Human Pathology (2019) **85**, 251–259

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Cortactin expression in non-Hodgkin B-cell lymphomas: a new marker for the differential diagnosis between chronic lymphocytic leukemia and mantle cell lymphoma ☆

Marco Pizzi MD, PhD [a,*], Livio Trentin MD [b], Andrea Visentin MD [b], Deborah Saraggi MD [a], Veronica Martini PhD [b], Vincenza Guzzardo BS [a], Simona Righi BS [c,d], Federica Frezzato PhD [b], Francesco Piazza MD [b], Elena Sabattini MD [c,d], Gianpietro Semenzato MD [b], Massimo Rugge MD, FACG [a]

[a] *General Pathology and Cytopathology Unit, Department of Medicine—DIMED, University of Padova, Padova, 35121 Italy*
[b] *Hematology and Clinical Immunology Unit, Department of Medicine—DIMED, University of Padova, Padova, 35128 Italy*
[c] *Hematopathology Unit, Department of Hematology and Oncology, Sant'Orsola University Hospital, Bologna, 40138 Italy*
[d] *Department of Experimental Diagnostic and Specialty Medicine, Sant'Orsola University Hospital, Bologna, 40138 Italy*

Received 2 June 2018; revised 24 October 2018; accepted 31 October 2018

Keywords:
Cortactin;
Non-Hodgkin lymphoma;
Mantle cell lymphoma;
Chronic lymphocytic leukemia;
Differential diagnosis

Summary Cortactin is a cytoskeletal-remodeling adaptor protein, playing an oncogenic role in solid tumors. Little is known on cortactin expression in non-Hodgkin B-cell lymphomas (B-NHLs). The present study aimed to characterize cortactin expression in B-NHLs and to assess its role in the differential diagnosis of such entities. Cortactin protein expression was first assessed by immunohistochemistry in a series of 131 B-NHLs, including B-cell chronic lymphocytic leukemia (CLL; n = 17), mantle cell lymphoma (MCL; n = 16), follicular lymphoma (FL; n = 25), marginal zone lymphoma (MZL; n = 30), hairy cell leukemia (HCL; n = 10), splenic diffuse red pulp small B-cell lymphomas (SDRPBL; n = 3), and diffuse large B-cell lymphoma (DLBCL; n = 30) cases. Cortactin was expressed in 14 of 17 CLLs, 10 of 10 HCLs, and 22 of 30 DLBCLs. MCLs, SDRPBLs, most FLs, and MZLs were cortactin negative. The immunohistochemical results were in keeping with in silico gene expression data. In CLL, cortactin positivity did correlate with LEF1 and CD200 expression, and the combined positivity for ≥2 markers strongly predicted CLL diagnosis. Such preliminary data suggested a role for cortactin in the differential diagnosis between CLL and MCL. This hypothesis was confirmed in a large validation set of 112 CLLs (n = 55) and MCLs (n = 57), which also disclosed rare cortactin-expressing MCLs. The immunohistochemical and gene expression results were

Abbreviations: B-NHL, non-Hodgkin B-cell lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; EMZL, extranodal marginal zone lymphoma; FDC, follicular dendritic cell; GC, germinal center; HCL, hairy cell leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MFI, mean fluorescence intensity; miRNA, microRNA; MZL, marginal zone lymphoma; NMZL, nodal marginal zone lymphoma; NPV, negative predictive value; PPV, positive predictive value; SDRPBL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma.

☆ Disclosures: None to declare.

* Corresponding author at: Surgical Pathology and Cytopathology Unit, Padova University Hospital, via A. Gabelli 61, 35121 Padova (PD), Italy.
E-mail address: marco.pizzi_01@aopd.veneto.it (M. Pizzi).

https://doi.org/10.1016/j.humpath.2018.10.038

sustained by flow cytometry and Western blot analysis on CLL and MCL cell lines. In conclusion, cortactin is mainly expressed in subsets of CLL and DLBCL and in HCL. Cortactin may represent a novel marker for the differential diagnosis between CLL and MCL.

1. Introduction

Cortactin is a cytoskeletal-remodeling adaptor protein, encoded by the *CTTN* gene on chromosome 11q13 [1]. Cortactin-mediated cytoskeletal rearrangements participate to several biological processes, including the following: (i) cell motility/migration, (ii) cell-cell adhesion, (iii) extracellular matrix degradation, and (iv) membrane trafficking [2-5]. These biological effects are largely dependent on the interaction with different cytosolic proteins, including the actin-nucleation complex Arp2/3 and proteins of the WASP family [6].

Cortactin is a key regulator of several physiological processes, such as embryonic development [7], neural morphogenesis [8], and trans-endothelial leukocyte extravasation [9]. It is also involved in a variety of pathologic conditions, including infectious, inflammatory, and neoplastic diseases. In particular, cortactin overexpression has been reported in head and neck squamous cell carcinoma [10], non–small cell lung cancer [11], and gastrointestinal, genitourinary, and breast carcinomas [5,12,13]. Its upregulation has also been documented in high-grade astrocytic tumors [14] and pediatric osteosarcomas [15]. In all such neoplasms, increased *CTTN* expression has been associated with aggressive clinical behavior and poor prognosis. This is probably due to cortactin-mediated promotion of malignant cell growth, invasion, and dissemination [1].

Few data on cortactin expression in hematopoietic and lymphoid cells are available. Seminal studies demonstrated a role for this protein in megakaryocytic differentiation [16], monocytic and dendritic cell function [17], and B-/T-cell activation [18]. Among lymphoproliferative disorders, cortactin upregulation has originally been reported in cell lines of Burkitt lymphoma, multiple myeloma, and T-cell acute lymphoblastic leukemia/lymphoma [19,20]. More recently, cortactin expression has been documented in primary CLL cells [21]. Of note, such studies mainly assessed cortactin positivity by Western blot and/or quantitative reverse transcription polymerase chain reaction analyses. Only preliminary results are instead available on the protein immunohistochemical expression [21].

The present study aimed to investigate cortactin immunohistochemical expression in a large series of B-NHLs/leukemias and to assess its role in the differential diagnosis of these entities. The obtained results were integrated by Western blot and flow cytometry analysis on primary and immortalized human lymphoma cell lines and by in silico assessment of *CTTN* gene expression.

2. Materials and methods

2.1. Case selection

This retrospective study assessed cortactin immunohistochemical expression in a test set of 131 clinically annotated B-NHLs/leukemias, retrieved from the archives of the Surgical Pathology and Cytopathology Unit of Padova University Hospital (Padova, Italy). These cases include the following: (i) 25 FLs of different grades (grade 1 FL, n = 7; grade 2 FL, n = 9; grade 3 FL, n = 9), (ii) 17 CLLs, (iii) 16 MCLs, (iv) 30 MZLs of different types (EMZL, 17 cases; NMZL, 6 cases; SMZL, 7 cases), (v) 10 HCLs, (vi) 3 SDRPBLs, and (vii) 30 DLBCLs, not otherwise specified (GCB type DLBCL, 16 cases; non-GCB type DLBCL, 14 cases). Such tumor cases included nodal (CLL, FL, NMZL, DLBCL), gastric (EMZL), splenic (SMZL, HCL, and SDRPBL), and bone marrow (HCL) tissue samples. All cases were diagnosed according to the revised fourth edition of the *WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues* [22]. In particular, all FLs stained positive for Bcl6, CD10, and Bcl2; all MCLs showed positivity for cyclin D1 (15 cases) or, in its absence, for SOX11 (1 case); and all CLLs did express CD5 and CD23. In CLL cases, data on CD200 and LEF1 expression were also available.

As a validation set for the differences highlighted between CLL and MCL, a further series of 112 CLL (n = 55) and MCL (n = 57) cases were retrieved from the archives of the Hematopathology Unit of Sant'Orsola University Hospital (Bologna, Italy). The immunohistochemical analysis was performed on tissue microarrays, prepared from donor samples with the *Manual Tissue Arrayer MTA-1* (Beecher Instruments, Sun Prairie, WI), as previously described [23].

Cortactin expression was also assessed in nonneoplastic lymphoid and hematopoietic tissue samples, including the following: (i) 5 lymph nodes with reactive follicular hyperplasia; (ii) 2 spleens, removed for procedure-related reasons during major abdominal surgery; and (iii) 5 nonneoplastic bone marrow samples. Each institute ethical regulation for research on human tissues was followed, consistent with the Declaration of Helsinki.

2.2. Immunohistochemical analysis

Immunohistochemistry was performed on 4-μm-thick FFPE sections, using a primary anticortactin rabbit polyclonal antibody (Abcam, Cambridge, United Kingdom; dilution:

1:100). Heat/EDTA-based antigen retrieval methods were applied, as previously described [23]. Antigen detection was performed with the Bond Polymer Refine Detection kit in an automated immunostainer (Bond maX; Menarini, Florence, Italy). Endothelial and stromal cells were used as positive internal controls. Cortactin immunostain was semiquantitatively scored in a 4-tiered scale, as follows: score 0, negative staining; score 1+, positive staining in ≤30% of neoplastic cells; score 2+, positive staining in 31% to 60% of neoplastic cells; and score 3+, positive staining in ≥61% of neoplastic cells. Immunohistochemical reactions were independently scored by 3 pathologists (M. P., D. S., and E. S.), with excellent intraobserver/interobserver agreement ($k > 0.80$). In case of discrepancy, a consensus opinion was rendered after joint sessions at the microscope.

Double immunostain for cortactin and PAX5 was performed on 4-μm-thick FFPE sections using the anticortactin rabbit polyclonal (Abcam; see above) and anti-PAX5 rabbit monoclonal (clone SP-34; Sigma-Aldrich, Milan, Italy) antibodies in the Bond maX automated immunostainer (Bond Polymer Refine Detection kit with peroxidase for cortactin; Bond Polymer Refine Red Detection kit with phosphatase for PAX5).

For LEF1 and CD200 immunohistochemical analysis, the primary anti-LEF1 rabbit monoclonal (Abcam; clone EPR2029Y, dilution: 1:100) and anti-CD200 goat polyclonal (R&D System, Minneapolis, MN; dilution: 1:80) antibodies were used. The EnVision Flex Target Retrieval Solution High pH-based antigen retrieval method was applied. Antigen detection was performed with the Alkaline REAL Detection System Alkaline Phosphatase/RED Rabbit/Mouse (DakoCytomation, Glostrup, Denmark; code K5005) for LEF1 and with the Goat-on-Rodent-HRP-Polymer (Biocare Medical, Pacheco-CA; code GHP516L) for CD200, in an automated immunostainer (Autostainer Plus; DakoCytomation).

2.3. cDNA microarray analysis

To confirm the immunohistochemical results at a messenger RNA (mRNA) level, the Oncomine database and gene microarray analysis tool was explored (September 2018) for *CTTN* expression in low-grade and high-grade peripheral B-cell lymphoma/leukemias (http//:www.oncomine.org). Inclusion criteria for the in silico analysis were set as follows: (i) gene expression studies had to assess *CTTN* mRNA levels in both CLL, MCL, FL, MZL, HCL, and DLBCL (both GCB and non-GCB type) and (ii) *CTTN* expression data had to be assessed in multiple cases for each considered entity. Such inclusion criteria were met by one data set for low-grade B-cell lymphomas [24] and one data set for DLBCL [25].

2.4. B-NHL lines

The immunohistochemical and gene expression results were further confirmed by Western blot analysis on the following: (i) 2 MCL cell lines (REC-1 and Jeko cells), (ii) 1 CLL immortalized cell line (MEC-1 cells), and (iii) primary CLL cells from the peripheral blood of untreated patients (n = 5 for Western blot studies, n = 20 for flow cytometry analysis). Cell lines were cultured in RPMI 1640 with 10% fetal bovine serum and 5% penicillin/streptavidin. They were routinely maintained at 37°C in a humidified atmosphere, containing 5% CO_2/95% air. Control, untouched CD19-positive B cells from 4 healthy donors were isolated from buffy coat by negative selection using the RosetteSep for B-cell isolation kit (StemCell Technologies, Vancouver, Canada).

2.5. Western blot analysis

For Western blot analysis, 5×10^5 cells for each assay were prepared by cell lyses with Tris 20 mM, NaCl 150 mM, EDTA 2 mM, EGTA 2 mM, and Triton X-100 0.5% supplemented with complete protease inhibitor cocktail (Roche, Mannheim, Germany) and sodium orthovanadate 1 mM (Calbiochem, Gibbstown, NJ). Total proteins were separated in SDS-PAGE, transferred to nitrocellulose membranes, and stained with a primary anticortactin polyclonal antibody (Abcam; dilution 1:1000) and an anti–β-actin monoclonal antibody (Sigma-Aldrich, St Louis, MO; dilution 1:1000). Cortactin expression was titrated on endogenous β-actin, and the cortactin/β-actin ratio was used to compare the protein levels in different cell lines. In detail, cortactin expression was stratified as follows: (i) low expression, cortactin/β-actin ratio <1.00; (ii) moderate expression, cortactin/β-actin ratio between 1.00 and 2.00; and (iii) high expression, cortactin/β-actin ratio >2.00.

2.6. Flow cytometry analysis

Flow cytometry was performed on the following: (i) purified CD5+/CD19+ cells from 20 CLL patients, (ii) CD19+ cells from 4 healthy donors, and (iii) CLL (ie, MEC-1) and MCL (ie, REC-1 and Jeko) cells lines. The FACS CANTO A cytometer (Becton Dickinson, Mountain View, CA) was used. In short, cells were fixed and permeabilized with the Fix & Perm kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The fluorescein isothiocyanate–conjugated anti-CD5 (Becton Dickinson Biosciences, San Jose, CA), the allophycocyanin-conjugated anti-CD19 (Becton Dickinson Biosciences, San Jose, CA), the Alexa488-conjugated anticortactin (Millipore, Burlington, MA), and the fluorescein isothiocyanate–conjugated anti-mouse IgG1 (Millipore) antibodies were used to stain the cell suspensions. The cytometric analysis was based on gating intact cells by forward light scatter *versus* right-angle light scatter and, as a second step, on assessing cortactin expression in CD19+/CD5+ CLL and control cells. The ratio between cortactin MFI and control IgG1 was also calculated and used to stratify each case according to cortactin positivity, as previously described [21]. A minimum of 30 000 total events were acquired per tube.

2.7. Statistical analysis

Differences in cortactin immunohistochemical expression rates were assessed using the Fisher exact test. Differences in Western blot and flow cytometry expression levels were tested using the analysis of variance test, whereas differences in mean *CTTN* mRNA levels from in silico gene expression data sets were assessed using the Student *t* test for independent means. Statistical analysis was performed with the R statistical software (http://www.r-project.org). For each test, differences were considered statistically significant for *P* values less than .05.

3. Results

3.1. Cortactin expression in normal lymphoid tissue and bone marrow

In lymph nodes with reactive lymphoid hyperplasia, cortactin was strongly expressed in the cytoplasm of endothelial and smooth muscle cells of both lymphatic sinuses and blood vessels. High-endothelial venules and stromal cells of the paracortical areas were also uniformly positive. Within GCs, cortactin immunostain disclosed an onion skin–like pattern, akin to the one observed with follicular dendritic cell (FDC) markers (CD21, CD23, and CD35; Fig. 1A). Scattered cells within GCs disclosed cytoplasmic positivity for cortactin, but double immunostaining for cortactin and PAX5 did exclude a B-cell origin for such elements (Fig. 1B). Mantle, marginal, and paracortical zones were consistently cortactin negative (Fig. 1A and B).

In nonneoplastic spleens, cortactin staining recapitulated the nodal findings. In the white pulp, cortactin was expressed by FDCs and stromal and endothelial cell. Lymphoid cells within the GCs and mantle and marginal zones were almost completely negative. In the red pulp, diffuse protein expression was observed in littoral cells of venous sinuses and endothelial and stromal cells (Fig. 1C).

In the bone marrow, cortactin expression was restricted to megakaryocytes and isolated myeloid/histoid cells. Scattered stromal cells were also positive, whereas interstitial lymphocytes did not show any protein positivity (Fig. 1D).

3.2. Cortactin expression in small B-NHLs/leukemias

Cortactin immunohistochemical expression was assessable in all small B-NHL cases (Table). In detail, diffuse cytoplasmic

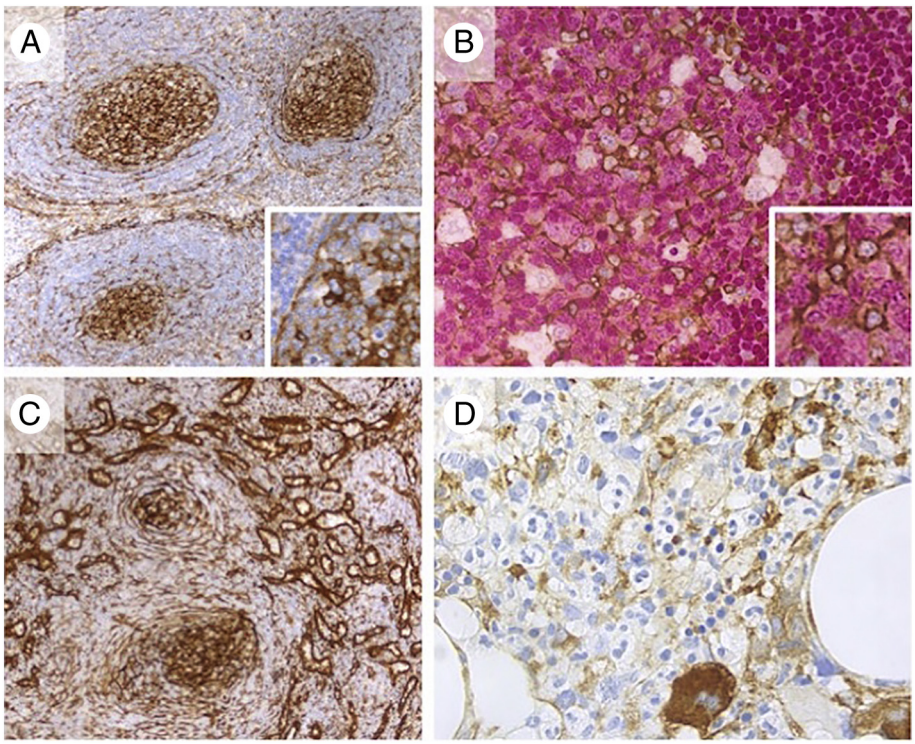

Fig. 1 Cortactin expression in normal lymphoid and hematopoietic tissue. A, In reactive lymph nodes, cortactin positivity was found in FDCs, endothelial cells, and paracortical stromal cells. Most lymphocytes were cortactin negative, with only scattered positivity in GC lymphoid cells (insert). B, Double immunostain for cortactin (brown) and PAX5 (red) disclosed that cortactin-positive lymphoid cells did not coexpress the pan–B-cell marker PAX5 (insert). C, In the nonneoplastic spleen, cortactin was expressed in both the white and the red pulp. The white pulp pattern paralleled the lymph node findings. The red pulp showed strong cortactin positivity in littoral cells (splenic sinusoids), endothelial cells (red pulp microvessels), and perivascular stromal cells. D, In normal bone marrow, cortactin expression was restricted to megakaryocytes (lower right), scattered stromal and rare myeloid cells. Immunoperoxidase and phosphatase stain, original magnifications ×10, ×20, and ×40.

Table Cortactin expression in a test set of 131 B-NHLs/leukemias

	Cortactin immunohistochemical score				Total (%)
	Score 0	Score 1+	Score 2+	Score 3+	
CLL	3 (17.6)	7 (41.2)	1 (5.9)	6 (35.3)	17 (100.0)
MCL	16 (100.0)	0 (–)	0 (–)	0 (–)	16 (100.0)
FL	23 (92.0)	0 (–)	0 (–)	2 (8.0)	25 (100.0)
NMZL	1 (16.7)	5 (85.7)	0 (–)	0 (–)	6 (100.0)
EMZL	10 (58.8)	7 (41.2)	0 (–)	0 (–)	17 (100.0)
SMZL	6 (85.7)	1 (14.3)	0 (–)	0 (–)	7 (100.0)
SDRPBL	3 (100.0)	0 (–)	0 (–)	0 (–)	3 (100.0)
HCL	0 (–)	0 (–)	0 (–)	10 (100)	10 (100)
DLBCL					
GCB type [a]	4 (13.3)	4 (13.3)	3 (10.0)	5 (16.7)	16 (53.3)
Non-GCB type [a]	4 (13.3)	2 (6.7)	3 (10.0)	5 (16.7)	14 (46.7)

NOTE. Values inside parentheses refer to the percentage of cases for each subgroup.
[a] According to Hans algorithm.

positivity was documented in 14 of 17 CLL cases (score 1+, 7/17 cases; 2+, 1/17 case; score 3+, 6/17 cases), with complete negativity (score 0) in the remaining 3 of 17 samples. The protein was positive in both the small cells and the paraimmunoblasts of proliferation centers. Cortactin positivity correlated with LEF1 and CD200 expression. Of note, cortactin immunostain did not correlate with any clinical, prognostic, molecular and/or cytogenetic feature of the analyzed cases. Moreover, no differences in the overall and progression-free survival were noted between cortactin-positive and cortactin-negative cases (Supplementary Table 1 and Supplementary Fig. 1).

A subset of MZLs (score 1+, 13/30 cases) stained positive for the protein, whereas none of the MCL and SDRPBL cases turned out to be positive. Of note, cortactin was expressed (score 3+) in all HCL cases (both splenic and bone marrow samples) and in 2 of 25 FLs (Fig. 2A-M; Table). The remaining 23/25 FL cases only disclosed scattered cortactin-positive lymphoid elements of non–B-cell lineage (Supplementary Fig. 2).

Statistical analysis disclosed higher cortactin expression rates in CLL and HCL compared with MCL and FL (Fisher exact test, $P < .05$). The protein was also more frequently expressed in CLL compared with MZL (considered as a group), but statistical differences according to the MZL subtype did not provide any consistent result.

From a diagnostic point of view, the immunohistochemical results suggested a role for cortactin mainly in the differential diagnosis between CLL and MCL. To confirm these preliminary results, cortactin expression was assessed in a validation set of 112 CLL (n = 55) and MCL (n = 57) cases, retrieved from the archives of the Hematopathology Unit of Sant'Orsola University Hospital. Cortactin positivity was documented in 18 of 55 CLL (score 1+, 13/55; score 2+, 4/55; score 3+, 1/55) and in 5 of 57 MCL (score 1+, 4/57; score 3+, 1/57) cases. The differences in protein expression rates were statistically significant (Fisher exact test, $P < .01$), and cortactin was confirmed as a highly specific marker (91.2%) of CLL. The sensitivity (32.7%), positive predictive value (PPV; 78.3%), and negative predictive value (NPV; 58.4%) of such marker were, however, lower than in the test set.

These results prompted to investigate the hypothesis that the joint assessment of cortactin and other CLL-specific markers (ie, LEF1 and CD200) better differentiates CLL from MCL. Indeed, the expression of 2 or more of such proteins disclosed very high sensitivity (89.1%), specificity (100%), PPV (100%), and NPV (90.5%) for the diagnosis of CLL. Overall, these results indicate cortactin as a novel CLL-specific marker, significantly contributing to the differential diagnosis with MCL.

3.3. Cortactin immunohistochemical expression in DLBCL

Cortactin expression was documented in 22 of 30 DLBCLs, not otherwise specified (12/16 GCB-type and 10/14 non–GCB-type cases; Table). In detail, the protein was expressed with moderate (score 2+) to strong (score 3+) intensity in 16 of 30 cases, without statistically significant differences between GCB and non-GCB cases. Weak (score 1+)

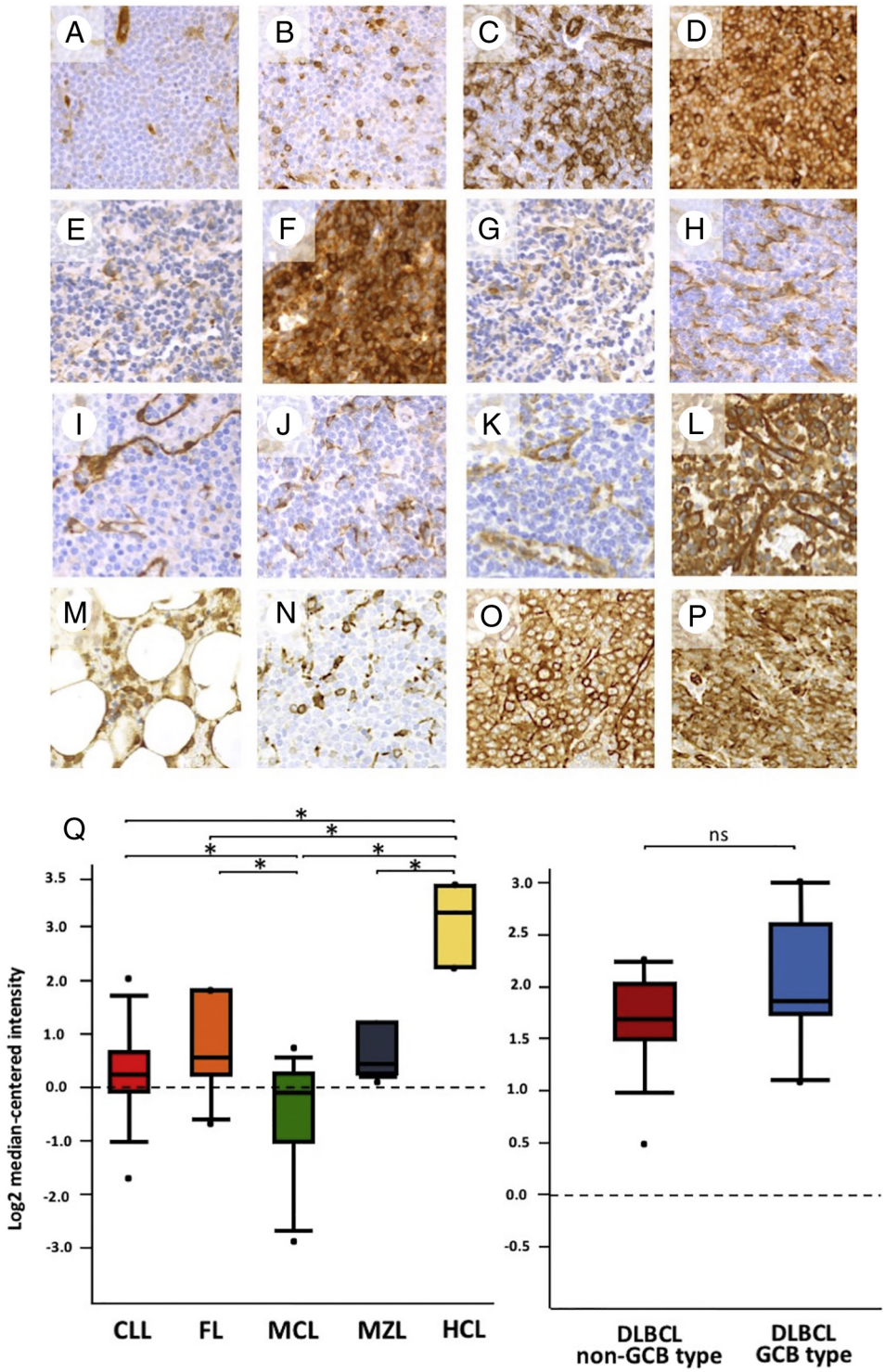

Fig. 2 Cortactin expression in non-Hodgkin small B-cell lymphomas. A-D, CLL was characterized by variable cortactin expression, spanning from complete negativity (A) to weak (B), moderate (C), and strong (D) positivity. E-F, The majority of FL was cortactin negative (E), with only rare cases featuring strong protein expression (F). G-K, Nodal (G), extranodal (H), and splenic (I) MZLs were characterized by low-to-absent cortactin expression. Similar results were reported in MCL (J) and SDRPBL (K). L and M, All tested HCL cases disclosed strong and diffuse cortactin positivity in both splenic (L) and marrow (M) samples. N-P, DLBCL showed variable cortactin expression, from complete negativity (N) to strong positivity in both GCB (O) and non-GCB cases (P). In silico gene expression analysis (Oncomine database) disclosed the highest *CTTN* mRNA levels in HCL. CLL and FL were characterized by higher *CTTN* expression compared with MCL. Differences with MZL were not statistically significant. Parallel studies in DLBCL showed high *CTTN* mRNA levels in both non-GCB and GCB samples, without statistically significant differences between the subgroups. A-P, Immunoperoxidase stain, original magnification ×20. Q, **P* < .05. ns, not statistically significant.

cortactin positivity was instead detected in 4 of 16 GCB-type and in 2 of 14 non GCB-type DLBCLs (Fig. 2N-P). Cortactin expression did not significantly correlate with any clinical-prognostic parameter.

3.4. *CTTN* gene expression profile in B-NHLs/leukemias

In silico gene expression analysis of the available data set of small B-cell lymphomas/leukemias [24] disclosed higher *CTTN* mRNA levels in CLL and FL compared with MCL (*t* test, $P = .02$, respectively). Differences between CLL and FL were not statistically significant. MZL also featured higher *CTTN* mRNA levels than MCL, but such a difference did not reach statistical significance (Student *t* test, $P = .86$). Of note, HCL was associated with the highest *CTTN* expression levels (Student *t* test, $P < .05$; Fig. 2Q).

As for the DLBCL cases [25], *CTTN* mRNA levels were significantly higher compared with control samples, whereas differences between GCB and non-GCB cases were not statistically significant (Fig. 2Q).

3.5. Western blot and flow cytometry analysis on MCL and CLL cells

Cortactin expression was further assessed by Western blot analysis on immortalized MCL (REC-1 and Jeko) and CLL (MEC-1) lines, and on primary lymphocytes from the peripheral blood of 5 CLL patients and 3 healthy donors. In keeping with the immunohistochemical data, REC-1, Jeko, and nonneoplastic B cells were consistently cortactin negative. By contrast, 4 of 5 primary CLL cells and the MEC-1 line disclosed variable protein positivity (Fig. 3A and B).

In line with the immunohistochemical and Western blot results, flow cytometry disclosed very low to absent cortactin expression (MFI < 300) in normal B cells, MCL lines (ie, REC-1 and Jeko), and in a subset of primary CLL samples (n = 7). By contrast, MEC-1 cells and most primary CLL samples (n = 13) featured moderate to strong protein positivity (MFI > 445; Fig. 3C).

4. Discussion

The present study highlights remarkable differences in cortactin expression among B-NHLs/leukemias and, for the first time, pinpoints immunohistochemistry as a valuable tool for cortactin assessment in lymphoid tissues.

In nonneoplastic lymphoid samples, cortactin expression was largely restricted to stromal cells, FDCs, and scattered GC lymphocytes of non–B-cell lineage. Among low- and high-grade B-cell lymphomas/leukemias, cortactin was expressed in HCLs, in most CLLs and DLBCLs, in a proportion of MZLs (mostly at low scores), and in rare cases of FL. Although CLL positivity is in keeping with previously reported data [21], cortactin expression in HCL, DLBCL, FL, and MZL has not been reported in the literature so far.

In our series, cortactin expression did not correlate with any clinical/prognostic features of CLL and DLBCL (ie,

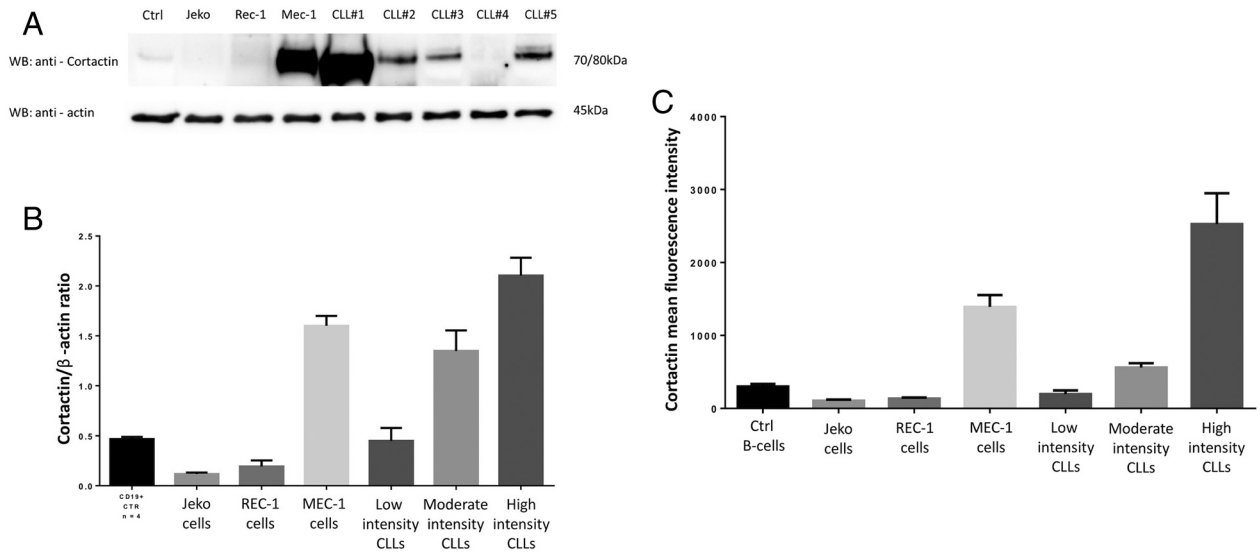

Fig. 3 Western blot and flow cytometry analysis in MCL and CLL lines. A, Western blot analysis disclosed consistent negativity for cortactin in both MCL cell lines (Jeko and REC-1) and control B-lymphocytes. In line with the immunohistochemical results, primary (CLL#1, CLL#2, CLL#3, CLL#4, CLL#5) and immortalized (MEC-1) CLL cells showed variable protein expression. B, Densitometric analysis (cortactin/β-actin ratio) in normal B lymphocytes (Ctrl B cell; n = 4), Jeko, REC-1, and MEC-1 cell lines, and in CD19+/CD5+ B cells from CLL patients with low (n = 7), moderate (n = 10), and high (n = 3) cortactin expression. Data obtained were evaluated for their statistical significance with the analysis of variance test (****$P < .0001$). C, By flow cytometry, MFI for cortactin was evaluated in normal B lymphocytes (Ctrl B cell; n = 4), Jeko, REC-1, and MEC-1 cell lines, and in CD19+/CD5+ B cells from CLL patients with low-intensity (n = 7), moderate-intensity (n = 10), and high-intensity (n = 3) cortactin expression. Data obtained were evaluated for their statistical significance with the analysis of variance test (****$P < .0001$).

neoplasms with the highest variability of protein expression). Although these results limit the prognostic value of cortactin expression in B-NHL, our data pinpoint important diagnostic implications for such marker. In particular, cortactin may represent a novel, additional marker for (i) the diagnosis of HCL (together with CD103, CD123, CD11c, annexin A1, CD25, DBA44, cyclin D1, and BRAFV600E) [26] and (ii) the differential diagnosis between CLL and MCL.

CLL and MCL are B-NHLs characterized by similar morphologic features (small- to medium-sized mature B cells) and partially overlapping immunophenotype (eg, aberrant positivity for CD5) [27]. The differential diagnosis between these entities relies on a limited number of immunohistochemical markers (CD23, LEF1, and CD200 positivity in CLL; cyclin D1 and SOX11 positivity in MCL), but aberrant phenotypes have been reported [28,29]. In this context, cortactin expression would strongly sustain a diagnosis of CLL over MCL, particularly in association with other CLL markers (ie, LEF1 and CD200). The positivity for 2 or more of such proteins is indeed associated with very high sensitivity (89.1%), specificity (100%), PPV (100%), and NPV (90.5%).

To further confirm the immunohistochemical results, we investigated cortactin expression on immortalized MCL and CLL cell lines and in circulating lymphocytes from CLL patients. Western blot analysis confirmed consistent cortactin negativity in MCL cells (Jeko and REC-1) and disclosed moderate to strong protein positivity in most of the analyzed CLL cells. The immunohistochemical and Western blot results were sustained by flow cytometry and in silico gene expression data.

The latter analysis showed higher *CTTN* levels in CLL compared with MCL. In keeping with the immunohistochemical results, HCL was associated with the highest *CTTN* mRNA levels, whereas differences between MZL and all other B-cell lymphomas were not statistically significant. Of note, FL was characterized by higher *CTTN* expression than MCL. This finding is in contrast with the immunohistochemical results and suggests the existence of posttranscriptional regulatory mechanisms for cortactin expression. Although the nature of such molecular events is still unknown, an explanatory hypothesis may come from deranged microRNA (miRNA) expression profiles. miRNA are a class of small noncoding RNAs that bind to target mRNAs, blocking their translation [30]. Aberrant miRNA expression profiles have been reported in several B-cell lymphomas, including FL [31]. Of note, 3 of the most deregulated miRNAs in FL have high affinity scores for the *CTTN* gene (miR-96, miR-221, and miR-502-3p) [32]. The aberrant expression of these molecules might thus explain the observed discrepancy between cortactin immunohistochemical expression and *CTTN* mRNA levels. By contrast, the rare cortactin-positive FL of our series may belong to a recently described subset of cases, characterized by low miR-96, miR-221, and/or miR-502-3p expression [32]. Additional studies will elucidate these possible pathobiological mechanisms.

Another intriguing finding of our study is the heterogeneous relation of cortactin expression in B-NHL and their nonneoplastic counterpart. FL and MCL were characterized by complete protein negativity or weak positivity, akin to benign GC and mantle zone cells. On the contrary, CLL disclosed more variable expression patterns, possibly stemming from the heterogeneous biological origin of this tumor. CLL is indeed characterized by distinct molecular subtypes (ie, CLL with unmutated and mutated *IGVH* genes), putatively originating from different nonneoplastic counterparts (ie, pre-GC and post-GC mature B cells, respectively) [27,33]. This heterogeneity (together with the variety of CLL-associated cytogenetic and molecular derangements) may explain the variability in cortactin expression of this tumor.

In conclusion, the present study highlights cortactin as a new marker of CLL, HCL, and DLBCL. In particular, cortactin may contribute to the differential diagnosis between CLL and MCL, 2 neoplasms with similar histologic features but very different clinical outcome [27]. Further studies are needed to clarify the molecular mechanisms of deranged cortactin expression in MCL and CLL and to investigate any possible relationship between cortactin status and the biological features of these lymphomas.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.038.

CRediT authorship contribution statement

Marco Pizzi: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Livio Trentin:** Conceptualization, Funding acquisition, Investigation. **Andrea Visentin:** Conceptualization, Data curation, Formal analysis. **Deborah Saraggi:** Data curation. **Veronica Martini:** Investigation, Writing - original draft. **Vincenza Guzzardo:** Methodology. **Simona Righi:** Methodology. **Federica Frezzato:** Investigation. **Francesco Piazza:** Data curation. **Elena Sabattini:** Investigation, Validation. **Gianpietro Semenzato:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing. **Massimo Rugge:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

References

[1] Kirkbride KC, Sung BH, Sinha S, Weaver AM. Cortactin: a multifunctional regulator of cellular invasiveness. Cell Adh Migr 2011;5:187-98.
[2] Bryce NS, Clark ES, Leysath JL, Currie JD, Webb DJ, Weaver AM. Cortactin promotes cell motility by enhancing lamellipodial persistence. Curr Biol 2005;15:1276-85.
[3] Kowalski JR, Egile C, Gil S, Snapper SB, Li R, Thomas SM. Cortactin regulates cell migration through activation of N-WASP. J Cell Sci 2005;118:79-87.

[4] Shen HL, Liu QJ, Yang PQ, Tian Y. Protein interactions of cortactin in relation to invadopodia formation in metastatic renal clear cell carcinoma. Tumour Biol 2015;36:3417-22.
[5] Lin CW, Sun MS, Liao MY, et al. Podocalyxin-like 1 promotes invadopodia formation and metastasis through activation of Rac1/Cdc42/cortactin signaling in breast cancer cells. Carcinogenesis 2014;35:2425-35.
[6] Uruno T, Liu J, Zhang P, et al. Activation of Arp2/3 complex–mediated actin polymerization by cortactin. Nat Cell Biol 2001;3:259-66.
[7] Yu D, Zhang H, Blanpied TA, Smith E, Zhan X. Cortactin is implicated in murine zygotic development. Exp Cell Res 2010;316:848-58.
[8] MacGillavry HD, Kerr JM, Kassner J, Frost NA, Blanpied TA. Shank-cortactin interactions control actin dynamics to maintain flexibility of neuronal spines and synapses. Eur J Neurosci 2016;43:179-93.
[9] Schnoor M. Endothelial actin-binding proteins and actin dynamics in leukocyte transendothelial migration. J Immunol 2015;194:3535-41.
[10] Timpson P, Wilson AS, Lehrbach GM, Sutherland RL, Musgrove EA, Daly RJ. Aberrant expression of cortactin in head and neck squamous cell carcinoma cells is associated with enhanced cell proliferation and resistance to the epidermal growth factor receptor inhibitor gefitinib. Cancer Res 2007;67:9304-14.
[11] Noh SJ, Baek HA, Park HS, et al. Expression of SIRT1 and cortactin is associated with progression of non–small cell lung cancer. Pathol Res Pract 2013;209:365-70.
[12] Ni QF, Yu JW, Qian F, Sun NZ, Xiao JJ, Zhu JW. Cortactin promotes colon cancer progression by regulating ERK pathway. Int J Oncol 2015;47:1034-42.
[13] Hou H, Zhao L, Chen W, et al. Expression and significance of cortactin and HDAC6 in human prostatic foamy gland carcinoma. Int J Exp Pathol 2015;96:248-54.
[14] Wang L, Zhao K, Ren B, et al. Expression of cortactin in human gliomas and its effect on migration and invasion of glioma cells. Oncol Rep 2015;34:1815-24.
[15] Folio C, Zalacain M, Zandueta C, et al. Cortactin (CTTN) overexpression in osteosarcoma correlates with advanced stage and reduced survival. Cancer Biomark 2011;10:35-41.
[16] Zhan X, Haudenschild CC, Ni Y, Smith E, Huang C. Upregulation of cortactin expression during the maturation of megakaryocytes. Blood 1997;89:457-64.
[17] Woollard SM, Li H, Singh S, Yu F, Kanmogne GD. HIV-1 induces cytoskeletal alterations and Rac1 activation during monocyte-blood-brain barrier interactions: modulatory role of CCR5. Retrovirology 2014;11:20.
[18] von Holleben M, Gohla A, Janssen KP, Iritani BM, Beer-Hammer S. Immunoinhibitory adapter protein Src homology domain 3 lymphocyte protein 2 (SLy2) regulates actin dynamics and B cell spreading. J Biol Chem 2011;286:13489-501.
[19] Vacca A, Ria R, Presta M, et al. alpha(v)beta(3) integrin engagement modulates cell adhesion, proliferation, and protease secretion in human lymphoid tumor cells. Exp Hematol 2001;29:993-1003.
[20] Passaro D, Irigoyen M, Catherinet C, et al. CXCR4 is required for leukemia-initiating cell activity in T cell acute lymphoblastic leukemia. Cancer Cell 2015;27:769-79.
[21] Martini V, Gattazzo C, Frezzato F, et al. Cortactin, a Lyn substrate, is a checkpoint molecule at the intersection of BCR and CXCR4 signalling pathway in chronic lymphocytic leukaemia cells. Br J Haematol 2017;178:81-93.
[22] Swerdlow SH, Campo E, Harris NL, et al. World Health Organization, International Agency for Research on Cancer. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: International Agency for Research on Cancer; 2017.
[23] Pizzi M, Agostinelli C, Righi S, et al. Aberrant expression of CD10 and BCL6 in mantle cell lymphoma. Histopathology 2017;71:769-77.
[24] Fernandez V, Salamero O, Espinet B, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res 2010;70:1408-18.
[25] Compagno M, Lim WK, Grunn A, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 2009;459:717-21.
[26] Troussard X, Cornet E. Hairy cell leukemia 2018: update on diagnosis, risk-stratification, and treatment. Am J Hematol 2017;92:1382-90.
[27] Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. Blood 2018;131(21):2283-96.
[28] Kelemen K, Peterson LC, Helenowski I, et al. CD23+ mantle cell lymphoma: a clinical pathologic entity associated with superior outcome compared with CD23-disease. Am J Clin Pathol 2008;130:166-77.
[29] Seto M. Cyclin D1–negative mantle cell lymphoma. Blood 2013;121:1249-50.
[30] Musilova K, Mraz M. MicroRNAs in B-cell lymphomas: how a complex biology gets more complex. Leukemia 2015;29:1004-17.
[31] Gebauer N, Gollub W, Stassek B, et al. MicroRNA signatures in subtypes of follicular lymphoma. Anticancer Res 2014;34:2105-11.
[32] Wang W, Corrigan-Cummins M, Hudson J, et al. MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. Haematologica 2012;97:586-94.
[33] Visentin A, Facco M, Frezzato F, et al. Integrated CLL scoring system, a new and simple index to predict time to treatment and overall survival in patients with chronic lymphocytic leukemia. Clin Lymphoma Myeloma Leuk 2015;15:612-620.e611–615.