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From Cell Biology to Tissue Engineering

Aberrant expression of napsin A in a subset of malignant lymphomas

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Summary. Background: Napsin A is commonly expressed in pulmonary adenocarcinomas and some renal cell carcinomas. However, napsin A expression in lymphoid neoplasms has never been reported. Methods: Glycoproteomic analyses of lymphoma-derived cell lines revealed napsin A expression in anaplastic large cell lymphoma (ALCL) cells. We thus investigated napsin A expression in lymphoid neoplasms. A variety of lymphomas (n=672) and histiocytic tumors (n=55) was immunostained for napsin A using patient tissues. Results: In reactive lymphoid tissues, only a few histiocytes were positive for napsin A. ALK-positive ALCLs most frequently expressed napsin A (34.4%, 11/32 cases) at a rate that was significantly higher compared with ALK-negative ALCL (8.6%, 3/35; P=0.015). Napsin A expression was also observed in 13.4% (20/149) of diffuse large B-cell lymphomas (DLBCL), 11.1% (15/134) of Hodgkin lymphomas, 4.9% (2/41) of follicular lymphomas, 6% (4/67) of peripheral T-cell lymphomas, and 3.8% (1/26) of plasma cell neoplasms. Otherwise, napsin A was not detected in any other types of lymphomas or histiocytic neoplasms. Napsin A expression in systemic ALCL was associated with a higher international prognostic index. ALCL and DLBCL patients with napsin A expression tended to have poor prognosis. Conclusion: These results demonstrated that napsin A is aberrantly expressed in a subset of lymphomas. The biological significance of napsin A in lymphomas warrants further study.

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Introduction

Napsin A is a recently identified molecule belonging to the aspartic protease family, which includes pepsin, gastricsin, renin, cathepsin D, and cathepsin E (Cronshaw et al., 2003). In humans, napsin A expression is restricted to the pneumocytes of the lung and the proximal and convoluted tubular epithelial cells of the kidney under physiological conditions (Schauer-Vukasinovic et al., 1999). Alveolar macrophages of the lung also ingest napsin A secreted by the pneumocytes and show immunoreactivity for napsin A (Mori et al., 2002). Napsin A plays an important role in the production and maturation of surfactant B protein by type II pneumocytes in the lung and functions as a lysosomal protease involved in protein catabolism in the kidney (Mori et al., 2002; Brasch et al., 2003). Moreover, napsin A is expressed in a majority of pulmonary adenocarcinomas and some renal cell carcinomas (Ordonez, 2012). Therefore, napsin A is considered a relatively specific and sensitive marker for pulmonary adenocarcinoma. Although napsin A has been observed in sporadic cases of adenocarcinomas other than those of lung and kidney origin, its expression in hematolymphoid neoplasm has not been previously addressed.

Protein glycosylation is one of the most common post-translational modifications, which takes the form of O-linked and N-linked glycosylation. Among these modifications, N-linked glycosylation at the N-X-S/TT

(N, asparagine; X, any amino acid other than proline; S, serine; T, threonine) motif of peptides destines proteins to be expressed on cellular membranes or to be secreted (Spiro, 2002). Therefore, N-linked glycoproteins are regarded as useful diagnostic and therapeutic biomarkers (Durand and Seta, 2000; Zhang et al., 2003). To discover new diagnostic, prognostic, and potential therapeutic markers in malignant lymphoma, we performed unbiased N-linked glycoprotein analysis using a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based glycoproteomic approach in a variety of lymphoma-derived cell lines. We observed that napsin A is expressed in several anaplastic large cell lymphoma cell lines and a follicular lymphoma cell line. We thus hypothesized that napsin A might be aberrantly expressed in a subset of malignant lymphomas. Therefore, we comprehensively investigated napsin A expression in a large cohort of lymphoid, dendritic, and histiocytic neoplasms using primary tumor tissues and analyzed the clinicopathological relevance of napsin A expression in these tumors.

Materials and methods

Glycoproteomic analysis of lymphoma-derived cell lines

A variety of lymphoma-derived cell lines including NPM-ALK-positive anaplastic large cell lymphoma (ALCL; DEL, Karpas299, SR786, SUDHL1, and SupM2), ALK-negative ALCL (MAC1 and MAC2A), cutaneous T-cell lymphoma (HH and Hut78), T-acute lymphoblastic lymphoma (Jurkat), extranodal NK/T-cell lymphoma (NKTCL; YT and NK-92MI), Burkitt lymphoma (BL; BJAB, Raji, and Ramos), follicular lymphoma (FL; FL18, FL218, FL318, and FL518), diffuse large B-cell lymphoma (DLBCL; OCI-LY1, SUDHL4, and OCI-LY2), mantle cell lymphoma (MCL; Granta, Jeko1, NCEB1, REC1, and UPN1), classical Hodgkin lymphoma (HL; KMH2, L1238, and L428), nodular lymphocyte predominant Hodgkin lymphoma (NLPHL; DEV) and primary mediastinal large B-cell lymphoma (PMBL; K1106 and MEDB1) cell lines, were subjected to glycoproteomic analysis at the University of Michigan, Ann Arbor (Professor MS Lim and KSJ Elenitoba-Johnson's laboratory). In brief, the N-linked glycopeptides were enriched using a protocol for the solid-phase extraction of glycoproteins/peptides (SPEG), as described previously, with appropriate modification for large-scale analysis (Tian et al., 2007). Peptide identification was performed by LC-MS/MS using an LTQ OrbitrapXL (ThermoFisher, San Jose, CA) in-line with a Paradigm MS2 HPLC (MichromBioresources, Auburn, CA). Subsequently, through proteomic database searches and bioinformatic processes using PeptideProphet and ProteinProphet, lists of unique peptides containing at least one N-glycosylation site and glycoproteins were obtained. Finally, the biological and technical triplicates of each cell line were merged, and a spectral count for each glycoprotein was extracted using Abacus software (Fermin et al., 2011).

Patients and tissue samples

Tissue samples from 672 malignant lymphoma cases and 55 dendritic and histiocytic neoplasm cases were retrieved. All of the available pathologic materials were reviewed by two hematopathologists (Y.K.J. and C.W.K.) according to the current World Health Organization (WHO) criteria (Swerdlow et al., 2008). The tissue samples included the following types of tumor: HL (n=134), ALCL (n=84), DLBCL (n=149), BL (n=11), FL (n=41), MCL (n=46), marginal zone B-cell lymphoma (MZBCL; n=56), chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL; n=46), plasmablastic lymphoma (PBL; n=6), plasma cell neoplasm (PCN; n=26), angioimmunoblastic T-cell lymphoma (AITL; n=37), peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS; n=30), NKTCL (n=20), T-lymphoblastic lymphoma (T-LBL; n=19), B-lymphoblastic lymphoma (B-LBL; n=10), follicular dendritic cell sarcoma (FDCS; n=28), histiocytic sarcoma (HS; n=5), and Langerhans cell histiocytosis (LCH; n=22). The clinical data were extracted from the medical records. Samples of reactive lymph nodes (n=4), tonsils (n=2), thymus (n=2), and spleen (n=2) were also examined as control tissues.

Immunohistochemistry

A tissue microarray (TMA) containing samples 2 mm in diameter was constructed using formalin-fixed paraffin-embedded (FFPE) tumor blocks from B-cell non-Hodgkin lymphomas, HLs, LBLs, FDCSs, and a portion of the ALCLs. The TMA was subjected to immunohistochemical staining. Otherwise, representative FFPE blocks containing excised or biopsied tissue were used. Immunohistochemistry (IHC) was performed using napsin A (clone IP64, 1:200, Novocastra, Leica Microsystems, Wetzlar, Germany) and ALK (clone ALK1, DAKO Cytomation, Glostrup, Denmark) antibodies with the *Leica* BOND-MAX automated immunostainer (Leica microsystems). The proportion of napsin A-expressing lymphoma cells in each sample was semi-quantitatively assessed by two pathologists (S.J.N. and Y.K.J) using the following 5 categories; 0, 1-25%, 26-50%, 51-75%, and 76-100%, and consensus was made in cases with discrepancy.

Statistical analysis

All of the statistical analyses were performed using SPSS 21 (IBM Corp., New York, USA). To evaluate the differences between the variables, either Pearson's χ^2 test or Fisher's exact test was used. In addition, the correlations between variables were evaluated using Spearman's test. Survival analyses were exclusively performed for the 44 patients with systemic ALCL and the 149 patients with DLBCL using the Kaplan-Meier model with a log rank test or Taron-Ware test. Two-sided P-values of <0.05 were considered statistically significant.

Results

Detection of napsin A in lymphoma cell lines using glycoproteomic analysis

N-linked glycoproteomic analysis identified napsin A peptides in three (DEL, SR786 and SupM2) of the five ALK-positive ALCL cell lines and one (MAC2A) of the two ALK-negative cell lines with spectral count ranging from 19 to 90 (Fig. 1A). Additionally, one FL cell line (FL518) exhibited napsin A expression with a low spectral count. Napsin A protein was found to have several N-linked glycosylation motifs within its amino acid sequence. Consistent with the glycoproteomic data, Western blotting analysis revealed increased napsin A protein expression in the DEL and SupM2 cell lines and reduced expression in the Karpas299 cell line compared with the levels observed in the lung adenocarcinoma cell lines A549 and HCC827, which were used as positive controls (Fig. 1B).

Napsin A expression pattern in non-neoplastic lymphoid tissues

In non-neoplastic lymphoid tissues, napsin A expression was rarely positive, only a few histiocytes in the tonsils and lymph nodes with a weak cytoplasmic dot-like pattern (Fig. 2A,B). No napsin A-positive cell was observed in the spleen or the thymus. Napsin A was not expressed in the reactive lymphocytes in the follicular or paracortical zone of the tonsils and lymph nodes.

Napsin A expression pattern in various malignant lymphomas and dendritic and histiocytic neoplasms

Among all the lymphoid and histiocytic neoplasms, napsin A expression was observed in a subset of ALCL, classical HL, and DLBCL samples, and rarely observed in FL, PCN, AITL and PTCL-NOS cases (Table 1). Napsin A expression demonstrated a granular or dot-like paranuclear pattern in the cytoplasm in variable proportions of the tumor cells (Fig. 2C-H). In general, a positive correlation was observed between the percentage of immunoreactive tumor cells and the labeling intensity. Few, if any, non-neoplastic reactive cells exhibited napsin A expression.

Napsin A expression was most prevalent in ALCLs; 16.7% (14/84) of all of the ALCL cases expressed napsin A (Table 1, Fig. 2C,D). Of note, napsin A was expressed in 20.9% (14/67) of the systemic ALCLs but not in any of the cutaneous ALCLs (n=17) (P=0.062)

Table 1. Napsin A expression pattern in various lymphomas and histiocytic neoplasms.

	Total	Napsin A- positive n (%)	P	Proportion of napsin A-positive tumor cells				
				0	1-25%	26-50%	51-75%	76-100%
ALCLs	84	14 (16.7)		70	6	1	3	4
Cutaneous ALCL	17	0	0.062*	17	0	0	0	0
Systemic ALCL	67	14 (20.9)		53	6	1	3	4
ALK (+)	32	11 (34.4)	0.015†	21	4	1	3	3
ALK (-)	35	3 (8.6)		32	2	0	0	1
HL	134	15 (11.1)		119	4	7	2	2
DLBCL	149	20 (13.4)		129	13	4	2	1
BL	11	0		11	0	0	0	0
FL	41	2 (4.9)		39	2	0	0	0
MCL	46	0		46	0	0	0	0
MZBCL	56	0		56	0	0	0	0
CLL/SLL	46	0		46	0	0	0	0
PBL	6	0		6	0	0	0	0
PCN	26	1 (3.8)		25	1	0	0	0
AITL	37	2 (5.4)		35	2	0	0	0
PTCL-NOS	30	2 (6.7)		28	2	0	0	0
NKTCL	20	0		20	0	0	0	0
T-LBL	19	0		19	0	0	0	0
B-LBL	10	0		10	0	0	0	0
FDCS	28	0		28	0	0	0	0
HS	5	0		5	0	0	0	0
LCH	22	0		22	0	0	0	0

ALCL, anaplastic large cell lymphoma; HL, Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZBCL, extranodal marginal zone lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; PBL, plasmablastic lymphoma; PCN, plasma cell neoplasm; AITL, angioimmunoblastic T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; NKTCL, extranodal NK/T-cell lymphoma, nasal type; T-LBL, T-lymphoblastic leukemia/lymphoma; B-LBL, B-lymphoblastic leukemia/lymphoma; FDCS, follicular dendritic cell sarcoma; HS, histiocytic sarcoma; LCH, Langerhans cell histiocytosis, *: Cutaneous ALCL vs. systemic ALCL, †: ALK (+) systemic ALCL vs. ALK (-) systemic ALCL.

(Table 1). In particular, among the systemic ALCLs, napsin A expression was observed in 34.4% (11/32) of the ALK-positive ALCLs, the highest frequency of all the lymphoid malignancies; in contrast, only 8.6% (3/35) of the ALK-negative ALCLs expressed napsin A (P=0.015) (Table 1).

Of the HL cases, 11.1% (15/134) expressed napsin A in the tumor cells, i.e., the Hodgkin and Reed-Sternberg (HRS) cells or their variants, and all napsin A-positive HLs were classical HLs (Table 1, Fig. 2E,F). Strong napsin A expression, as defined by immunopositivity in greater than 75% of the neoplastic cells, was observed in 1.5% (2/134) of the HLs.

Napsin A expression was also observed in 13.4% (20/149) of the DLBCLs in a variable proportion of the tumor cells (Table 1, Fig. 2G,H). In contrast, only two cases (4.9%) among the 41 FLs, one case (3.8%) among the 26 PCNs, two cases (5.4%) among the 37 AITLs, and two cases (6.7%) among the 30 PTCL-NOSs exhibited napsin A positivity. Furthermore, the percentage of tumor cells expressing napsin A was low with 25% or less in these lymphomas (Table 1). Notably, none of the other mature or immature B-cell or T/NK-cell lymphomas or the histiocytic neoplasms showed napsin A expression (Table 1).

The clinicopathological features and prognostic implications of napsin A expression in ALCLs and DLBCLs

The clinical characteristics of the ALCLs according to napsin A expression are summarized in Fig. 3 and Table 2. As shown in Fig. 3, the positivity of napsin A and the proportion of napsin A-expressing tumor cells were increased in ALCL patients with a higher IPI, an advanced-stage tumor, a poor performance status, and

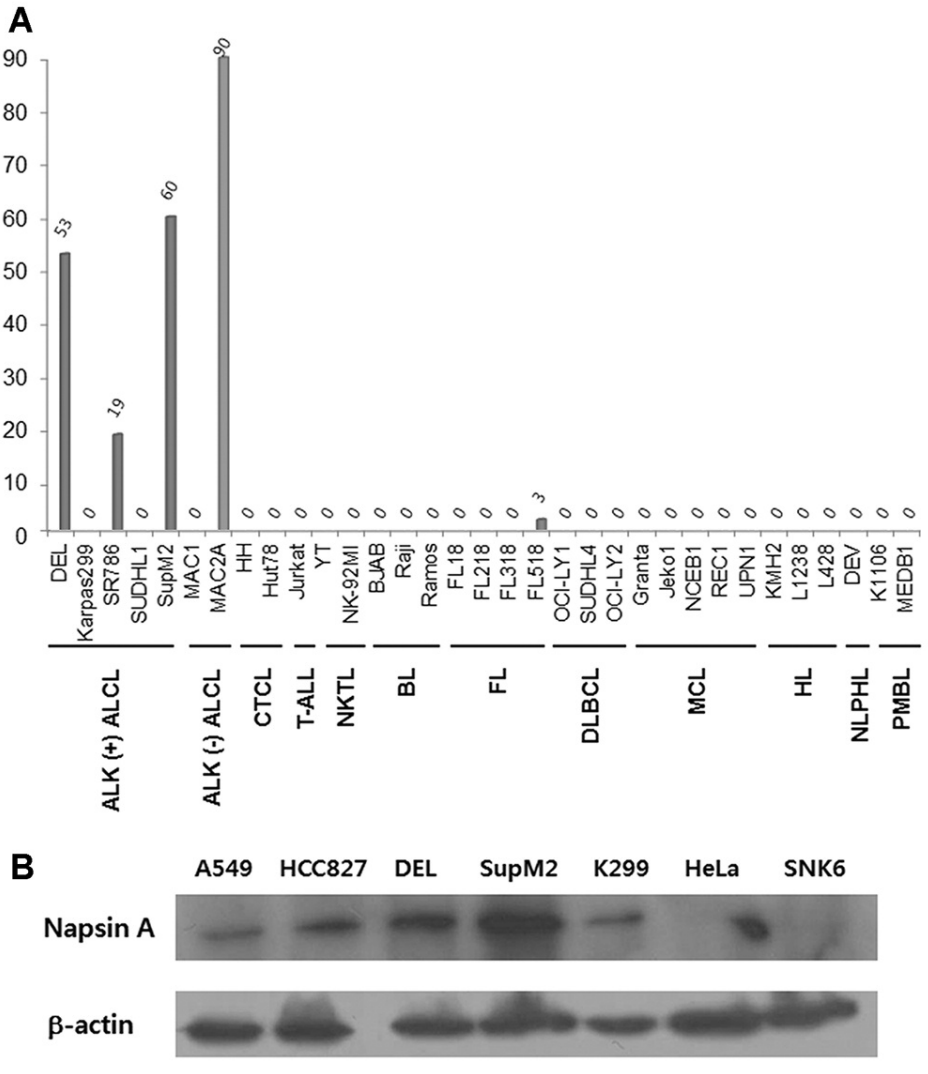

Fig. 1. Detection of napsin A in lymphoma cell lines using glycoproteomics. **A.** N-linked glycoprotein analysis using a liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based glycoproteomic approach was performed in a variety of lymphoma-derived cell lines. The histogram reveals the spectral count of the napsin A peptides, which were detected in three (DEL, SR786, and SupM2) of the five ALK-positive ALCL cell lines, one (MAC2A) of the two ALK-negative ALCL cell lines, and one follicular lymphoma cell line (FL518). Napsin A was not identified in other lymphoma-derived cell lines. **B.** Western blotting analysis revealed an increased level of napsin A protein in the DEL and SupM2 cell lines and a low level of expression in the Karpas299 (K299) cell line. Lung adenocarcinoma cell lines (A549 and HCC827) were used as positive controls, and HeLa and SNK6 cell lines were used as negative controls.

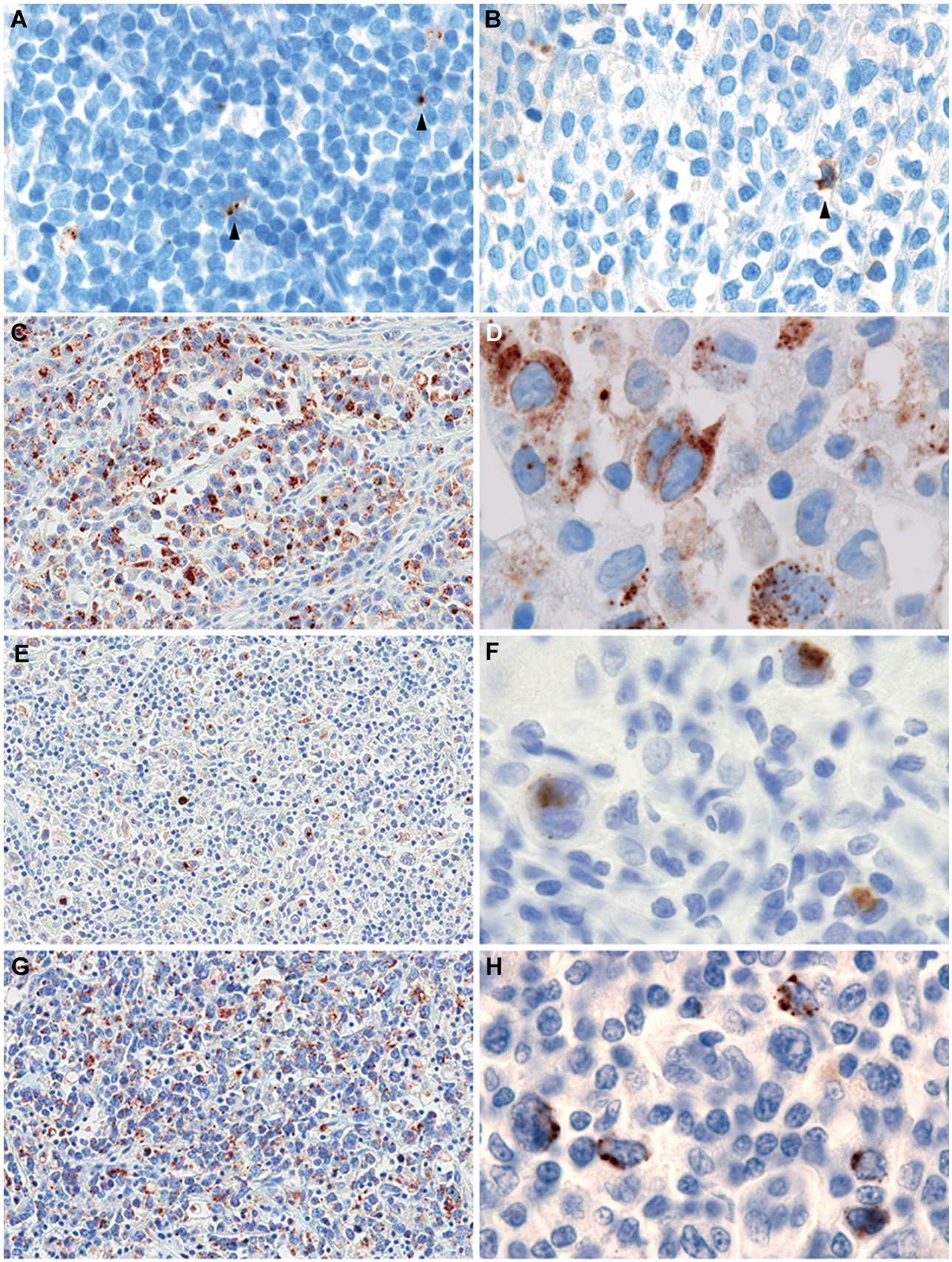

Fig. 2. Napsin A expressions in non-neoplastic and neoplastic lymphoid tissues assessed by immunohistochemistry. **A, B.** Napsin A was positive in only a few histiocytes with a weak cytoplasmic dot-like pattern in a non-neoplastic lymph node **(A)** and tonsil **(B)** (arrows). **C-H.** Representative images demonstrating napsin A expression in the tumor cells in a subset of lymphomas. Napsin A expression demonstrated a granular or dot-like paranuclear pattern in the cytoplasm. Strong napsin A expression was observed in ALK-positive anaplastic large cell lymphoma **(C, D)**, Hodgkin lymphoma **(E, F)**, and diffuse large B-cell lymphoma **(G, H)**. A, B, × 600; C, E, G, × 200; D, F, H, ×1000.

bone marrow involvement. Within the patients with systemic ALCLs (n=67) (Table 2), napsin A positivity was significantly associated with a higher IPI score (P=0.034). Of the systemic ALK-positive ALCL patients with available clinical data, those with napsin A-positive tumors presented with B symptom, a higher IPI score, and advanced stage; however, the differences were statistically insignificant compared with those negative for napsin A (Table 2).

The survival curves for the ALCL and DLBCL patients according to napsin A expression are displayed in Fig. 4. The patients with cutaneous ALCL exhibited better overall survival (OS) and progression-free survival (PFS) compared with those with systemic ALCL. In the latter group, the ALK-positive ALCL patients tended to survive longer than the ALK-negative ALCL patients (Fig. 4A). These clinical outcomes were consistent with previously published data (ten Berge et al., 2003), although statistical significance was not achieved in the present study. Interestingly, systemic ALCL patients with napsin A-positive tumors demonstrated a reduced OS (P=0.061) (Fig. 4B). Moreover, strong napsin A expression was significantly associated with a worse OS (Fig. 4C). Furthermore, when the ALK-positive and ALK-negative ALCL patients were separately analyzed, patients with napsin

Table 2. Clinical features of systemic ALCLs according to napsin A expression.

Variables*		Systemic ALCL			ALK-positive systemic ALCL			ALK-negative systemic ALCL		
		n/total	Napsin A-positive (%)	P	n/total	Napsin A-positive (%)	P	n/total	Napsin A-positive (%)	P
Age (yr)	≤18	13/44	4 (30.8)	0.471	11/26	4 (36.4)	0.353	2/18	0	0.383
	>18 to ≤ 60	25/44	4 (16)		15/26	3 (20)		10/18	1 (10)	
	> 61	6/44	2 (33.3)		0	0		6/18	2 (33.3)	
Sex	Male	32/44	8 (25)	0.702	20/26	6 (30)	1.000	12/18	2 (16.7)	1.000
	Female	12/44	2 (16.7)		6/26	1 (16.7)		6/18	1 (16.7)	
B symptom	Present	25/39	7 (28)	0.218	20/24	6 (30)	0.539	5/15	1 (20)	1.000
IPI	3 - 5	26/40	8 (30.8)	0.034	17/25	6 (35.3)	0.129	9/15	2 (22.2)	0.486
Stage	III, IV	19/38	6 (31.6)	0.232	17/24	6 (35.3)	0.130	2/14	0	1.000
ECOG PS	3 - 5	6/40	2 (33.3)	0.580	4/25	2 (50)	0.234	2/15	0	1.000
LDH	Elevated	16/37	4 (25)	0.705	11/23	4 (36.4)	0.371	5/14	0	0.505
BM involvement	Present	4/34	2 (50)	0.171	3/22	2 (66.7)	0.169	1/13	0	1.000
Bulky disease	Present	4/41	2 (50)	0.165	1/25	1 (100)	0.240	3/16	1 (33.3)	0.350
No. of EN site	≥ 2	2/42	0	0.720	2/25	0	0.631	0/17	0	1.000
Therapeutic response*	CR	26/37	4 (15.4)	0.203	14/21	3 (21.4)	1.000	12/16	1 (8.3)	0.136
	PR, SD, PD	11/37	4 (36.4)		7/21	2 (28.6)		4/16	2 (50)	

n.s., not significant; IPI, International prognostic index; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; BM, bone marrow; No. number; EN, extranodal; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; *: Some variables contain missing values due to the lack of relevant information or could not be classified into the categories of systemic or cutaneous disease.

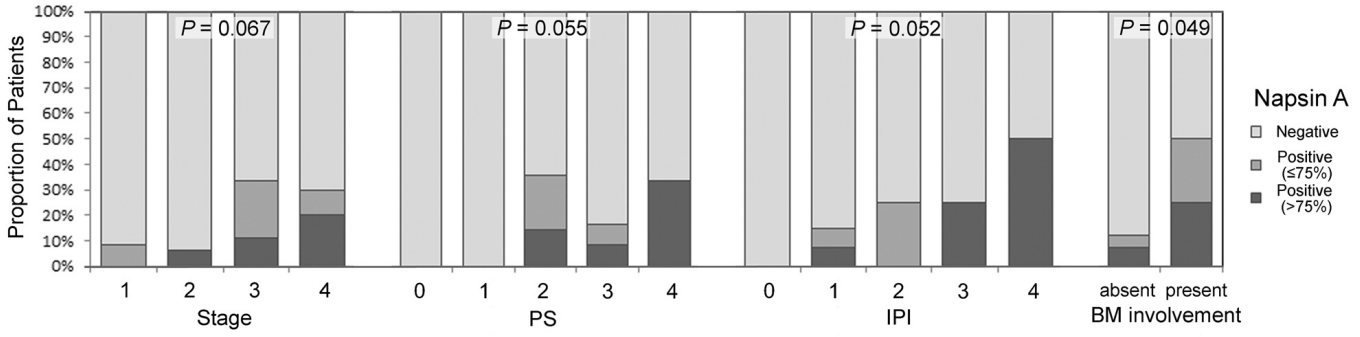

Fig. 3. Correlation of Napsin A expression and clinical variables in ALCLs. The proportion of ALCL patients who were negative for napsin A and positive for napsin A in ≤75% or greater than 75% of the tumor cells are plotted according to the clinical variables. The statistical significance of the correlations between napsin A expression and variables were assessed using Spearman's rho test. Napsin A expression was associated with a more advanced stage, a higher performance status (PS), bone marrow (BM) involvement, and a higher IPI score.

A-expressing tumors tended to have a poor prognosis in each group (Fig. 4D,E), although this was statistically insignificant.

No significant relationship was noted between clinicopathological features and napsin A expression in the DLBCL patients (data not shown). However, DLBCL patients with napsin A-positive tumors tended to have poor prognosis (Fig. 4F,G).

Discussion

Through an unbiased glycoproteomic analysis of lymphoma-derived cell lines and validation using immunohistochemistry on a large number of patient tumor tissues, we demonstrated for the first time that napsin A is aberrantly expressed in a subset of malignant lymphomas. Moreover, the present study indicates that napsin A expression is largely restricted to certain lymphoma subtypes, including ALK-positive ALCL, HL, and DLBCL. These data provide novel information on napsin A in lymphomas and its potential prognostic utility in ALCLs and DLBCL.

Napsin A was initially found to be exclusively expressed in pulmonary adenocarcinomas and some renal cell carcinomas (Ordonez, 2012). Therefore, napsin A has been considered an excellent diagnostic marker for determining the origin of adenocarcinomas and the histologic subtype of lung cancers (Kim et al., 2014). However, recently, other tumors have been shown to express napsin A, including endometrial and ovarian clear cell adenocarcinomas, adrenal cortical neoplasms, and anaplastic or poorly differentiated thyroid carcinomas (Ballard et al., 2013; Chernock et al., 2013; Skirnisdottir et al., 2013; Fadare et al., 2014). These observations emphasized the need to use multiple immunomarkers in combination to make a pathologic diagnosis in equivocal cases and raised a potential role of napsin A in the pathogenesis of cancers other than lung and kidney.

Although an initial study on napsin A using polyclonal antibodies revealed only a few scattered napsin A-immunostained cells in the human spleen, no additional information has been available on napsin A expression in the lymphoreticular system (Schauer-Vukasinovic et al., 1999). The present study demonstrates that several lymphoma types should be

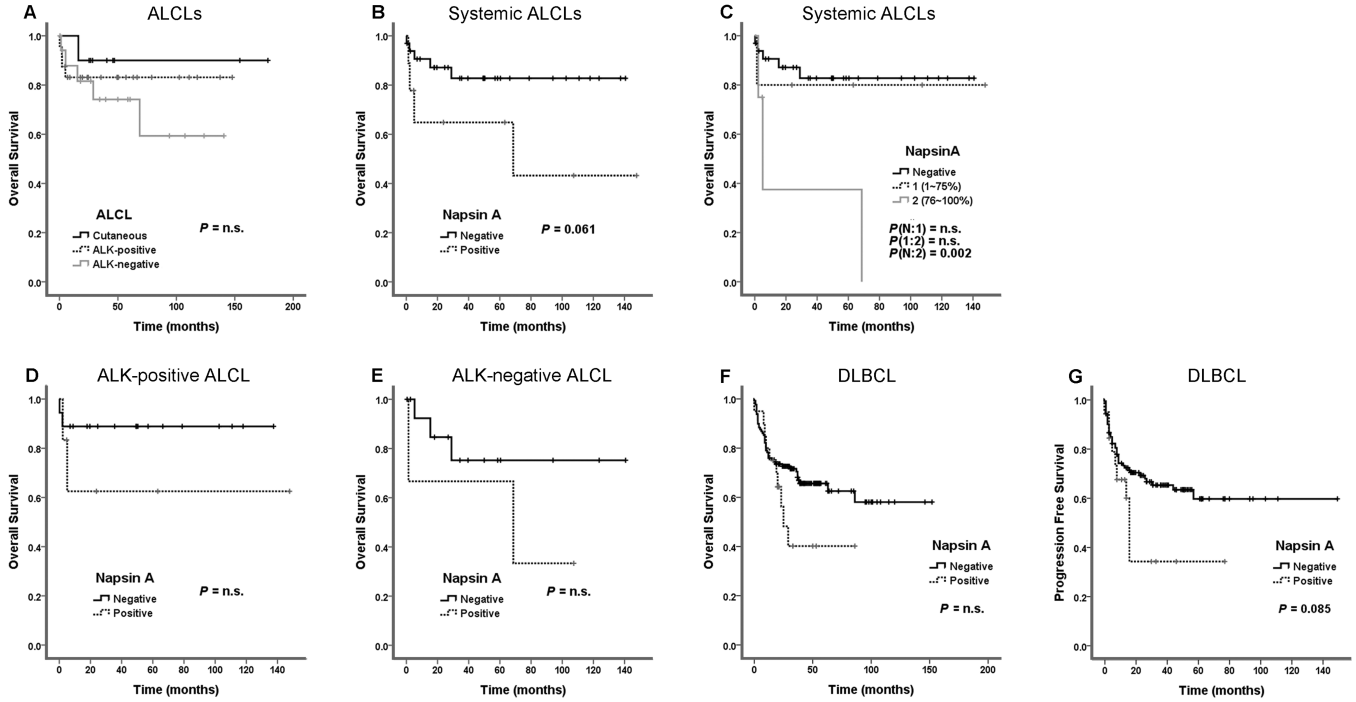

Fig. 4. Survival analyses according to napsin A expression. **A**. Kaplan-Meir survival curves with a log rank test for the overall survival (OS) of ALCL patients according to the subtypes of ALCLs, including cutaneous, systemic ALK-positive, and systemic ALK-negative ALCLs, are shown. **B, C**. Kaplan-Meir survival curves with a log rank test for the overall survival (OS) of systemic ALCL patients. In the patients with systemic ALCL, the patients whose tumor expressed napsin A exhibited a shorter OS. Strong napsin A expression was significantly related with a worse OS. **D, E.** Kaplan-Meir survival curves with a log rank test for the OS of ALK-positive and negative ALCL patients. In the group with ALK-positive or ALK-negative ALCLs, patients whose tumors showed napsin A expression had a tendency toward a poor prognosis. **F, G.** Kaplan-Meir survival curves with a Taron-Ware test for the OS and progression-free survival (PFS). Patients with DLBCLs positive for napsin A also exhibited a shorter survival compared to those negative for napsin A, but with no statistical significance.

included in the differential diagnosis of napsin A-positive tumors. Moreover, it is noteworthy that 34% of the systemic ALK-positive ALCLs expressed napsin A. Given that ALK-positive ALCLs often lose expression of leukocyte-associated molecules, such as CD45 and CD3, while often expressing epithelial membrane antigen (EMA) and that approximately 5% of non-small cell lung cancers express ALK (Park et al., 2012), napsin A- expressing ALK-positive ALCL may be included as a differential diagnosis of ALK-positive pulmonary adenocarcinoma. The incorporation of additional markers, including CD30, cytotoxic molecules, TTF-1, and cytokeratin, and recognizing that ALK-positive ALCLs often express napsin A would be helpful for pathologic diagnosis of napsin A-positive tumors.

A limited number of studies have addressed the prognostic implications of napsin A expression, mainly in carcinomas originating from the lungs and kidneys. The absence of napsin A expression was found to be an independent poor prognostic factor for patients with surgically resected lung adenocarcinomas (Lee et al., 2012). In renal cell carcinomas, napsin A expression was inversely related to aggressive local tumor characteristics, such as advanced pathological stage and an increased nuclear grade (Xu et al., 2013). Furthermore, an in vitro study revealed that napsin A inhibits the growth of the tumorigenic HEK293 cell line (Ueno et al., 2008). In contrast, in the present study, napsin A expression tended to be associated with a higher IPI score and poor clinical outcome in patients with systemic ALCLs. Although statistical significance was not reached, napsin A expression was correlated with the presence of B symptom, a higher IPI, and an advanced stage in ALK-positive ALCLs. Moreover, patients with napsin A-positive DLBCL exhibited a tendency toward shorter survival compared to those with napsin A-negative DLBCL. However, this study has a limitation in that the number of ALCL patients is relatively small, which made it difficult to draw a significant conclusion regarding the clinical and prognostic implication of napsin A. Moreover, the difference in the survival of DLBCL patients according to the napsin A expression was statistically insignificant. Thus, an investigation using a larger number of ALCL or DLBCL patients would be needed to clarify the prognostic implication of napsin A expression.

In this study, napsin A expression was confined to tumor cells and not bystander cells. Napsin A expression in lymphoid or histiocytic cells other than pulmonary alveolar macrophages has not been observed. Thus, we think that napsin A expression in some lymphoma cells would be aberrantly induced. The lymphoma subtypes that exhibited napsin A expression in greater than 10% of the cases included ALK-positive ALCL, HL, and DLBCL. These tumors vary in terms of the cell of origin (i.e., T cells or B cells). ALK-positive ALCL and HL share some morphological characteristics, including a large size, a generous cytoplasm, and anaplastic features. Given that napsin A potentially plays a role in protein catabolism in renal tubules and in the maturation of surfactant proteins in the lung (Mori et al., 1997; Brasch et al., 2003), it is conceivable that napsin A might be involved in the catabolism of proteins that lead to the unique morphology shared by ALK-positive ALCL and HL. However, despite the similar cytomorphology of tumor cells in ALK-negative ALCL and cutaneous ALCL, napsin A was rarely expressed in these lymphomas. The potential role of ALK in regulation of napsin A expression is of interest because ALK-positive ALCLs exhibited the most frequent and the strongest expression of napsin A among all of the lymphoid malignancies. However, at present, the mechanism underlying the aberrant napsin A expression in subset of lymphoma cells and the functional role of napsin A in lymphoma remain unclear.

In summary, the present study demonstrated that napsin A is aberrantly expressed in a subset of lymphomas, particularly in ALK-positive ALCL, HL, and DLBCL; therefore, these lymphomas should be included in the differential diagnosis of napsin A-positive tumors. The biological and functional relevance of napsin A in lymphoma warrants further study.

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