become untethered[7] and mislocalized to aberrant subcellular sites where they could act on unnatural substrates.

Although most somatic mutations in cancer are passengers that are unnecessary for neoplastic transformation, new and unexpected driver mutations continue to be discovered.[35,36] We believe that our data are highly suggestive of a heretofore-unsuspected connection between spectrin expression and lymphomagenesis. This is based on the patterns of spectrin loss that affect some, but not all, B-cell malignant lymphomas and the lack of expression of specific isoforms in particular lymphoma subtypes. Nevertheless, definitive proof of a causal link between loss of spectrin expression and lymphomagenesis must await additional studies that are beyond the scope of the current investigation.

References

1 Broderick MJ, Winder SJ. Spectrin, alpha-actinin, and dystrophin. Adv Protein Chem 2005;70:203–246.
2 Bennett V, Baines AJ. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev 2001;81:1353–1392.
3 Winkelmann JC, Forget BG. Erythroid and nonerythroid spectrins. Blood 1993;81:3173–3185.
4 Pradhan D, Morrow J. The spectrin-ankyrin skeleton controls CD45 surface display and interleukin-2 production. Immunity 2002;17:303–315.
5 Williams MW, Resneck WG, Kaysser T, *et al.* Na,K-ATPase in skeletal muscle: two populations of beta-spectrin control localization in the sarcolemma but not partitioning between the sarcolemma and the transverse tubules. J Cell Sci 2001;114:751–762.
6 Bennett PM, Baines AJ, Lecomte MC, *et al.* Not just a plasma membrane protein: in cardiac muscle cells alpha-II spectrin also shows a close association with myofibrils. J Muscle Res Cell Motil 2004;25:119–126.
7 De Matteis MA, Morrow JS. Spectrin tethers and mesh in the biosynthetic pathway. J Cell Sci 2000;113 (Part 13):2331–2343.

8 Goodman SR, Zagon IS. The neural cell spectrin skeleton: a review. Am J Physiol 1986;250:C347–C360.
9 Baines AJ, Pinder JC. The spectrin-associated cytoskeleton in mammalian heart. Front Biosci 2005;10: 3020–3033.
10 Evans SS, Wang WC, Gregorio CC, *et al.* Interferon-alpha alters spectrin organization in normal and leukemic human B lymphocytes. Blood 1993;81: 759–766.
11 Repasky EA, Symer DE, Bankert RB. Spectrin immunofluorescence distinguishes a population of naturally capped lymphocytes *in situ*. J Cell Biol 1984;99: 350–355.
12 Dubielecka P, Potoczek S, Jazwiec B, *et al.* The effect of chemotherapy with fluodarabine/mitoxantrone/dexamethasone on the distribution of spectrin in lymphocytes of non-Hodgkin lymphoma patients. Cell Mol Biol Lett 2001;6:200.
13 Dubielecka PM, Jazwiec B, Potoczek S, *et al.* Changes in spectrin organisation in leukaemic and lymphoid cells upon chemotherapy. Biochem Pharmacol 2005;69:73–85.
14 Maggio E, van den Berg A, Diepstra A, *et al.* Chemokines, cytokines and their receptors in Hodgkin's lymphoma cell lines and tissues. Ann Oncol 2002;13(Suppl 1):52–56.
15 Malec M, Soderqvist M, Sirsjo A, *et al.* Real-time polymerase chain reaction determination of cytokine mRNA expression profiles in Hodgkin's lymphoma. Haematologica 2004;89:679–685.
16 Marshall NA, Christie LE, Munro LR, *et al.* Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. Blood 2004;103:1755–1762.
17 Gandhi MK, Lambley E, Duraiswamy J, *et al.* Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. Blood 2006;108:2280–2289.
18 Ishida T, Ishii T, Inagaki A, *et al.* Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. Cancer Res 2006;66:5716–5722.
19 Atayar C, Poppema S, Visser L, *et al.* Cytokine gene expression profile distinguishes CD4+/CD57+ T cells of the nodular lymphocyte predominance type of Hodgkin's lymphoma from their tonsillar counterparts. J Pathol 2006;208:423–430.
20 Browne P, Petrosyan K, Hernandez A, *et al.* The B-cell transcription factors BSAP, Oct-2, and BOB.1 and the pan-B-cell markers CD20, CD22, and CD79a are useful in the differential diagnosis of classic Hodgkin lymphoma. Am J Clin Pathol 2003;120:767–777.
21 Boudova L, Torlakovic E, Delabie J, *et al.* Nodular lymphocyte-predominant Hodgkin lymphoma with nodules resembling T-cell/histiocyte-rich B-cell lymphoma: differential diagnosis between nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte-rich B-cell lymphoma. Blood 2003;102: 3753–3758.
22 Frost M, Newell J, Lones MA, *et al.* Comparative immunohistochemical analysis of pediatric Burkitt lymphoma and diffuse large B-cell lymphoma. Am J Clin Pathol 2004;121:384–392.
23 Gormley RP, Madan R, Dulau AE, *et al.* Germinal center and activated B-cell profiles separate Burkitt lymphoma and diffuse large B-cell lymphoma in AIDS and non-AIDS cases. Am J Clin Pathol 2005;124: 790–798.
24 Zhang K, Prichard JW, Yoder S, *et al.* Utility of SKP2 and MIB-1 in grading follicular lymphoma using quantitative imaging analysis. Hum Pathol 2007;38:878–882.
25 Mestre-Escorihuela C, Rubio-Moscardo F, Richter JA, *et al.* Homozygous deletions localize novel tumor suppressor genes in B-cell lymphomas. Blood 2007; 109:271–280.
26 Berglund M, Enblad G, Thunberg U, *et al.* Genomic imbalances during transformation from follicular lymphoma to diffuse large B-cell lymphoma. Mod Pathol 2007;20:63–75.
27 Gamberi B, Gaidano G, Parsa N, *et al.* Microsatellite instability is rare in B-cell non-Hodgkin's lymphomas. Blood 1997;89:975–979.
28 Nagy M, Balazs M, Adam Z, *et al.* Genetic instability is associated with histological transformation of follicle center lymphoma. Leukemia 2000;14:2142–2148.
29 Fulop Z, Csernus B, Timar B, *et al.* Microsatellite instability and hMLH1 promoter hypermethylation in Richter's transformation of chronic lymphocytic leukemia. Leukemia 2003;17:411–415.
30 Pasqualucci L, Neumeister P, Goossens T, *et al.* Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 2001;412: 341–346.
31 Tse WT, Tang J, Jin O, *et al.* A new spectrin, beta IV, has a major truncated isoform that associates with promyelocytic leukemia protein nuclear bodies and the nuclear matrix. J Biol Chem 2001;276: 23974–23985.
32 McMahon LW, Walsh CE, Lambert MW. Human alpha spectrin II and the Fanconi anemia proteins FANCA and FANCC interact to form a nuclear complex. J Biol Chem 1999;274:32904–32908.
33 Tang Y, Katuri V, Dillner A, *et al.* Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. Science 2003;299: 574–577.
34 Kim SS, Shetty K, Katuri V, *et al.* TGF-beta signaling pathway inactivation and cell cycle deregulation in the development of gastric cancer: role of the beta-spectrin, ELF. Biochem Biophys Res Commun 2006; 344:1216–1223.
35 Greenman C, Stephens P, Smith R, *et al.* Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153–158.
36 Mullighan CG, Goorha S, Radtke I, *et al.* Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature 2007;446:758–764.